

Standard Methods

FOR THE

Examination
of Water and
Wastewater

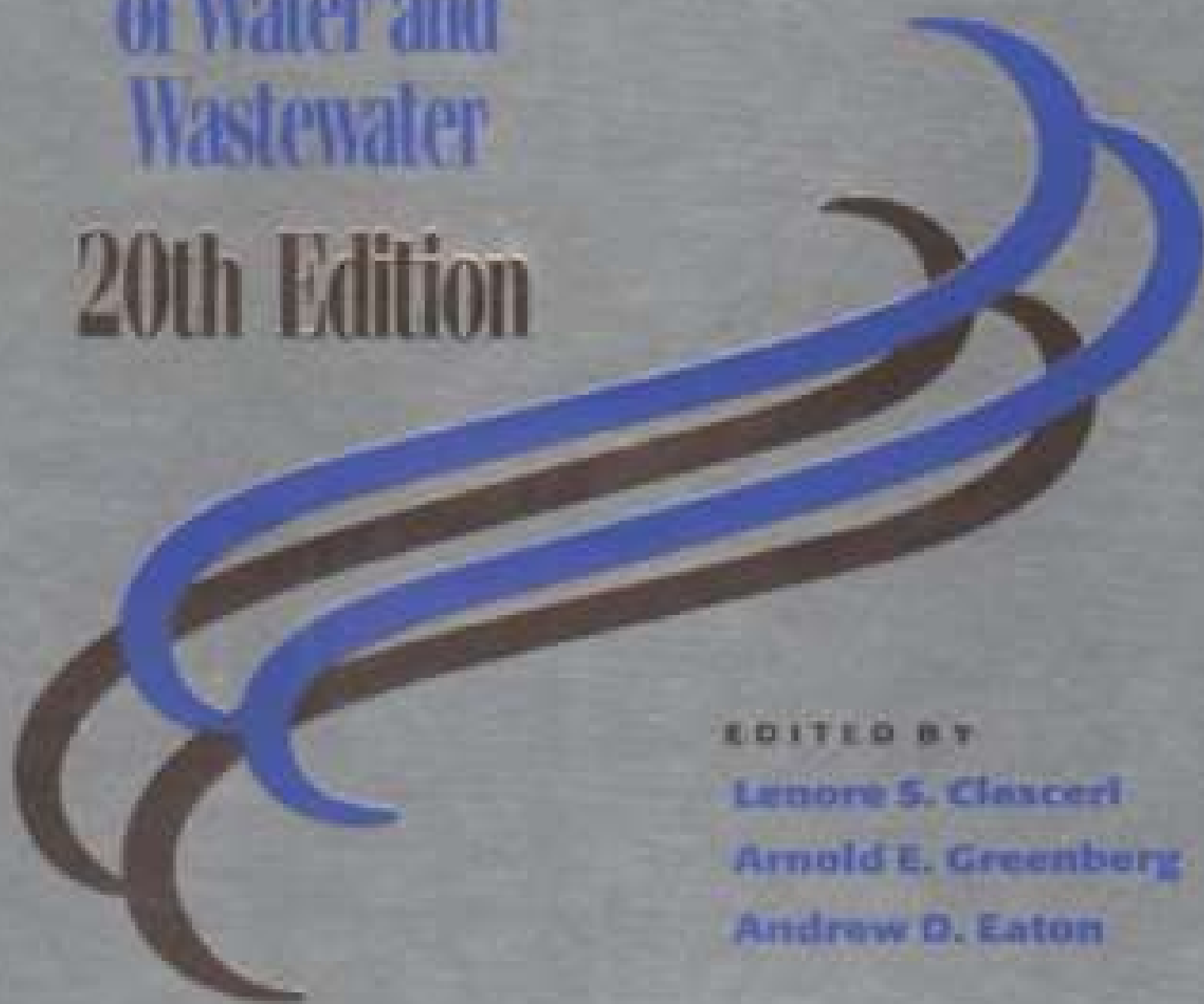
20th Edition

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Standard Methods for the Examination of Water and Wastewater

PREFACE TO THE TWENTIETH EDITION

The Nineteenth and Earlier Editions

The first edition of *Standard Methods* was published in 1905. Each subsequent edition presented significant improvements of methodology and enlarged its scope to include techniques suitable for examination of many types of samples encountered in the assessment and control of water quality and water pollution.

A brief history of *Standard Methods* is of interest because of its contemporary relevance. A movement for “securing the adoption of more uniform and efficient methods of water analysis” led in the 1880’s to the organization of a special committee of the Chemical Section of American Association for the Advancement of Science. A report of this committee, published in 1889, was entitled: A Method, in Part, for the Sanitary Examination of Water, and for the Statement of Results, Offered for General Adoption.*#(1) Five topics were covered: (1) “free” and “albuminoid” ammonia; (2) oxygen-consuming capacity; (3) total nitrogen as nitrates and nitrites; (4) nitrogen as nitrites; and (5) statement of results.

In 1895, members of the American Public Health Association, recognizing the need for standard methods in the bacteriological examination of water, sponsored a convention of bacteriologists to discuss the problem. As a result, an APHA committee was appointed “to draw up procedures for the study of bacteria in a uniform manner and with special references to the differentiation of species.” Submitted in 1897,†#(2) the procedures found wide acceptance.

In 1899, APHA appointed a Committee on Standard Methods of Water Analysis, charged with the extension of standard procedures to all methods involved in the analysis of water. The committee report, published in 1905, constituted the first edition of *Standard Methods* (then entitled *Standard Methods of Water Analysis*). Physical, chemical, microscopic, and bacteriological methods of water examination were included. In its letter of transmittal, the Committee stated:

The methods of analysis presented in this report as “Standard Methods” are believed to represent the best current practice of American water analysts, and to be generally applicable in connection with the ordinary problems of water purification, sewage disposal and sanitary investigations. Analysts working on widely different problems manifestly cannot use methods which are identical, and special problems obviously require the methods best adapted to them; but, while recognizing these facts, it yet remains true that sound progress in analytical work will advance in proportion to the general adoption of methods which are reliable, uniform and adequate.

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It is said by some that standard methods within the field of applied science tend to stifle investigations and that they retard true progress. If such standards are used in the proper spirit, this ought not to be so. The Committee strongly desires that every effort shall be continued to improve the techniques of water analysis and especially to compare current methods with those herein recommended, where different, so that the results obtained may be still more accurate and reliable than they are at present.

Revised and enlarged editions were published by APHA under the title *Standard Methods of Water Analysis* in 1912 (Second Edition), 1917 (Third), 1920 (Fourth), and 1923 (Fifth). In 1925, the American Water Works Association joined APHA in publishing the Sixth Edition, which had the broader title, *Standard Methods of the Examination of Water and Sewage*. Joint publication was continued in the Seventh Edition, dated 1933.

In 1935, the Federation of Sewage Works Associations (now the Water Environment Federation) issued a committee report, “Standard Methods of Sewage Analysis.”³ With minor modifications, these methods were incorporated into the Eighth Edition (1936) of *Standard Methods*, which was thus the first to provide methods for the examination of “sewages, effluents, industrial wastes, grossly polluted waters, sludges, and muds.” The Ninth Edition, appearing in 1946, likewise contained these methods, and in the following year the Federation became a full-fledged publishing partner. Since 1947, the work of the *Standard Methods* committees of the three associations—APHA, AWWA, and WEF—has been coordinated by a Joint Editorial Board, on which all three are represented.

The Tenth Edition (1955) included methods specific for examination of industrial wastewaters; this was reflected by a new title: *Standard Methods for the Examination of Water, Sewage and Industrial Wastes*. To describe more accurately and concisely the contents of the Eleventh Edition (1960), the title was shortened to *Standard Methods for the Examination of Water and Wastewater*. It remained unchanged in the Twelfth Edition (1965), the Thirteenth Edition (1971), the Fourteenth Edition (1976), and the Fifteenth Edition (1981).

In the Fourteenth Edition, the separation of test methods for water from those for wastewater was discontinued. All methods for a given component or characteristic appeared under a single heading. With minor differences, the organization of the Fourteenth Edition was retained for the Fifteenth and Sixteenth (1985) Editions. Two major policy decisions of the Joint Editorial Board were implemented for the Sixteenth Edition. First, the International System of Units (SI) was adopted except where prevailing field systems or practices require English units. Second, the use of trade names or proprietary materials was eliminated insofar as possible, to avoid potential claims regarding restraint of trade or commercial favoritism.

The organization of the Seventeenth Edition (1989) reflected a commitment to develop and retain a permanent numbering system. New numbers were assigned to all sections, and unused numbers were reserved for future use. All part numbers were expanded to multiples of 1000 instead of 100. The parts retained their identity from the previous edition, with the exception of Part 6000, which contained methods for the measurement of specific organic compounds. The

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more general procedures for organics were found in Part 5000.

The Seventeenth Edition also underwent a major revision in the introductory Part 1000. Sections dealing with statistical analysis, data quality, and methods development were greatly expanded. The section on reagent water was updated to include a classification scheme for various types of reagent water. At the beginning of each of the subsequent parts of the manual, sections were included that discussed quality assurance and other matters of general application within the specific subject area, to minimize repetition in the succeeding text.

The Eighteenth Edition (1992) underwent only minor revisions in the format from the 17th edition. A number of new methods were added in each section. The 18th Edition has many of its methods cited for compliance monitoring of both drinking water and wastewater.

In the Nineteenth Edition (1995), sections were added on laboratory safety and waste management in Part 1000. Substantial changes occurred throughout, adding new methodology and revisions to many of the sections.

The Twentieth Edition

The Twentieth Edition has maintained the trend of the Nineteenth Edition in continued renewal of Part 1000. Significant revision has occurred in the sections on data quality (1030), sampling (1060) and reagent water (1080).

In Part 2000 (physical and aggregate properties), odor (2150) has been revised to supply new tables for odor identification. The salinity (2520) formula has been made compatible with conductivity nomenclature and quality control procedures have been updated and strengthened.

Significant reworking of the introductory material has occurred in Part 3000 (metals); the introduction now includes a user guide to appropriate methods of metal analysis. A new section, inductively coupled plasma/mass spectrometry (ICP/MS), has been added. Anodic stripping voltammetry (3130) has been expanded to include zinc. The sections on ICP, sample preparation, and specific metal analyses have been revised.

Part 4000 (inorganic nonmetallic constituents) has been reviewed and includes new methods on flow injection analysis (4130), potassium permanganate (4500-KMnO₄), and capillary ion electrophoresis (4140). Ozone (4500-O₃) methods have been updated. Significant revisions also have been made in the nitrogen sections. Other sections have undergone minor revisions.

Part 5000 (aggregate organic constituents) has significantly revised sections on chemical oxygen demand (5220), total organic carbon (5310) (from the Nineteenth Edition supplement), and dissolved organic halogen (5320). Freon has been mostly replaced by hexane in the oil and grease section (5520).

In Part 6000 (individual organic compounds), a new section on volatile organic compounds has replaced a number of old sections and a major section on quality control has been added.

Various editorial changes were made in Part 7000 (radioactivity) and a revision in gamma-emitting radionuclides (7120) was made.

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Part 8000 (toxicity testing) underwent major changes with new protocols for quality assurance (8020), P450 methodology (8070) from the Nineteenth Edition supplement, pore water test procedures (8080), protozoa (8310), rotifers (8420), *Daphnia* (8711), *Ceriodaphnia* (8712), mysids (8714), decapods (8740), echinoderm fertilization and development (8810), and fathead minnows (8911). Other sections have been revised significantly and illustrations of many test organisms have been added.

Part 9000 (microbiological examination) has had major revisions to quality assurance and pathogenic bacteria (9260) and minor revisions in several other sections.

Part 10000 (biological examination) has undergone minor revisions. Some new figures and illustrations of organisms have been added.

Making Reagents

Following the instructions for making reagents may result in preparation of quantities larger than actually needed. In some cases these materials are toxic. To promote economy and minimize waste, the analyst should review needs and scale down solution volumes where appropriate. This conservative attitude also should extend to purchasing policies so that unused chemicals do not accumulate or need to be discarded as their shelf lives expire.

Selection and Approval of Methods

For each new edition both the technical criteria for selection of methods and the formal procedures for their approval and inclusion are reviewed critically. In regard to the approval procedures, it is considered particularly important to assure that the methods presented have been reviewed and are supported by the largest number of qualified people, so that they may represent a true consensus of expert opinion.

For the Fourteenth Edition a Joint Task Group was established for each test. This scheme has continued for each subsequent edition. Appointment of an individual to a Joint Task Group generally was based on the expressed interest or recognized expertise of the individual. The effort in every case was to assemble a group having maximum available expertise in the test methods of concern.

Each Joint Task Group was charged with reviewing the pertinent methods in the Nineteenth Edition along with other methods from the literature, recommending the methods to be included in the Twentieth Edition, and presenting those methods in the form of a proposed section manuscript. Subsequently, each section manuscript (except for Part 1000) was ratified by vote of those members of the Standard Methods Committee who asked to review sections in that part. Every negative vote and every comment submitted in the balloting was reviewed by the Joint Editorial Board. Relevant suggestions were referred appropriately for resolution. When negative votes on the first ballot could not be resolved by the Joint Task Group or the Joint Editorial Board, the section was reballoted among all who voted affirmatively or negatively on the

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original ballot. Only a few issues could not be resolved in this manner and the Joint Editorial Board made the final decision.

The general and quality assurance information presented in Part 1000 was treated somewhat differently. Again, Joint Task Groups were formed, given a charge, and allowed to produce a consensus draft. This draft was reviewed by the Joint Editorial Board Liaison and subsequently by the Joint Editorial Board. The draft sections were sent to the Standard Methods Committee and comments resulting from this review were used to develop the final draft.

The methods presented here, as in previous editions, are believed to be the best available and generally accepted procedures for the analysis of water, wastewaters, and related materials. They represent the recommendations of specialists, ratified by a large number of analysts and others of more general expertise, and as such are truly consensus standards, offering a valid and recognized basis for control and evaluation.

The technical criteria for selection of methods were applied by the Joint Task Groups and by the individuals reviewing their recommendations, with the Joint Editorial Board providing only general guidelines. In addition to the classical concepts of precision, bias, and minimum detectable concentration, selection of a method also must recognize such considerations as the time required to obtain a result, needs for specialized equipment and for special training of the analyst, and other factors related to the cost of the analysis and the feasibility of its widespread use.

Status of Methods

All methods in the Twentieth Edition are dated to assist users in identifying those methods that have been changed significantly between editions. The year the section was approved by the Standard Methods Committee is indicated in a footnote at the beginning of each section. Sections or methods that appeared in the Nineteenth Edition that are unchanged, or changed only editorially in the Twentieth Edition, show an approval date of 1993 or 1994. Sections or methods that were changed significantly, or that were reaffirmed by general balloting of the Standard Methods Committee, are dated 1996 or 1997. If an individual method within a section was revised, that method carries an approval date different from that of the rest of the section.

Methods in the Twentieth Edition are divided into fundamental classes: PROPOSED, SPECIALIZED, STANDARD, AND GENERAL. None of the methods in the Twentieth Edition have the specialized designation. Regardless of assigned class, all methods must be approved by the Standard Methods Committee. The four classes are described below:

1. PROPOSED—A PROPOSED method must undergo development and validation that meets the requirements set forth in Section 1040A of *Standard Methods*.
2. SPECIALIZED—A procedure qualifies as a SPECIALIZED method in one of two ways:
 - a) The procedure must undergo development and validation and collaborative testing that meet the requirements set forth in Section 1040B and C of *Standard Methods*, respectively; or
 - b) The procedure is the “METHOD OF CHOICE” of the members of the Standard Methods

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Committee actively conducting the analysis and it has appeared in TWO PREVIOUS EDITIONS of *Standard Methods*.

3. STANDARD—A procedure qualifies as a STANDARD method in one of two ways: a) The procedure must undergo development and validation and collaborative testing that meet the requirements set forth in Section 1040B and C of *Standard Methods*, respectively, and it is “WIDELY USED” by the members of the Standard Methods Committee; or b) The procedure is “WIDELY USED” by the members of the Standard Methods Committee and it has appeared in TWO PREVIOUS EDITIONS of *Standard Methods*.

4. GENERAL—A procedure qualifies as a GENERAL method if it has appeared in TWO PREVIOUS EDITIONS of *Standard Methods*.

Assignment of a classification to a method is done by the Joint Editorial Board. When making method classifications, the Joint Editorial Board evaluates the results of the survey on method use by the Standard Methods Committee that is conducted at the time of general balloting of the method. In addition, the Joint Editorial Board considers recommendations offered by Joint Task Groups and the Part Coordinator.

Methods categorized as “PROPOSED,” “SPECIALIZED,” and “GENERAL” are so designated in their titles; methods with no designation are “STANDARD.”

Technical progress makes advisable the establishment of a program to keep *Standard Methods* abreast of advances in research and general practice. The Joint Editorial Board has developed the following procedure for effecting interim changes in methods between editions:

1. Any method given proposed status in the current edition may be elevated by action of the Joint Editorial Board, on the basis of adequate published data supporting such a change as submitted to the Board by the appropriate Joint Task Group. Notification of such a change in status shall be accomplished by publication in the official journals of the three associations sponsoring *Standard Methods*.

2. No method may be abandoned or reduced to a lower status during the interval between editions.

3. A new method may be adopted as proposed, specialized, or standard by the Joint Editorial Board between editions, such action being based on the usual consensus procedure. Such new methods may be published in supplements to editions of *Standard Methods*. It is intended that a supplement be published midway between editions.

Even more important to maintaining the current status of these standards is the intention of the sponsors and the Joint Editorial Board that subsequent editions will appear regularly at reasonably short intervals.

Reader comments and questions concerning this manual should be addressed to: Standard Methods Manager, American Water Works Association, 6666 West Quincy Avenue, Denver, CO 80235.

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Acknowledgments

For the work in preparing the methods for the Twentieth Edition, the Joint Editorial Board gives full credit to the Standard Methods Committees of the American Water Works Association and of the Water Environment Federation and to the Committee on Laboratory Standards and Practices of the American Public Health Association. Full credit also is given to those individuals who were not members of the sponsoring societies. A list of all committee members follows these pages. Herbert J. Brass, U.S. Environmental Protection Agency, served as a liaison from EPA to the Joint Editorial Board; thanks are due for his interest and help.

The Joint Editorial Board expresses its appreciation to Fernando M. Trevino, former Executive Director, and Mohammad N. Akhter, M.D., current Executive Director, American Public Health Association, to John B. Mannion, former Executive Director, and Jack W. Hoffbuhr, current Executive Director, American Water Works Association, and to Quincalee Brown, Executive Director, Water Environment Federation, for their cooperation and advice in the development of this publication. Steven J. Posavec, Standard Methods Manager and Joint Editorial Board Secretary, provided a variety of important services that are vital to the preparation of a volume of this type. Ellen Meyer, Director of Publications, American Public Health Association, functioned as publisher. Judy Castagna, also with APHA, served as production manager. Special recognition for her valuable services is due to Mary Ann H. Franson, Managing Editor, who discharged most efficiently the extensive and detailed responsibilities on which this publication depends.

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Endnotes

1 (Popup - Footnote)

* *J. Anal. Chem.* 3:398 (1889).

2 (Popup - Footnote)

† *Proc. Amer. Pub. Health Assoc.* 23:56 (1897).

3 (Popup - Footnote)

‡ *Sewage Works J.* 7:444 (1935).

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Part 1000 INTRODUCTION

1010 INTRODUCTION

1010 A. Scope and Application of Methods

The procedures described in these standards are intended for the examination of waters of a wide range of quality, including water suitable for domestic or industrial supplies, surface water, ground water, cooling or circulating water, boiler water, boiler feed water, treated and untreated municipal or industrial wastewater, and saline water. The unity of the fields of water supply, receiving water quality, and wastewater treatment and disposal is recognized by presenting methods of analysis for each constituent in a single section for all types of waters.

An effort has been made to present methods that apply generally. Where alternative methods are necessary for samples of different composition, the basis for selecting the most appropriate method is presented as clearly as possible. However, samples with extreme concentrations or otherwise unusual compositions or characteristics may present difficulties that preclude the direct use of these methods. Hence, some modification of a procedure may be necessary in specific instances. Whenever a procedure is modified, the analyst should state plainly the nature of modification in the report of results.

Certain procedures are intended for use with sludges and sediments. Here again, the effort has been to present methods of the widest possible application, but when chemical sludges or slurries or other samples of highly unusual composition are encountered, the methods of this manual may require modification or may be inappropriate.

Most of the methods included here have been endorsed by regulatory agencies. Procedural modification without formal approval may be unacceptable to a regulatory body.

The analysis of bulk chemicals received for water treatment is not included herein. A committee of the American Water Works Association prepares and issues standards for water treatment chemicals.

Part 1000 contains information that is common to, or useful in, laboratories desiring to produce analytical results of known quality, that is, of known accuracy and with known uncertainty in that accuracy. To accomplish this, apply the quality assurance methods described herein to the standard methods described elsewhere in this publication. Other sections of Part 1000 address laboratory equipment, laboratory safety, sampling procedures, and method development and validation, all of which provide necessary information.

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1010 B. Statistics

1. Normal Distribution

If a measurement is repeated many times under essentially identical conditions, the results of each measurement, x , will be distributed randomly about a mean value (arithmetic average) because of uncontrollable or experimental error. If an infinite number of such measurements were to be accumulated, the individual values would be distributed in a curve similar to those shown in Figure 1010:1. The left curve illustrates the Gaussian or normal distribution, which is described precisely by the mean, μ , and the standard deviation, σ . The mean, or average, of the distribution is simply the sum of all values divided by the number of values so summed, i.e., $\mu = (\sum_i x_i)/n$. Because no measurements are repeated an infinite number of times, an *estimate* of the mean is made, using the same summation procedure but with n equal to a finite number of repeated measurements (10, or 20, or . . .). This estimate of μ is denoted by $\bar{x}$. The standard deviation of the normal distribution is defined as $\sigma = [\sum(x-\mu)^2/n]^{1/2}$. Again, the analyst can only estimate the standard deviation because the number of observations made is finite; the estimate of σ is denoted by s and is calculated as follows:

$$s = [\sum(x - \bar{x})^2 / (n - 1)]^{1/2}$$

The standard deviation fixes the width, or spread, of the normal distribution, and also includes a fixed fraction of the values making up the curve. For example, 68.27% of the measurements lie between $\mu \pm 1\sigma$, 95.45% between $\mu \pm 2\sigma$, and 99.70% between $\mu \pm 3\sigma$. It is sufficiently accurate to state that 95% of the values are within $\pm 2\sigma$ and 99% within $\pm 3\sigma$. When values are assigned to the $\pm\sigma$ multiples, they are confidence limits. For example, 10 ± 4 indicates that the confidence limits are 6 and 14, while values from 6 to 14 represent the confidence interval.

Another useful statistic is the standard error of the mean, σ_μ , which is the standard deviation divided by the square root of the number of values, or $\sigma/\sqrt{n}$. This is an estimate of the accuracy of the mean and implies that another sample from the same population would have a mean within some multiple of this. Multiples of this statistic include the same fraction of the values as stated above for σ . In practice, a relatively small number of average values is available, so the confidence intervals of the mean are expressed as $\bar{x} \pm ts/\sqrt{n}$ where t has the following values for 95% confidence intervals:

n	t
2	12.71
3	4.30

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<i>n</i>	<i>t</i>
4	3.18
5	2.78
10	2.26
∞	1.96

The use of *t* compensates for the tendency of a small number of values to underestimate uncertainty. For $n > 15$, it is common to use $t = 2$ to estimate the 95% confidence interval.

Still another statistic is the relative standard deviation, σ/μ , with its estimate s/x , also known as the coefficient of variation (CV), which commonly is expressed as a percentage. This statistic normalizes the standard deviation and sometimes facilitates making direct comparisons among analyses that include a wide range of concentrations. For example, if analyses at low concentrations yield a result of 10 ± 1.5 mg/L and at high concentrations 100 ± 8 mg/L, the standard deviations do not appear comparable. However, the percent relative standard deviations are $100 (1.5/10) = 15\%$ and $100 (8/100) = 8\%$, which indicate the smaller variability obtained by using this parameter.

2. Log-Normal Distribution

In many cases the results obtained from analysis of environmental samples will not be normally distributed, i.e., a graph of the data will be obviously skewed, as shown at right in Figure 1010:1, with the mode, median, and mean being distinctly different. To obtain a nearly normal distribution, convert the results to logarithms and then calculate $\bar{x}$ and s . The antilogarithms of these two values are estimates of the geometric mean and the geometric standard deviation, x_g and s_g .

3. Rejection of Data

Quite often in a series of measurements, one or more of the results will differ greatly from the other values. Theoretically, no result should be rejected, because it may indicate either a faulty technique that casts doubt on all results or the presence of a true variant in the distribution. In practice, reject the result of any analysis in which a known error has occurred. In environmental studies, extremely high and low concentrations of contaminants may indicate the existence of areas with problems or areas with no contamination, so they should not be rejected arbitrarily.

An objective test for outliers has been described.¹ If a set of data is ordered from low to high: $x_L, x_2 \dots x_H$, and the average and standard deviation are calculated, then suspected high or low outliers can be tested by the following procedure. First, calculate the statistic *T* :

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$$T = (x_H - \bar{x})/s \text{ for a high value, or}$$
$$T = (\bar{x} - x_L)/s \text{ for a low value.}$$

Second, compare the value of T with the value from Table 1010:I for either a 5% or 1% level of significance. If the calculated T is larger than the table value for the number of measurements, n , then the x_H or x_L is an outlier at that level of significance.

Further information on statistical techniques is available elsewhere.^{2,3}

4. References

1. BARNETT, V. & T. LEWIS. 1984. Outliers in Statistical Data. John Wiley & Sons, New York, N.Y.
2. NATRELLA, M.G. 1963. Experimental Statistics. National Bur. Standards Handbook 91, Washington, D.C.
3. SNEDECOR, G.W. & W.G. COCHRAN. 1980. Statistical Methods. Iowa State University Press, Ames.

1010 C. Glossary

1. Definition of Terms

The purpose of this glossary is to define concepts, not regulatory terms; it is not intended to be all-inclusive.

Accuracy—combination of bias and precision of an analytical procedure, which reflects the closeness of a measured value to a true value.

Bias—consistent deviation of measured values from the true value, caused by systematic errors in a procedure.

Calibration check standard—standard used to determine the state of calibration of an instrument between periodic recalibrations.

Confidence coefficient—the probability, %, that a measurement result will lie within the confidence interval or between the confidence limits.

Confidence interval—set of possible values within which the true value will lie with a specified level of probability.

Confidence limit—one of the boundary values defining the confidence interval.

Detection levels—Various levels in increasing order are:

Instrumental detection level (IDL)—the constituent concentration that produces a signal greater than five times the signal/ noise ratio of the instrument. This is similar, in many respects, to “critical level” and “criterion of detection.” The latter level is stated as

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1.645 times the s of blank analyses.

Lower level of detection (LLD)—the constituent concentration in reagent water that produces a signal $2(1.645)s$ above the mean of blank analyses. This sets both Type I and Type II errors at 5%. Other names for this level are “detection level” and “level of detection” (LOD).

Method detection level (MDL)—the constituent concentration that, when processed through the complete method, produces a signal with a 99% probability that it is different from the blank. For seven replicates of the sample, the mean must be $3.14s$ above the blank where s is the standard deviation of the seven replicates. Compute MDL from replicate measurements one to five times the actual MDL. The MDL will be larger than the LLD because of the few replications and the sample processing steps and may vary with constituent and matrix.

Level of quantitation (LOQ)/minimum quantitation level (MQL)—the constituent concentration that produces a signal sufficiently greater than the blank that it can be detected within specified levels by good laboratories during routine operating conditions. Typically it is the concentration that produces a signal $10s$ above the reagent water blank signal.

Duplicate—usually the smallest number of replicates (two) but specifically herein refers to duplicate samples, i.e., two samples taken at the same time from one location.

Internal standard—a pure compound added to a sample extract just before instrumental analysis to permit correction for inefficiencies.

Laboratory control standard—a standard, usually certified by an outside agency, used to measure the bias in a procedure. For certain constituents and matrices, use National Institute of Standards and Technology (NIST) Standard Reference Materials when they are available.

Precision—measure of the degree of agreement among replicate analyses of a sample, usually expressed as the standard deviation.

Quality assessment—procedure for determining the quality of laboratory measurements by use of data from internal and external quality control measures.

Quality assurance—a definitive plan for laboratory operation that specifies the measures used to produce data of known precision and bias.

Quality control—set of measures within a sample analysis methodology to assure that the process is in control.

Random error—the deviation in any step in an analytical procedure that can be treated by standard statistical techniques.

Replicate—repeated operation occurring within an analytical procedure. Two or more analyses for the same constituent in an extract of a single sample constitute replicate extract analyses.

Surrogate standard—a pure compound added to a sample in the laboratory just before processing so that the overall efficiency of a method can be determined.

Type I error—also called alpha error, is the probability of deciding a constituent is present when

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it actually is absent.

Type II error—also called beta error, is the probability of not detecting a constituent when it actually is present.

1020 QUALITY ASSURANCE

1020 A. Introduction

This section applies primarily to chemical analyses. See Section 9020 for quality assurance and control for microbiological analyses.

Quality assurance (QA) is the definitive program for laboratory operation that specifies the measures required to produce defensible data of known precision and accuracy. This program will be defined in a documented laboratory quality system.

The laboratory quality system will consist of a QA manual, written procedures, work instructions, and records. The manual should include a quality policy that defines the statistical level of confidence used to express the precision and bias of data, as well as the method detection limits. Quality systems, which include QA policies and all quality control (QC) processes, must be in place to document and ensure the quality of analytical data produced by the laboratory and to demonstrate the competence of the laboratory. Quality systems are essential for any laboratory seeking accreditation under state or federal laboratory certification programs. Included in quality assurance are quality control (Section 1020B) and quality assessment (Section 1020C). See Section 1030 for evaluation of data quality.

1. Quality Assurance Planning

Establish a QA program and prepare a QA manual or plan. Include in the QA manual and associated documents the following items¹⁻⁴: cover sheet with approval signatures; quality policy statement; organizational structure; staff responsibilities; analyst training and performance requirements; tests performed by the laboratory; procedures for handling and receiving samples; sample control and documentation procedures; procedures for achieving traceability of measurements; major equipment, instrumentation, and reference measurement standards used; standard operating procedures (SOPs) for each analytical method; procedures for generation, approval, and control of policies and procedures; procedures for procurement of reference materials and supplies; procedures for procurement of subcontractors' services; internal quality control activities; procedures for calibration, verification, and maintenance of instrumentation and equipment; data-verification practices including interlaboratory comparison and proficiency-testing programs; procedures to be followed for feedback and corrective action whenever testing discrepancies are detected; procedures for exceptions that permit departure from documented policies; procedures for system and performance audits and reviews; procedures for assessing data precision and accuracy and determining method detection limits;

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procedures for data reduction, validation, and reporting; procedures for records archiving; procedures and systems for control of the testing environment; and procedures for dealing with complaints from users of the data. Also define and include the responsibility for, and frequency of, management review and updates to the QA manual and associated documents.

On the title page, include approval signatures and a statement that the manual has been reviewed and determined to be appropriate for the scope, volume, and range of testing activities at the laboratory,⁴ as well as an indication that management has made a commitment to assure that the quality systems defined in the QA manual are implemented and followed at all times.

In the QA manual, clearly specify and document the managerial responsibility, authority, quality goals, objectives, and commitment to quality. Write the manual so that it is clearly understood and ensures that all laboratory personnel understand their roles and responsibilities.

Implement and follow chain-of-custody procedures to ensure that chain of custody is maintained and documented for each sample. Institute procedures to permit tracing a sample and its derivatives through all steps from collection through analysis to reporting final results to the laboratory's client and disposal of the sample. Routinely practice adequate and complete documentation, which is critical to assure data defensibility and to meet laboratory accreditation/certification requirements, and ensure full traceability for all tests and samples.

Standard operating procedures (SOPs) describe the analytical methods to be used in the laboratory in sufficient detail that a competent analyst unfamiliar with the method can conduct a reliable review and/or obtain acceptable results. Include in SOPs, where applicable, the following items²⁻⁵: title of referenced, consensus test method; sample matrix or matrices; method detection level (MDL); scope and application; summary of SOP; definitions; interferences; safety considerations; waste management; apparatus, equipment, and supplies; reagents and standards; sample collection, preservation, shipment, and storage requirements; specific quality control practices, frequency, acceptance criteria, and required corrective action if acceptance criteria are not met; calibration and standardization; details on the actual test procedure, including sample preparation; calculations; qualifications and performance requirements for analysts (including number and type of analyses); data assessment/data management; references; and any tables, flowcharts, and validation or method performance data. At a minimum, validate a new SOP before use by first determining the MDL and performing an initial demonstration of capability using relevant regulatory guidelines.

Use and document preventive maintenance procedures for instrumentation and equipment. An effective preventive maintenance program will reduce instrument malfunctions, maintain more consistent calibration, be cost-effective, and reduce downtime. Include measurement traceability to National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs) or commercially available reference materials certified traceable to NIST SRMs in the QA manual or SOP to establish integrity of the laboratory calibration and measurement program. Formulate document-control procedures, which are essential to data defensibility, to cover the complete process of document generation, approval, distribution, storage, recall, archiving, and disposal. Maintain logbooks for each test or procedure performed

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with complete documentation on preparation and analysis of each sample, including sample identification, associated standards and QC samples, method reference, date/time of preparation/analysis, analyst, weights and volumes used, results obtained, and any problems encountered. Keep logbooks that document maintenance and calibration for each instrument or piece of equipment. Calibration procedures, corrective actions, internal quality control activities, performance audits, and data assessments for precision and accuracy (bias) are discussed in Section 1020B and Section 1020C.

Data reduction, validation, and reporting are the final steps in the data-generation process. The data obtained from an analytical instrument must first be subjected to the data reduction processes described in the applicable SOP before the final result can be obtained. Specify calculations and any correction factors, as well as the steps to be followed in generating the sample result, in the QA manual or SOP. Also specify all of the data validation steps to be followed before the final result is made available. Report results in standard units of mass, volume, or concentration as specified in the method or SOP. Report results below the MDL in accordance with the procedure prescribed in the SOP. Ideally, include a statement of uncertainty with each result. See references and bibliography for other useful information and guidance on establishing a QA program and developing an effective QA manual.

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1020 B. Quality Control

Include in each analytical method or SOP the minimum required QC for each analysis. A good quality control program consists of at least the following elements, as applicable: initial demonstration of capability, ongoing demonstration of capability, method detection limit determination, reagent blank (also referred to as method blank), laboratory-fortified blank (also referred to as blank spike), laboratory-fortified matrix (also referred to as matrix spike), laboratory-fortified matrix duplicate (also referred to as matrix spike duplicate) or duplicate sample, internal standard, surrogate standard (for organic analysis) or tracer (for radiochemistry), calibration, control charts, and corrective action, frequency of QC indicators, QC acceptance criteria, and definitions of a batch. Section 1010 and Section 1030 describe calculations for evaluating data quality.

1. Initial Demonstration of Capability

The laboratory should conduct an initial demonstration of capability (IDC) at least once, by each analyst, before analysis of any sample, to demonstrate proficiency to perform the method and obtain acceptable results for each analyte. The IDC also is used to demonstrate that modifications to the method by the laboratory will produce results as precise and accurate as results produced by the reference method. As a minimum, include a reagent blank and at least four laboratory-fortified blanks (LFBs) at a concentration between 10 times the method detection level (MDL) and the midpoint of the calibration curve or other level as specified in the method.

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Run the IDC after analyzing all required calibration standards. Ensure that the reagent blank does not contain any analyte of interest at a concentration greater than half the MQL or other level as specified in the method. See Section 1010C, for definition of MQL. Ensure that precision and accuracy (percent recovery) calculated for the LFBs are within the acceptance criteria listed in the method of choice. If no acceptance criteria are provided, use 80 to 120% recovery and $\leq 20\%$ relative standard deviation (RSD), as a starting point. If details of initial demonstration of capability are not provided in the method of choice, specify and reference the method or procedure used for demonstrating capability.

2. Ongoing Demonstration of Capability

The ongoing demonstration of capability, sometimes referred to as a “laboratory control sample or laboratory control standard,” “quality control check sample,” or “laboratory-fortified blank,” is used to ensure that the laboratory remains in control during the period when samples are analyzed, and separates laboratory performance from method performance on the sample matrix. See ¶ 5 below for further details on the laboratory-fortified blank. Preferably obtain this sample from an external source (not the same stock as the calibration standards). Analyze QC check samples on a quarterly basis, at a minimum.

3. Method Detection Level Determination and Application

Determine the method detection level (MDL) for each analyte of interest and method to be used before data from any samples are reported, using the procedure described in Section 1030C. As a starting point for determining the concentration to use in MDL determination, use an estimate of five times the estimated detection limit. Perform MDL determinations as an iterative process. If calculated MDL is not within a factor of 10 of the value for the known addition, repeat determinations at a more suitable concentration. Conduct MDL determinations at least annually (or other specified frequency) for each analyte and method in use at the laboratory. Perform or verify MDL determination for each instrument. Perform MDL determinations over a period of at least 3 d for each part of the procedure. Calculate recoveries for MDL samples. Recoveries should be between 50 and 150% and %RSD values $\leq 20\%$ or repeat the MDL determination. Maintain MDL and IDC data and have them available for inspection.

Apply the MDL to reporting sample results as follows:

- Report results below the MDL as “not detected.”
- Report results between the MDL and MQL with qualification for quantitation.
- Report results above the MQL with a value and its associated error.

4. Reagent Blank

A reagent blank or method blank consists of reagent water (See Section 1080) and all reagents that normally are in contact with a sample during the entire analytical procedure. The reagent blank is used to determine the contribution of the reagents and the preparative analytical steps to error in the measurement. As a minimum, include one reagent blank with each sample set (batch) or on a 5% basis, whichever is more frequent. Analyze a blank after the daily

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calibration standard and after highly contaminated samples if carryover is suspected. Evaluate reagent blank results for the presence of contamination. If unacceptable contamination is present in the reagent blank, identify and eliminate source of contamination. Typically, sample results are suspect if analyte(s) in the reagent blank are greater than the MQL. Samples analyzed with an associated contaminated blank must be re-prepared and re-analyzed. Refer to the method of choice for specific acceptance criteria for the reagent blank. Guidelines for qualifying sample results with consideration to reagent blank results are as follows:

- If the reagent blank is less than the MDL and sample results are greater than the MQL, then no qualification is required.
- If the reagent blank is greater than the MDL but less than the MQL and sample results are greater than the MQL, then qualify the results to indicate that analyte was detected in the reagent blank.
- If the reagent blank is greater than the MQL, further corrective action and qualification is required.

5. Laboratory-Fortified Blank

A laboratory-fortified blank is a reagent water sample to which a known concentration of the analytes of interest has been added. A LFB is used to evaluate laboratory performance and analyte recovery in a blank matrix. As a minimum, include one LFB with each sample set (batch) or on a 5% basis, whichever is more frequent. The definition of a batch is typically method-specific. Process the LFB through all of the sample preparation and analysis steps. Use an added concentration of at least 10 times the MDL, the midpoint of the calibration curve, or other level as specified in the method. Prepare the addition solution from a different reference source than that used for calibration. Evaluate the LFB for percent recovery of the added analytes. If LFB results are out of control, take corrective action, including re-preparation and re-analysis of associated samples if required. Use the results obtained for the LFB to evaluate batch performance, calculate recovery limits, and plot control charts (see ¶ 12 below). Refer to the method of choice for specific acceptance criteria for the LFB.

6. Laboratory-Fortified Matrix

A laboratory-fortified matrix (LFM) is an additional portion of a sample to which known amounts of the analytes of interest are added before sample preparation. The LFM is used to evaluate analyte recovery in a sample matrix. As a minimum, include one LFM with each sample set (batch) or on a 5% basis, whichever is more frequent. Add a concentration of at least 10 times the MRL, the midpoint of the calibration curve, or other level as specified in the method to the selected sample(s). Preferably use the same concentration as for the LFB to allow the analyst to separate the effect of matrix from laboratory performance. Prepare the LFM from a reference source different from that used for calibration. Make the addition such that sample background levels do not adversely affect the recovery (preferably adjust LFM concentrations if the known sample is above five times the background level). For example, if the sample contains the analyte of interest, make the LFM sample at a concentration equivalent to the concentration found in the

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known sample. Evaluate the results obtained for LFM for accuracy or percent recovery. If LFM results are out of control, take corrective action to rectify the effect or use another method or the method of standard addition. Refer to the method of choice for specific acceptance criteria for LFM until the laboratory develops statistically valid, laboratory-specific performance criteria. Base sample batch acceptance on results of LFB analyses rather than LFM alone, because the matrix of the LFM sample may interfere with the method performance.

7. Laboratory-Fortified Matrix Duplicate/Duplicate Sample

A LFM duplicate is a second portion of the sample described in ¶ 6 above to which a known amount of the analyte of interest is added before sample preparation. If sufficient sample volume is collected, this second portion of sample is added and processed in the same way as the LFM. If sufficient sample volume is not collected to analyze a LFM duplicate, use an additional portion of an alternate sample to obtain results for a duplicate sample to gather data on precision. As a minimum, include one LFM duplicate or one duplicate sample with each sample set (batch) or on a 5% basis, whichever is more frequent. Evaluate the results obtained for LFM duplicates for precision and accuracy (precision alone for duplicate samples). If LFM duplicate results are out of control, take corrective action to rectify the effect or use another method or the method of standard addition. If duplicate results are out of control, reprepare and reanalyze the sample and take additional corrective action as needed (such as reanalysis of sample batch). Refer to the method of choice for specific acceptance criteria for LFM duplicates or duplicate samples until the laboratory develops statistically valid, laboratory-specific performance criteria. If no limits are included in the method of choice, calculate preliminary limits from initial demonstration of capability. Base sample batch acceptance on results of LFB analyses rather than LFM duplicates alone, because the matrix of the LFM sample may interfere with the method performance.

8. Internal Standard

Internal standards (IS) are used for organic analyses by GC/MS, some GC analyses, and some metals analyses by ICP/MS. An internal standard is an analyte included in each standard and added to each sample or sample extract/digestate just before sample analysis. Internal standards should mimic the analytes of interest but not interfere with the analysis. Choose an internal standard having retention time or mass spectrum separate from the analytes of interest and eluting in a representative area of the chromatogram. Internal standards are used to monitor retention time, calculate relative response, and quantify the analytes of interest in each sample or sample extract/digestate. When quantifying by the internal standard method, measure all analyte responses relative to this internal standard, unless interference is suspected. If internal standard results are out of control, take corrective action, including reanalysis if required. Refer to the method of choice for specific internal standards and their acceptance criteria.

9. Surrogates and Tracers

Surrogates are used for organic analyses; tracers are used for radiochemistry analyses. Surrogates and tracers are used to evaluate method performance in each sample. A surrogate

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standard is a compound of a known amount added to each sample before extraction. Surrogates mimic the analytes of interest and are compound(s) unlikely to be found in environmental samples, such as fluorinated compounds or stable, isotopically labeled analogs of the analytes of interest. Tracers are a different isotope of the analyte or element of interest. Surrogates and tracers are introduced to samples before extraction to monitor extraction efficiency and percent recovery in each sample. If surrogate or tracer results are out of control, take corrective action, including reparation and reanalysis if required. Refer to the method of choice for specific surrogates or tracers and their acceptance criteria, until the laboratory develops statistically valid, laboratory-specific performance criteria.

10. Calibration

a. Instrument calibration: Perform instrument calibration, as well as maintenance, according to instrument manual instructions. Use instrument manufacturer's recommendations for calibration. Perform instrument performance checks, such as those for GC/MS analyses, according to method or SOP instructions.

b. Initial calibration: Perform initial calibration with a minimum of three concentrations of standards for linear curves, a minimum of five concentrations of standards for nonlinear curves, or as specified by the method of choice. Choose a lowest concentration at the reporting limit, and highest concentration at the upper end of the calibration range. Ensure that the calibration range encompasses the analytical concentration values expected in the samples or required dilutions. Choose calibration standard concentrations with no more than one order of magnitude between concentrations.

Use the following calibration functions as appropriate: response factor for internal standard calibration, calibration factor for external standard calibration, or calibration curve. Calibration curves may be linear through the origin, linear not through the origin, or nonlinear through or not through the origin. Some nonlinear functions can be linearized through mathematical transformations, e.g., log. The following acceptance criteria are recommended for the various calibration functions.

If response factors or calibration factors are used, the calculated %RSD for each analyte of interest must be less than the method-specified value. When using response factors (e.g., for GC/MS analysis), evaluate the performance or sensitivity of the instrument for the analyte of interest against minimum acceptance values for the response factors. Refer to the method of choice for the calibration procedure and acceptance criteria on the response factors or calibration factors for each analyte.

If linear regression is used, use the minimum correlation coefficient specified in the method. If the minimum correlation coefficient is not specified, then a minimum value of 0.995 is recommended. Compare each calibration point to the curve and recalculate. If any recalculated values are not within the method acceptance criteria, identify the source of outlier(s) and correct before sample quantitation. Alternately, a method's calibration can be judged against a reference method by measuring the method's "calibration linearity" or %RSD among the "response

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factors’’ at each calibration level or concentration.²

Use initial calibration, with any of the above functions (response factor, calibration factor, or calibration curve), for quantitation of the analytes of interest in samples. Use calibration verification, described in the next section, only for checks on the initial calibration and not for sample quantitation, unless otherwise specified by the method of choice. Perform initial calibration when the instrument is set up and whenever the calibration verification criteria are not met.

c. Calibration verification: Calibration verification is the periodic confirmation by analysis of a calibration standard that the instrument performance has not changed significantly from the initial calibration. Base this verification on time (e.g., every 12 h) or on the number of samples analyzed (e.g., after every 10 samples). Verify calibration by analyzing a single standard at a concentration near or at the midpoint of the calibration range. The evaluation of the calibration verification analysis is based either on allowable deviations from the values obtained in the initial calibration or from specific points on the calibration curve. If the calibration verification is out of control, take corrective action, including reanalysis of any affected samples. Refer to the method of choice for the frequency of calibration verification and the acceptance criteria for calibration verification.

11. QC Calculations

The following is a compilation of equations frequently used in QC calculations.

a. Initial calibrations:

Relative response factor (RRF):

$$RRF(x) = \frac{A_x}{A_{is}} \times \frac{C_{is}}{C_x}$$

where:

RRF = relative response factor,

A = peak area or height of characteristic ion measured,

C = concentration,

is = internal standard, and

x = analyte of interest.

Response factor (RF):

$$RF(x) = \frac{A_x}{C_x}$$

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where:

RF = response factor,
 A = peak area or height,
 C = concentration, and
 x = analyte of interest.

Calibration factor (CF):

$$CF = \frac{\text{peak area (or height) of standards}}{\text{mass injected}}$$

Relative standard deviation (%RSD):

$$\% RSD = \frac{s}{\bar{x}} \times 100\%$$

$$s = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{(n-1)}}$$

where:

s = standard deviation,
 n = total number of values,
 x_i = each individual value used to calculate mean, and
 $\bar{x}$ = mean of n values.

b. Calibration verification:

% Difference (%D) for response factor:

$$\% D = \frac{\overline{RF}_i - RF_c}{RF_i} \times 100\%$$

where:

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$\overline{RF}_i$ = average RF or RRF from initial calibration, and
 RF_c = relative RF or RRF from calibration verification standard.

% Difference (%D) for values:

$$\%D = \frac{\text{true value} - \text{found value}}{\text{true value}} \times 100\%$$

% Recovery:

$$\% \text{ Recovery} = \frac{\text{found value}}{\text{true value}} \times 100\%$$

c. *Laboratory-fortified blank (laboratory control sample):*

$$\% \text{ Recovery} = \frac{\text{found value}}{\text{true value}} \times 100\%$$

d. *Surrogates:*

$$\% \text{ Recovery} = \frac{\text{quantity measured}}{\text{quantity added}} \times 100\%$$

e. *Laboratory-fortified matrix (LFM) sample (matrix spike sample):*

$$\% \text{ Recovery} = \frac{(\text{LFM sample result} - \text{sample result})}{\text{known LFM added concentration}} \times 100\%$$

f. *Duplicate sample:*

Relative percent difference (RPD):

$$RPD = \frac{(\text{sample result} - \text{duplicate result})}{(\text{sample result} + \text{duplicate result})/2} \times 100\%$$

g. *Method of standards addition:*

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$$\text{Sample concentrations} \times \text{mg/L} = \frac{S_2 \times V_1 \times C}{(S_1 - S_2) \times V_2}$$

where:

- C = concentration of the standard solution, mg/L,
- S_1 = signal for fortified portion,
- S_2 = signal for unfortified portion,
- V_1 = volume of standard addition, L, and
- V_2 = volume of sample portion used for method of standard addition, L.

12. Control Charts

Two types of control charts commonly used in laboratories are as follows: accuracy or means charts for QC samples, including reagent blanks, laboratory control standards, calibration check standards, laboratory fortified blanks, laboratory fortified matrices, and surrogates; and precision or range charts, %RSD or relative percent difference (RPD), for replicate or duplicate analyses. These charts are essential tools for quality control. Computer-generated and maintained lists or databases with values, limits, and trending may be used as an alternate to control charts.

a. Accuracy (means) chart: The accuracy chart for QC samples is constructed from the average and standard deviation of a specified number of measurements of the analyte of interest. The accuracy chart includes upper and lower warning levels (WL) and upper and lower control levels (CL). Common practice is to use $\pm 2s$ and $\pm 3s$ limits for the WL and CL, respectively, where s represents standard deviation. These values are derived from stated or measured values for reference materials. The number of measurements, n or $n-1$, used to determine the standard deviation, s , is specified relative to statistical confidence limits of 95% for WLs and 99% for CLs. Set up an accuracy chart by using either the calculated values for mean and standard deviation or the percent recovery. Percent recovery is necessary if the concentration varies. Construct a chart for each analytical method. Enter results on the chart each time the QC sample is analyzed. Examples of control charts for accuracy are given in Figure 1020:1.

b. Precision (range) chart: The precision chart also is constructed from the average and standard deviation of a specified number of measurements of the analyte of interest. If the standard deviation of the method is known, use the factors from Table 1020:I to construct the central line and warning and control limits as in Figure 1020:2. Perfect agreement between replicates or duplicates results in a difference of zero when the values are subtracted, so the baseline on the chart is zero. Therefore for precision charts, only upper warning limits and upper control limits are meaningful. The standard deviation is converted to the range so that the analyst need only subtract the two results to plot the value on the precision chart. The mean range is computed as:

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$$\bar{R} = D_2 s$$

the control limit as

$$CL = \bar{R} \pm 3s(R) = D_4 \bar{R}$$

and the warning limit as

$$WL = \bar{R} \pm 2s(R) = \bar{R} \pm 2/3(D_4 \bar{R} - \bar{R})$$

where:

D_2 = factor to convert s to the range (1.128 for duplicates, as given in Table 1020:I),

$s(R)$ = standard deviation of the range, and

D_4 = factor to convert mean range to $3s(R)$ (3.267 for duplicates, as given in Table 1020:I).

A precision chart is rather simple when duplicate analyses of a standard are used (Figure 1020:2). For duplicate analyses of samples, the plot will appear different because of the variation in sample concentration. If a constant relative standard deviation in the concentration range of interest is assumed, then R , $D_4 R$ etc., may be computed as above for several concentrations, a smooth curve drawn through the points obtained, and an acceptable range for duplicates determined. Figure 1020:3 illustrates such a chart. A separate table, as suggested below the figure, will be needed to track precision over time.

More commonly, the range can be expressed as a function of the relative standard deviation (coefficient of variation). The range can be normalized by dividing by the average. Determine the mean range for the pairs analyzed by

$$\bar{R} = (\sum R_i)/n$$

and the variance (square of the standard deviation) as

$$s_R^2 = (\sum R_i^2 - n\bar{R}^2)/(n - 1)$$

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Then draw lines on the chart at $R + 2s_R$ and $R + 3s_R$ and, for each duplicate analysis, calculate normalized range and enter the result on the chart. Figure 1020:4 is an example of such a chart.

c. *Chart analyses:* If the warning limits (WL) are at the 95% confidence level, 1 out of 20 points, on the average, would exceed that limit, whereas only 1 out of 100 would exceed the control limits (CL). Use the following guidelines, based on these statistical parameters, which are illustrated in Figure 1020:5 :

Control limit—If one measurement exceeds a CL, repeat the analysis immediately. If the repeat measurement is within the CL, continue analyses; if it exceeds the CL, discontinue analyses and correct the problem.

Warning limit—If two out of three successive points exceed a WL, analyze another sample. If the next point is within the WL, continue analyses; if the next point exceeds the WL, evaluate potential bias and correct the problem.

Standard deviation—If four out of five successive points exceed $1s$, or are in decreasing or increasing order, analyze another sample. If the next point is less than $1s$, or changes the order, continue analyses; otherwise, discontinue analyses and correct the problem.

Trending—If seven successive samples are on the same side of the central line, discontinue analyses and correct the problem.

The above considerations apply when the conditions are either above or below the central line, but not on both sides, e.g., four of five values must exceed either $+1s$ or $-1s$. After correcting the problem, reanalyze the samples analyzed between the last in-control measurement and the out-of-control one.

Another important function of the control chart is assessment of improvements in method precision. In the accuracy and precision charts, if measurements never or rarely exceed the WL, recalculate the WL and CL using the 10 to 20 most recent data points. Trends in precision can be detected sooner if running averages of 10 to 20 are kept. Trends indicate systematic error; random error is revealed when measurements randomly exceed warning or control limits.

13. QC Evaluation for Small Sample Sizes

Small sample sizes, such as for field blanks and duplicate samples, may not be suitable for QC evaluation with control charts. QC evaluation techniques for small sample sizes are discussed elsewhere.³

14. Corrective Action

Quality control data outside the acceptance limits or exhibiting a trend are evidence of unacceptable error in the analytical process. Take corrective action promptly to determine and eliminate the source of the error. Do not report data until the cause of the problem is identified and either corrected or qualified. Example data qualifiers are listed in Table 1020:II. Qualifying data does not eliminate the need to take corrective actions, but allows for the reporting of data of known quality when it is either not possible or practical to reanalyze the sample(s). Maintain

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records of all out-of-control events, determined causes, and corrective action taken. The goal of corrective action is not only to eliminate such events, but also to reduce repetition of the causes.

Corrective action begins with the analyst, who is responsible for knowing when the analytical process is out of control. The analyst should initiate corrective action when a QC check exceeds the acceptance limits or exhibits trending and should report an out-of-control event to the supervisor. Such events include QC outliers, hold-time failures, loss of sample, equipment malfunctions, and evidence of sample contamination. Recommended corrective action to be used when QC data are unacceptable are as follows:

- Check data for calculation or transcription error. Correct results if error occurred.
- Check to see if sample(s) was prepared and analyzed according to the approved method and SOP. If it was not, prepare and/or analyze again.
- Check calibration standards against an independent standard or reference material. If calibration standards fail, reprepare calibration standards and/or recalibrate instrument and reanalyze affected sample(s).
- If a LFB fails, reanalyze another laboratory-fortified blank.
- If a second LFB fails, check an independent reference material. If the second source is acceptable, reprepare and reanalyze affected sample(s).
- If a LFM fails, check LFB. If the LFB is acceptable, qualify the data for the LFM sample or use another method or the method of standard addition.
- If a LFM and the associated LFB fail, reprepare and reanalyze affected samples.
- If reagent blank fails, analyze another reagent blank.
- If second reagent blank fails, reprepare and reanalyze affected sample(s).
- If the surrogate or internal standard known addition fails and there are no calculation or reporting errors, reprepare and reanalyze affected sample(s).

If data qualifiers are used to qualify samples not meeting QC requirements, the data may or may not be usable for the intended purposes. It is the responsibility of the laboratory to provide the client or end-user of the data with sufficient information to determine the usability of qualified data.

15. References

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1020 C. Quality Assessment

Quality assessment is the process used to ensure that quality control measures are being performed as required and to determine the quality of the data produced by the laboratory. It includes such items as proficiency samples, laboratory intercomparison samples, and performance audits. These are applied to test the precision, accuracy, and detection limits of the methods in use, and to assess adherence to standard operating procedure requirements.

1. Laboratory Check Samples (Internal Proficiency)

The laboratory should perform self-evaluation of its proficiency for each analyte and method in use by periodically analyzing laboratory check samples. Check samples with known amounts of the analytes of interest supplied by an outside organization or blind additions can be prepared independently within the laboratory to determine percent recovery of the analytes of interest by each method.

In general, method performance will have been established beforehand; acceptable percent recovery consists of values that fall within the established acceptance range. For example, if the acceptable range of recovery for a substance is 85 to 115%, then the analyst is expected to achieve a recovery within that range on all laboratory check samples and to take corrective action if results are outside of the acceptance range.

2. Laboratory Intercomparison Samples

A good quality assessment program requires participation in periodic laboratory intercomparison studies. Commercial and some governmental programs supply laboratory intercomparison samples containing one or multiple constituents in various matrices. The frequency of participation in intercomparison studies should be adjusted relative to the quality of results produced by the analysts. For routine procedures, semi-annual analyses are customary. If failures occur, take corrective action and analyze laboratory check samples more frequently until acceptable performance is achieved.

3. Compliance Audits

Compliance audits are conducted to evaluate whether the laboratory meets the applicable requirements of the SOP or consensus method claimed as followed by the laboratory. Compliance audits can be conducted by internal or external parties. A checklist can be used to document the manner in which a sample is treated from time of receipt to final reporting of the result. The goal of compliance audits is to detect any deviations from the SOP or consensus method so that corrective action can be taken on those deviations. An example format for a

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checklist is shown in Table 1020:III.

4. Laboratory Quality Systems Audits

A quality systems audit program is designed and conducted to address all program elements and provide a review of the quality system. Quality systems audits should be conducted by a qualified auditor(s) who is knowledgeable about the section or analysis being audited. Audit all major elements of the quality system at least annually. Quality system audits may be conducted internally or externally; both types should occur on a regular scheduled basis and should be handled properly to protect confidentiality. Internal audits are used for self-evaluation and improvement. External audits are used for accreditation as well as education on client requirements and for approval of the end use of the data. Corrective action should be taken on all audit findings and its effectiveness reviewed at or before the next scheduled audit.

5. Management Review

Review and revision of the quality system, conducted by laboratory management, is vital to its maintenance and effectiveness. Management review should assess the effectiveness of the quality system and corrective action implementation, and should include internal and external audit results, performance evaluation sample results, input from end user complaints, and corrective actions.

6. Bibliography

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1030 DATA QUALITY

1030 A. Introduction

The role of the analytical laboratory is to produce measurement-based information that is technically valid, legally defensible, and of known quality. Quality assurance is aimed at optimizing the reliability of the measurement process. All measurements contain error, which may be systematic (with an unvarying magnitude) or random (with equal probability of being positive or negative and varying in magnitude). Determination of the systematic and random error components of an analytical method uniquely defines the analytical performance of that

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method.¹ Quality control (QC) procedures identify and control these sources of error.

1. Measures of Quality Control

Random error (precision) and systematic error (bias) are two routine indicators of measurement quality used by analysts to assess validity of the analytical process. Precision is the closeness of agreement between repeated measurements. A measurement has acceptable precision if the random errors are low. Accuracy is the closeness of a measurement to the true value. A measurement is acceptably accurate when both the systematic and random errors are low. QC results outside the acceptance limits, as set by the data quality objectives, are evidence of an analytical process that may be out of control due to determinant errors such as contaminated reagents or degraded standards.

2. Measurement Error and Data Use

Measurement error, whether random or systematic, reduces the usability of laboratory data. As a measured value decreases, its relative error (e.g., relative standard deviation) may increase and its usable information decrease. Reporting tools, such as detection or quantitation limits, frequently are used to establish a lower limit on usable information content.

Laboratory data may be used for such purposes as regulatory monitoring, environmental decision-making, and process control. The procedures used to extract information for these different purposes vary and may be diametrically opposed. For example, a measurement for regulatory monitoring may be appropriately qualified when below the detection level because the error bar is relatively large and may preclude a statistically sound decision. Data collected over a period of time, however, may be treated by statistical methods to provide a statistically sound decision even when many of the data are below detection levels.²

3. The Analyst's Responsibility

The analyst must understand the measures of quality control and how to apply them to the data quality objectives of process control, regulatory monitoring, and environmental field studies. It is important that the quality objectives for the data be clearly defined and detailed before sample analysis so that the data will be technically correct and legally defensible.

4. Reference

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1030 B. Measurement Uncertainty

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1. Introduction

Even with the fullest possible extent of correction, every measurement has error that is ultimately unknown and unknowable. The description of this unknown error is “measurement uncertainty.”

Reporting uncertainty along with a measurement result is good practice, and may spare the user from making unwarranted or risky decisions based only on the measurement.

Whereas measurement error (E) is the actual, unknown deviation of the measurement (M) from the unknown true value (T), measurement uncertainty (U) is the state of knowledge about this unknown deviation, and is often expressed as U , as in $M \pm U$. U may be defined as an uncertainty expression.^{1,2} This section concerns the definition of U , how to compute it, a recommendation for reporting uncertainty, the interpretation and scope of uncertainty, and other ways of expressing measurement uncertainty.

2. Error

A measurement can be related to the unknown true value and unknown measurement error as follows:

$$M = T + E$$

This is a simple additive relationship. There are other plausible relationships between M and E , such as multiplicative or arbitrary functional relationships, which are not discussed here.

Because E is unknown, M must be regarded as an uncertain measurement. In some practical situations, a value may be treated as known. T^* may be, for example, a published reference value, a traceable value, or a consensus value. The purpose of the substitution may be for convenience or because the measurement process that produced T^* has less bias or variation than the one that produced M . For example, based on the average of many measurements, a vessel might be thought to contain $T^* = 50 \mu\text{g/L}$ of salt in water. It then may be sampled and routinely measured, resulting in a reported concentration of $M = 51 \mu\text{g/L}$. The actual concentration may be $T = 49.9 \mu\text{g/L}$, resulting in $E = 51 - 49.9 = 1.1 \mu\text{g/L}$.

To generalize the nature of uncertainty, measurement error may be negligible or large in absolute terms (i.e., in the original units) or relative terms (i.e., unitless, $E \div T$ or T^*). The perceived acceptability of the magnitude of an absolute error depends on its intended use. For example, an absolute error of $1.1 \mu\text{g/L}$ may be inconsequential for an application where any concentration over $30 \mu\text{g/L}$ will be sufficient. However, if it is to be used instead as a standard for precision measurement (e.g., of pharmaceutical ingredients), $1.1 \mu\text{g/L}$ too much could be unacceptable.

3. Uncertainty

Reported measurement uncertainty will contain the actual measurement error with a stated

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level of confidence. For example, if $M \pm U$ is presented as a 95% confidence interval, approximately 95% of the time, the measurement error E will fall within the range of $\pm U$.

4. Bias

Bias is the systematic component of error. It is defined as the signed deviation between the limiting average measured value and the true value being measured as the number of measurements in the average tends to infinity and the uncertainty about the average tends to zero. For example, the reason the $T = 49.9 \mu\text{g/L}$ salt solution is thought to be $T^* = 50 \mu\text{g/L}$ could be a bias, $B = 0.1 \mu\text{g/L}$. The “leftover” error, $1.1 - 0.1 = 1.0 \mu\text{g/L}$, is the random component. This random component (also called stochastic error) changes with each measurement. The bias is fixed, and may be related to the laboratory method used to produce T . * Usually, a recognized method will be used to produce or certify the traceable standard, a sample with a certificate stating the accepted true value T^* . * The method may be the best method available or simply the most widely accepted method. It is chosen to have very low error, both bias and random. Such a traceable standard may be purchased from a standards organization such as NIST.

5. Bias and Random Variation

Measurement error, E , (and measurement uncertainty) can be split into two components, random and systematic:

$$E = Z + B$$

Random error, Z , is the component of the measurement error that changes from one measurement to the next, under certain conditions. Random measurement errors are assumed to be independent and have a distribution, often assumed to be Gaussian (i.e., they are normally distributed). The normal distribution of Z is characterized by the distribution mean, μ , and standard deviation, σ_E . In discussion of measurement error distribution, μ is assumed to be zero because any non-zero component is part of bias, by definition. The population standard deviation, σ_E , can be used to characterize the random component of measurement error because the critical values of the normal distribution are well known and widely available. For example, about 95% of the normal distribution lies within the interval $\mu \pm 2\sigma_E$. Hence, if there is no measurement bias, and measurement errors are independent and normally distributed, $M \pm 2\sigma_E$ (95% confidence, assumed normal) is a suitable way to report a measurement and its uncertainty. More generally, normal probability tables and statistical software give the proportion of the normal distribution and thus the % confidence gained that is contained within $\pm k\sigma_E$ for any value of scalar k .

Usually, however, the population standard deviation, σ_E , is not known and must be estimated by the sample standard deviation, s_E . This estimate of the standard deviation is based on multiple observations and statistical estimation. In this case, the choice of the scalar k must be based not

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on the normal distribution function, but on the Student's t distribution, taking into account the number of degrees of freedom associated with s_E .

Systematic error (B) is all error that is not random, and typically is equated with bias. Systematic error also can contain outright mistakes (blunders) and lack of control (drifts, fluctuations, etc.).³ In this manual, the terms “systematic error” and “bias” are used interchangeably.

Systematic uncertainty often is more difficult to estimate and make useful than is random uncertainty. Knowledge about bias is likely to be hard to obtain, and once obtained it is appropriately and likely to be exploited to make the measurement less biased. If measurement bias is known exactly (or nearly so), the user can subtract it from M to reduce total measurement error.

If measurement bias is entirely unknown, and could take on any value from a wide but unknown distribution of plausible values, users may adopt a worst-case approach and report an extreme bound, or they may simply ignore the bias altogether. For example, historical data may indicate that significant interlaboratory biases are present, or that every time a measurement system is cleaned, a shift is observed in QC measurements of standards. In the absence of traceable standards, it is hard for laboratory management or analysts to do anything other than ignore the potential problem.

The recommended practice is to conduct routine QA/QC measurements with a suite of internal standards. Plot measurements on control charts, and when an out-of-control condition is encountered, recalibrate the system with traceable standards. This permits the laboratory to publish a boundary on bias, assuming that the underlying behavior of the measurement system is somewhat predictable and acceptably small in scale in between QA/QC sampling (e.g., slow drifts and small shifts).

6. Repeatability, Reproducibility, and Sources of Bias and Variation

a. Sources and measurement: The sources of bias and variability in measurements are many; they include sampling error, sample preparation, interference by matrix or other measurement quantities/qualities, calibration error variation, software errors, counting statistics, deviations from method by analyst, instrument differences (e.g., chamber volume, voltage level), environmental changes (temperature, humidity, ambient light, etc.), contamination of sample or equipment (e.g., carryover and ambient contamination), variations in purity of solvent, reagent, catalyst, etc., stability and age of sample, analyte, or matrix, and warm-up or cool-down effects, or a tendency to drift over time.

The simplest strategy for estimating typical measurement bias is to measure a traceable (known) standard, then compute the difference between the measured value M and the known value T , assumed to be the true value being measured.

$$M - T = B + Z$$

The uncertainty in the measurement of the traceable standard is assumed to be small,

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although in practice there may be situations where this is not an appropriate assumption. If random measurement uncertainty is negligible (i.e., $Z \approx 0$), the difference, $M - T$, will provide an estimate of bias (B). If random uncertainty is not negligible, it can be observed and quantified by making a measurement repeatedly on the same test specimen (if the measurement process is not destructive). This may be part of a QA/QC procedure.

b. Repeatability: As quantified by the repeatability standard deviation (σ_{RPT}), repeatability is the minimal variability of a measurement system obtained by repeatedly measuring the same specimen while allowing *no* controllable sources of variability to affect the measurement. Repeatability also can be obtained by pooling sample standard deviations of measurements of J different specimens, as follows:

$$\sigma_{RPT} = \sqrt{\frac{1}{J} \cdot \sum_{i=1}^J \sigma_{RPT,i}^2}$$

Repeatability also is called “intrinsic measurement variability,” and is considered an approximate lower boundary to the measurement standard deviation that will be experienced in practice. The repeatability standard deviation sometimes is used to compute uncertainty intervals, $\pm U$, that can be referred to as ultimate instrument variability, based on the Student’s t distribution function ($\pm U = \pm ks_{RPT}$).

Common sense and application experience demonstrate that repeatability is an overly optimistic estimate to report as measurement uncertainty for routine measurement. In routine use, measurements will be subject to many sources of bias and variability that are intentionally eliminated or restrained during a repeatability study. In routine use, uncertainty in both bias (B) and variability (Z) are greater.

c. Reproducibility: As quantified by the reproducibility standard deviation (σ_{RPD}), reproducibility is the variability of a measurement system obtained by repeatedly measuring a sample while allowing (or requiring) selected sources of bias or variability to affect the measurement. With σ_{RPD} , provide list of known applicable sources of bias and variability, and whether or not they were varied.

Barring statistical variation (i.e., variation in estimates of variability, such as the noisiness in sample standard deviations), the reproducibility standard deviation always is greater than the repeatability standard deviation, because it has additional components. Typically, one or more of the following is varied in a reproducibility study: instrument, analyst, laboratory, or day. Preferably design a study tailored to the particular measurement system (see 1030B.7). If the sample is varied, compute reproducibility standard deviations separately for each sample, then pool results if they are homogeneous. Treat factors varied in the study as random factors and assume them to be independent normal random variables with zero mean. However, this

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assumption often can be challenged, because the sample and possibly the target populations may be small (they may even be identical), and there may be a question of “representativeness.” For example, six laboratories (or analysts, or instruments) may report usable measurements out of a total population of twenty capable of doing tandem mass spectrometry for a particular analyte and matrix. It is hard to know how representative the six are of the twenty, especially after a ranking and exclusion process that can follow a study, and whether the biases of the twenty are normally distributed (probably not discernible from six measurements, even if the six are representative).

It may be more appropriate to treat each factor with few, known factor values (i.e., choices such as laboratories) as fixed factors, to use the statistical term. Fixed factors have fixed effects. That is, each laboratory has a different bias, as might each analyst, each instrument, and each day, but these biases are not assumed to have a known (or knowable) distribution. Therefore, a small sample cannot be used to estimate distribution parameters, particularly a standard deviation. For example, assuming that variables are random, normal, and have zero mean may be inappropriate in an interlaboratory round-robin study. It must be assumed that every laboratory has some bias, but it is difficult to characterize the biases because of laboratory anonymity, the small number of laboratories contributing usable data, and other factors.

Because of these concerns about assumptions and the potential ambiguity of its definition, do not report reproducibility unless it is accompanied with study design and a list of known sources of bias and variability and whether or not they were varied.

7. Gage Repeatability and Reproducibility, and the Measurement Capability Study

Combining the concepts of repeatability and reproducibility, the Gage Repeatability and Reproducibility (Gage R&R) approach has been developed.⁴ It treats all factors as random (including biases), and is based on the simplest nontrivial model:

$$Z = Z_{RPT} + Z_L$$

where:

Z_{RPT} = normally distributed random variable with mean equal to zero and variance equal to σ_{RPT}^2 , and

Z_L = normally distributed random variable with mean equal to zero and with the variance of the factor (e.g., interlaboratory) biases, σ_L^2 .

The overall measurement variation then is quantified by

$$\sigma_E = \sigma_{RPD} = \sqrt{\sigma_{RPT}^2 + \sigma_L^2}$$

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Estimates for σ_{RPT} and σ_{RPD} usually are obtained by conducting a nested designed study and analyzing variance components of the results. This approach can be generalized to reflect good practice in conducting experiments. The following measurement capability study (MCS) procedure is recommended. The objective of such studies is not necessarily to quantify the contribution of every source of bias and variability, but to study those considered to be important, through systematic error budgeting.

To perform a measurement capability study to assess measurement uncertainty through systematic error budgeting, proceed as follows:

Identify sources of bias and variation that affect measurement error. This can be done with a cause-and-effect diagram, perhaps with source categories of: equipment, analyst, method (i.e., procedure and algorithm), material (i.e., aspects of the test specimens), and environment.

Select sources to study, either empirically or theoretically. Typically, study sources that are influential, that can be varied during the MCS, and that cannot be eliminated during routine measurement. Select models for the sources. Treat sources of bias as fixed factors, and sources of variation as random factors.

Design and conduct the study, allowing (or requiring) the selected sources to contribute to measurement error. Analyze the data graphically and statistically (e.g., by regression analysis, ANOVA, or variance components analysis). Identify and possibly eliminate outliers (observations with responses that are far out of line with the general pattern of the data), and leverage points (observations that exert high, perhaps undue, influence).

Refine the models, if necessary (e.g., based on residual analysis), and draw inferences for future measurements. For random effects, this probably would be a confidence interval; for fixed effects, a table of estimated biases.

8. Other Assessments of Measurement Uncertainty

In addition to the strictly empirical MCS approach to assessing measurement uncertainty, there are alternative procedures, discussed below in order of increasing empiricism.

a. Exact theoretical: Some measurement methods are tied closely to exact first-principles models of physics or chemistry. For example, measurement systems that count or track the position and velocity of atomic particles can have exact formulas for measurement uncertainty based on the known theoretical behavior of the particles.

b. Delta method (law of propagation of uncertainty): If the measurement result can be expressed as a function of input variables with known error distributions, the distribution of the measurement result sometimes can be computed exactly.

c. Linearized: The mathematics of the delta method may be difficult, so a linearized form of $M = T + E$ may be used instead, involving a first-order Taylor series expansion about key variables that influence E :

$$(M + \delta M) = T + \delta M/\delta G_1 + \delta M/\delta G_2 + \delta M/\delta G_3 + \dots$$

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for sources G_1 , G_2 , G_3 , etc. of bias and variation that are continuous variables (or can be represented by continuous variables). The distribution of this expression may be simpler to determine, as it involves the linear combination of scalar multiples of the random variables.

d. Simulation: Another use of the delta method is to conduct computer simulation. Again assuming that the distributions of measurement errors in input variables are known or can be approximated, a computer (i.e., Monte Carlo) simulation can obtain empirically the distribution of measurement errors in the result. Typically, one to ten thousand sets of random deviates are generated (each set has one random deviate for each variable), and the value of M is computed and archived. The archived distribution is an empirical characterization of the uncertainty in M .

e. Sensitivity study (designed experiment): If the identities and distributions of sources of bias and variation are known and these sources are continuous factors, but the functional form of the relationship between them and M is not known, an empirical sensitivity study (i.e., MCS) can be conducted to estimate the low-order coefficients ($\delta M/\delta G$) for any factor G . This will produce a Taylor series approximation to the δM , which can be used to estimate the distribution of δM , as in ¶ c above.

f. Random effects study: This is the nested MCS and variance components analysis described in ¶ 7 above.

g. Passive empirical (QA/QC-type data): An even more empirical and passive approach is to rely solely on QA/QC or similar data. The estimated standard deviation of sample measurements taken on many different days, by different analysts, using different equipment, perhaps in different laboratories can provide a useful indication of uncertainty.

9. Statements of Uncertainty

Always report measurements with a statement of uncertainty and the basis for the statement.

Develop uncertainty statements as follows:⁴⁻⁶

Involve experts in the measurement principles and use of the measurement system, individuals familiar with sampling contexts, and potential measurement users to generate a cause-and-effect diagram for measurement error, with sources of bias and variation (“factors”) identified and prioritized. Consult literature quantifying bias and variation. If needed, conduct one or more measurement capability studies incorporating those sources thought to be most important. In some cases, Gage R&R studies may be sufficient. These studies will provide “snapshot” estimates of bias and variation.

Institute a QA/QC program in which traceable or internal standards are measured routinely and the results are plotted on $\bar{X}$ and R control charts (or equivalent charts). React to out-of-control signals on the control charts. In particular, re-calibrate using traceable standards when the mean control chart shows a statistically significant change. Use the control charts, relevant literature, and the MCSs to develop uncertainty statements that involve both bias and variation.

10. References

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1030 C. Method Detection Level

1. Introduction

Detection levels are controversial, principally because of inadequate definition and confusion of terms. Frequently, the instrumental detection level is used for the method detection level and *vice versa*. Whatever term is used, most analysts agree that the smallest amount that can be detected above the noise in a procedure and within a stated confidence level is the detection level. The confidence levels are set so that the probabilities of both Type I and Type II errors are acceptably small.

Current practice identifies several detection levels (see 1010C), each of which has a defined purpose. These are the instrument detection level (IDL), the lower level of detection (LLD), the method detection level (MDL), and the level of quantitation (LOQ). Occasionally the instrument detection level is used as a guide for determining the MDL. The relationship among these levels is approximately IDL:LLD:MDL:LOQ = 1:2:4:10.

2. Determining Detection Levels

An operating analytical instrument usually produces a signal (noise) even when no sample is present or when a blank is being analyzed. Because any QA program requires frequent analysis of blanks, the mean and standard deviation become well known; the blank signal becomes very precise, i.e., the Gaussian curve of the blank distribution becomes very narrow. The IDL is the constituent concentration that produces a signal greater than three standard deviations of the mean noise level or that can be determined by injecting a standard to produce a signal that is five times the signal-to-noise ratio. The IDL is useful for estimating the constituent concentration or amount in an extract needed to produce a signal to permit calculating an estimated method detection level.

The LLD is the amount of constituent that produces a signal sufficiently large that 99% of

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the trials with that amount will produce a detectable signal. Determine the LLD by multiple injections of a standard at near zero concentration (concentration no greater than five times the IDL). Determine the standard deviation by the usual method. To reduce the probability of a Type I error (false detection) to 5%, multiply s by 1.645 from a cumulative normal probability table. Also, to reduce the probability of a Type II error (false nondetection) to 5%, double this amount to 3.290. As an example, if 20 determinations of a low-level standard yielded a standard deviation of 6 $\mu\text{g/L}$, the LLD is $3.29 \times 6 = 20 \mu\text{g/L}$.¹

The MDL differs from the LLD in that samples containing the constituent of interest are processed through the complete analytical method. The method detection level is greater than the LLD because of extraction efficiency and extract concentration factors. The MDL can be achieved by experienced analysts operating well-calibrated instruments on a nonroutine basis. For example, to determine the MDL, add a constituent to reagent water, or to the matrix of interest, to make a concentration near the estimated MDL.² Prepare and analyze seven portions of this solution over a period of at least 3 d to ensure that MDL determination is more representative than measurements performed sequentially. Include all sample processing steps in the determination. Calculate the standard deviation and compute the MDL. The replicate measurements should be in the range of one to five times the calculated MDL. From a table of the one-sided t distribution select the value of t for $7 - 1 = 6$ degrees of freedom and at the 99% level; this value is 3.14. The product 3.14 times s is the desired MDL.

Although the LOQ is useful within a laboratory, the practical quantitation limit (PQL) has been proposed as the lowest level achievable among laboratories within specified limits during routine laboratory operations.³ The PQL is significant because different laboratories will produce different MDLs even though using the same analytical procedures, instruments, and sample matrices. The PQL is about five times the MDL and represents a practical and routinely achievable detection level with a relatively good certainty that any reported value is reliable.

3. Description of Levels

Figure 1030:1 illustrates the detection levels discussed above. For this figure it is assumed that the signals from an analytical instrument are distributed normally and can be represented by a normal (Gaussian) curve.⁴ The curve labeled B is representative of the background or blank signal distribution. As shown, the distribution of the blank signals is nearly as broad as for the other distributions, that is $\sigma_B = \sigma_I = \sigma_L$. As blank analyses continue, this curve will become narrower because of increased degrees of freedom.

The curve labeled I represents the IDL. Its average value is located $k\sigma_B$ units distant from the blank curve, and k represents the value of t (from the one-sided t distribution) that corresponds to the confidence level chosen to describe instrument performance. For a 95% level and $n = 14$, $k = 1.782$ and for a 99% limit, $k = 2.68$. The overlap of the B and I curves indicates the probability of not detecting a constituent when it is present (Type II error).

The curve at the extreme right of Figure 1030:1 represents the LLD. Because only a finite

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number of determinations is used for calculating the IDL and LLD, the curves are broader than the blank but are similar, so it is reasonable to choose $\sigma_I = \sigma_L$. Therefore, the LLD is $k\sigma_I + k\sigma_L = 2k\sigma_L$ from the blank curve.

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1030 D. Data Quality Objectives

1. Introduction

Data quality objectives are systematic planning tools based on the scientific method. They are used to develop data collection designs and to establish specific criteria for the quality of data to be collected. The process helps planners identify decision-making points for data collection activities, to determine the decisions to be made based on the data collected, and to identify the criteria to be used for making each decision. This process documents the criteria for defensible decision-making before an environmental data collection activity begins.

2. Procedure

The data quality objective process comprises the stages explained in this section.

a. Stating the issue: Sometimes the reason for performing analyses is straightforward, e.g., to comply with a permit or other regulatory requirement. However, at times the reason is far more subjective; e.g., to gather data to support remedial decisions, or to track the changes in effluent quality resulting from process changes. A clear statement of the reason for the analyses is integral to establishing appropriate data quality objectives; this should include a statement of how the data are to be used, e.g., to determine permit compliance, to support decisions as to whether additional process changes will be necessary, etc.

b. Identifying possible decisions and actions: Initially, express the principal study question. For example: Is the level of contaminant A in environmental medium B higher than regulatory

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level C? This example is relatively straightforward.

Other questions may be more complex, for example: How is aquatic life affected by discharges into receiving waters by publicly owned treatment works (POTWs)? Break such a question down into several questions that might then be used to develop several decisions; organize these questions in order of consensus priority of all participating parties.

Identify alternative actions, including the no-action alternative, that could result from the various possible answers to the principal study questions.

In the first example above, if the level of contaminant in the environmental medium is higher than the regulatory level, some cleanup or treatment action may be indicated. If it is lower, the no-action alternative may be indicated, or the study team may wish to look at other environmental media and regulatory levels.

Finally, combine the principal study question with alternative actions into a decision statement. For the first example, the decision statement might be: Determine whether the mean level of contaminant A in environmental medium B exceeds the regulatory level C and requires remediation.

A multi-tiered decision statement might be: . . . if not, determine whether the maximum level of contaminant A in environmental medium D exceeds the regulatory level E and requires remediation.

c. Identifying inputs: Identify the information needed to make the necessary decision. Inputs may include measurements (including measurements of physical and chemical characteristics), data sources (historical), applicable action levels, or health effects concerns.

Identify and list the sources of information: previous data, historical records, regulatory guidance, professional judgment, scientific literature, and new data. Evaluate qualitatively whether any existing data are appropriate for the study. Existing data will be evaluated quantitatively later. Identify information needed to establish the action level. Define the basis for setting the action levels: they may be based on regulatory thresholds or standards or may be derived from issue-specific considerations, such as risk analysis. Determine only the criteria that will be used to set the numerical value. The actual numerical action level is determined later.

Confirm that the appropriate measurement methods exist to provide the necessary data. Assure that there are analytical methods for the parameters or contaminants of interest, and that they are appropriate for the matrix to be sampled. Consider the samples to be collected and the analytical methods to determine the potential for matrix interferences for each method. Assure that the limits of the method (e.g., detection limit, quantitation limit, reporting limit) are appropriate for the matrix (e.g., drinking water, wastewater, groundwater, leachate, soil, sediment, hazardous waste) and the parameter to be measured. Ensure that a laboratory is available to perform the analyses; determine its capacity, turn-around time, data product, and cost. Include this information as input to the decision-making process.

d. Identifying study limits: Identify both the geographical area and the time frame to which the decision will apply. Also define the scale of decision-making. Identify the smallest, most appropriate subsets of the total population for which decisions will be made. These subsets could

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be based on spatial or temporal boundaries. For example, while spatial boundaries of the issue may be a 300-acre site, samples may be collected from, and decisions made for, each square of a grid made up of 50-ft squares drawn on a site map. Also, while temporal boundaries of the issue may be identified (as the duration of storm events), samples may be collected at, and decisions made for, 2-h increments during a storm event. A decision resulting from this type of study might be to construct a stormwater bypass structure that would carry the first flow, which might contain the highest nutrient load, but would not necessarily carry the peak flow.

Identify any practical constraints on data collection. Identify any logistical issues that might interfere with the data-collection process, including seasonal conditions, daily variations, meteorological conditions, access conditions, availability of personnel, time, equipment, project budget, regulatory limits, appropriate analytical methods, matrix interferences, detection limits, reporting limits, site access limitations, and expertise.

e. Developing a decision rule: Define the parameter of interest, specify an action level, and integrate outputs from the previous data quality objective process steps into a single statement that describes a logical basis for choosing among alternative actions. A decision rule may be worded as follows, substituting case-specific information for the underlined words:

If the factor of interest within the scale of decision making is greater than the action level, then take alternative action A; otherwise take alternative action B.

The factor of interest is a descriptive measure (such as an instantaneous value, a mean, a median, or a proportion) that specifies the characteristic (such as calcium level in water, PCB level in soil, radon level in air) that the decision-maker would like to know about the statistical population affected by the potential decision (such as rivers or streams within a specific watershed, the specified depth of soil within a site boundary, or in basements or crawlspaces within a metropolitan area).

The scale of decision-making is the smallest, most appropriate subset for which separate decisions will be made (such as each stream segment/river mile or each square of a grid identified on a site map, or each section of township X, range Y of county Z).

The action level is a measurement threshold value of the parameter of interest that provides the criterion for choosing among alternative actions (such as a stream standard to protect aquatic life, a published regulatory standard, or a health-effects-related level).

Alternative action A is the alternative of choice if the action level is exceeded (such as initiate non-point-source controls, initiate cleanup of the soil to a specified depth, or distribute technical information to property owners). Noncompliance with the action level is the alternative hypothesis. (Either alternative action can be labeled A without making the decision rule any less valid.)

Alternative action B is the alternative of choice if the action level is not exceeded (such as continue routine monitoring, leave the soil in place, or provide a summary of the data collection activity to potential developers). Compliance with the action level is the null hypothesis that is generally the no-action alternative or baseline condition. Either alternative action can be labeled B without making the decision rule any less valid.

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f. Specifying limits on decision errors: Establish limits on the decision error that the decision-maker will tolerate. Use these limits to establish performance goals for design of the data collection activity. Base limits on the consequences of making a wrong decision.

Decision-makers are interested in knowing the true state of some feature of the environment. Environmental data can be only an estimate of this true state; decisions therefore are based on environmental data that are in some degree of error. The goal is to develop a data-collection design that reduces the chances of making a decision error to a level that is acceptable to the decision-maker. Sources of uncertainty include sample design error and measurement error; when combined, they represent the total study error.

Sample design error refers to the error inherent in using a portion of a population to represent the whole population. It is not practical, for example, to measure and record the concentration of an analyte at every point in a stream on a continuous basis; instead, measure analyte concentration at well-defined locations and time intervals to represent this analyte concentration continuum.

Measurement error refers to the error inherent in the measurement process. A measurement system does not measure, on a molecular level, the amount of an analyte in a sample; it measures an indicator of the amount of an analyte in a sample. This indicator might be the amount of a specific wavelength of light absorbed by a sample, the change in conductivity of a solution containing the analyte, or the amount of an analyte, in a gaseous or ionized form, that passes through a membrane.

Use data to choose between the one condition of the environment (the null hypothesis, H_0) and an alternative condition (the alternative hypothesis, H_a). A decision error occurs when the decision-maker rejects the null hypothesis when it is true (false-positive decision error) or fails to reject the null hypothesis when it is false (false-negative decision error).*(1)

The null hypothesis usually is treated as the baseline condition that is presumed to be true in the absence of strong evidence to the contrary. Either condition may be selected as the null hypothesis, but if the null hypothesis is chosen carefully, it provides a way to guard against making the decision error that the decision-maker considers to have the more undesirable consequences.

While the possibility of a decision error never can be totally eliminated, it can be controlled by various means, including collecting a large number of samples (to control sampling design error), analyzing individual samples several times, or using more precise laboratory methods (to control measurement error). Better sampling designs also can be developed to collect data that more accurately represent the population of interest. Every study will use a different method of controlling decision errors, depending on the source of the largest components of total decision error in the data set and the ease of reducing those error components.

Reducing the probability of making decision errors generally increases study costs. In many cases, however, it is not necessary to control decision error within very small limits to meet the decision-maker's needs. If the consequences of decision errors are minor, a reasonable decision could be made on the basis of relatively crude data. If, on the other hand, consequences of

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decision errors are severe, the decision-maker will want to control sampling design and measurements within very small limits.

Factors used to judge data quality include precision, bias, representativeness, completeness, and comparability. Precision, bias, and completeness can be applied to the measurement (field and laboratory) system. Most analytical laboratories have systems to quantify these factors. Laboratory precision can be estimated through the analysis of laboratory replicates. Laboratory bias can be estimated by the analysis of standards, known additions, and performance evaluation (PE) samples. There is no common system in place to estimate field bias. A combination of field and laboratory completeness can be estimated through comparison of the number of analytical results provided by the laboratory with the number of analytical results specified in the sample design. Laboratory representativeness and comparability involve the analytical method used and the performance of the laboratory as compared to the performance of other laboratories (PE studies), which are not commonly quantified.

Precision, bias, representativeness, completeness, and comparability can be applied to the sample design: Precision would indicate how precisely this sample design reflects the total population. Bias would indicate how accurately this sample design reflects the total population. Representativeness would indicate to what extent the sample design is representative of the total population. Completeness would indicate how well the sample design reflects the complete population. Comparability would indicate the similarity of the sample design to other sample designs for similar situations. None of these usually is measured.

While data quality factors provide some insight into sample measurement errors, they do not provide any indication of sample design errors. These errors are additive, so that if precision were $\pm 90\%$, bias were $\pm 90\%$, and representativeness were $\pm 90\%$, combined uncertainty could be up to $\pm 27\%$:

$$(100\% \times 0.1) + (90\% \times 0.1) + (81\% \times 0.1) = 10\% + 9\% + 8\% = 27\%$$

Because most errors are not quantifiable, a study usually is designed with a balance between acceptable decision errors and acceptable study cost.

g. Optimizing the design for collection: Identify the most resource-effective design for the study that will achieve the data quality objectives (DQOs). Use statistical techniques to develop alternative data collection designs and evaluate their efficiency in meeting the DQOs. To develop the optimal study design, it may be necessary to work through this step more than once after revisiting previous steps of the process.

Review the DQO outputs and existing environmental data, develop general data collection design alternatives, and formulate the mathematical expressions needed to solve the design issue for each data collection design alternative. Develop the following three mathematical expressions:

- A method for testing the statistical hypothesis and a sample size formula that corresponds to the method (e.g., Student's *t* test),

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- A statistical model that describes the relationship of the measured value to the “true” value. Often the model will describe the components of error or bias believed to exist in the measured value, and
- A cost function that relates the number of samples to the total cost of sampling and analysis.

Select the optimal sample size that satisfies the DQOs for each data collection design alternative. Using the mathematical expressions specified above, calculate the optimal sample size that satisfies the DQOs. If no design will meet the limits on decision errors within the budget or other constraints, relax one or more constraints by, for example, increasing the budget for sampling and analysis, increasing the width of the region of uncertainty, increasing the tolerable decision error rates, relaxing other project constraints such as the schedule, or changing the boundaries; it may be possible to reduce sampling and analysis costs by changing or eliminating subgroups that will require separate decisions.

Select the most resource-effective data collection design that satisfies all of the DQOs and document the operational details and theoretical assumptions of the selected design in the sampling and analysis plan.

3. Bibliography

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1994. Guidance for the Data Quality Objectives Process. EPA QA/G-4, Quality Assurance Management Staff, U.S. Environmental Protection Agency, Washington, D.C.

1030 E. Checking Correctness of Analyses

The following procedures for checking correctness of analyses are applicable specifically to water samples for which relatively complete analyses are made.¹ These include pH, conductivity, total dissolved solids (TDS), and major anionic and cationic constituents that are indications of general water quality.

The checks described do not require additional laboratory analyses. Three of the checks require calculation of the total dissolved solids and conductivity from measured constituents. Sum concentrations (in milligrams per liter) of constituents to calculate the total dissolved solids are as follows:

$$\begin{aligned} \text{Total dissolved solids} = & 0.6 (\text{alkalinity} \times 2) + \text{Na}^+ + \text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+} + \text{Cl}^- \\ & + \text{SO}_4^{2-} + \text{SiO}_3^{2-} + \text{NO}_3^- + \text{F}^- \end{aligned}$$

Calculate electrical conductivity from the equation:

$$G = \lambda C - (k_1 \lambda + k_2) (C)^{3/2}$$

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where:

G = conductivity of salt solution,

C = concentration of salt solution,

λ = equivalent conductance of salt solution at infinite dilution,

k_1, k_2 = constants for relaxation of ion cloud effect and electrophoretic effect relative to ion mobility.¹

1. Anion-Cation Balance²

The anion and cation sums, when expressed as milliequivalents per liter, must balance because all potable waters are electrically neutral. The test is based on the percentage difference defined as

follows:

$$\% \text{ difference} = 100 \frac{\Sigma \text{ cations} - \Sigma \text{ anions}}{\Sigma \text{ cations} + \Sigma \text{ anions}}$$

and the typical criteria for acceptance are as follows:

Anion Sum meq/L	Acceptable Difference
0–3.0	±0.2 meq/L
3.0–10.0	± 2%
10.0–800	5%

2. Measured TDS = Calculated TDS²

The measured total dissolved solids concentration should be higher than the calculated one because a significant contributor may not be included in the calculation. If the measured value is less than the calculated one, the higher ion sum and measured value are suspect; the sample should be reanalyzed. If the measured solids concentration is more than 20% higher than the calculated one, the low ion sum is suspect and selected constituents should be reanalyzed. The acceptable ratio is as follows:

$$1.0 < \frac{\text{measured TDS}}{\text{calculated TDS}} < 1.2$$

3. Measured EC = Calculated EC

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If the calculated electrical conductivity (EC) is higher than the measured value, reanalyze the higher ion sum. If the calculated EC is less than the measured one, reanalyze the lower ion sum. The acceptable ratio is as follows:

$$0.9 < \frac{\text{calculated EC}}{\text{measured EC}} < 1.1$$

Some electrical conductivity values for ions commonly found in water are given in Table 1030:I.

4. Measured EC and Ion Sums

Both the anion and cation sums should be $\frac{1}{100}$ of the measured EC value. If either of the two sums does not meet this criterion, that sum is suspect; reanalyze the sample. The acceptable criteria are as follows:

$$100 \times \text{anion (or cation) sum, meq/L} = (0.9\text{--}1.1) \text{ EC}$$

5. Calculated TDS to EC Ratio

If the ratio of calculated TDS to conductivity falls below 0.55, the lower ion sum is suspect; reanalyze it. If the ratio is above 0.7, the higher ion sum is suspect; reanalyze it. If reanalysis causes no change in the lower ion sum, an unmeasured constituent, such as ammonia or nitrite, may be present at a significant concentration. If poorly dissociated calcium and sulfate ions are present, the TDS may be as high as 0.8 times the EC. The acceptable criterion is as follows:

$$\text{calculated TDS/conductivity} = 0.55\text{--}0.7$$

6. Measured TDS to EC Ratio

The acceptable criteria for this ratio are from 0.55 to 0.7. If the ratio of TDS to EC is outside these limits, measured TDS or measured conductivity is suspect; reanalyze.

A more complete exposition³ of the above quality-control checks has been published.

7. References

1. ROSSUM, J.R. 1975. Checking the accuracy of water analyses through the use of conductivity. *J. Amer. Water Works Assoc.* 67:204.
2. FRIEDMAN, L.C. & D.E. ERDMANN. 1982. Quality Assurance Practices for Analyses of Water and Fluvial Sediments. Tech. Water Resources Inc., Book 5, Chapter A6. U.S. Government Printing Off., Washington, D.C.
3. OPPENHEIMER, J. & A.D. EATON. 1986. Quality control and mineral analysis. *In Proc. Water Quality Technology Conference* (Houston, Texas, December 8-11, 1985). American Water Works Assoc., Denver, Colo.

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1040 METHOD DEVELOPMENT AND EVALUATION

1040 A. Introduction

Although standard methods are available from many nationally recognized sources, there may be occasions when they cannot be used or when no standard method exists for a particular constituent or characteristic. Therefore, method development may be required. Method development is the set of experimental procedures devised for measuring a known amount of a constituent in various matrices, in the case of chemical analyses; or a known characteristic (e.g., biological or toxicological) of various matrices.

1040 B. Method Validation

Whether an entirely new method is developed by accepted research procedures or an existing method is modified to meet special requirements, validation by a three-step process is required: determination of single-operator precision and bias, analysis of independently prepared unknown samples, and determination of method ruggedness.

1. Single-Operator Characteristics

This part of the validation procedure requires determining the method detection level (MDL) as in Section 1030; the bias of the method, i.e., the systematic error of the method; and the precision obtainable by a single operator, i.e., the random error introduced in using the method. To make these determinations, analyze at least 7 but preferably 10 or more portions of a standard at each of several concentrations in each matrix that may be used. Use one concentration at, or slightly above, the MDL and one relatively high so that the range of concentrations for which the method is applicable can be specified.

The use of several concentrations to determine bias and precision will reveal the form of the relationship between these method characteristics and the concentration of the substance, the characteristic toxicity of the substance, or the biological factor of interest. This relationship may be constant, linear, or curvilinear and is a significant characteristic of the method that should be explained clearly. Table 1040:I shows calculation of precision and bias for a single concentration in a single matrix from eight replicate analyses of a standard with a known concentration of 1.30 mg/L.

The bias is $0.49/8 = 0.06$ mg/L and the precision is the square root of $0.2335/(8-1) = \sqrt{0.03336}$, or 0.18 mg/L (note that this is similar to the calculation for standard deviation).

2. Analysis of Unknown Samples

This step in the method validation procedure requires analysis of independently prepared standards where the value is unknown to the analyst. Analyze each unknown in replicate by

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following the standard operating procedure for the method. The mean amount recovered should be within three standard deviations (s) of the mean value of the standard but preferably within 2 s .

Obtain the unknowns from other personnel in the analyst's laboratory using either purchased analytical-grade reagents or standards available from National Institute of Standards and Technology (NIST). If available for the particular constituent, performance evaluation samples from EPA-Cincinnati are particularly useful.

3. Method Ruggedness

A test of the ruggedness, i.e., stability of the result produced when steps in the method are varied, is the final validation step. It is especially important to determine this characteristic of a method if it is to be proposed as a standard or reference method. A properly conducted ruggedness test will point out those procedural steps in which rigor is critical and those in which some leeway is permissible.

The Association of Official Analytical Chemists¹ has suggested a method for this test in which eight separate analyses can be used to determine the effect of varying seven different steps in an analytical procedure. To illustrate, suppose the effect of changing the factors in Table 1040:II is to be determined. To make the determination, denote the nominal factors by capital letters A through G and the variations by the corresponding lower-case letters. Then set up a table of the factors as in Table 1040:III.

If combination 1 is analyzed, the result will be s . If combination 2 is analyzed, the result will be t , and so on until all eight combinations have been analyzed. To determine the effect of varying a factor, find the four results where the factor was nominal (all caps) and the four where it was varied (all lower case) and compare the averages of the two groups. For example, to compare the effect of changing C to c , use results $(s + u + w + y)/4$ and $(t + v + x + z)/4$. Calculate all seven pairs to get seven differences, which can then be ranked to reveal those with a significant effect on the results. If there is no outstanding difference, calculate the average and standard deviation of the eight results s through z . The standard deviation is a realistic estimate of the precision of the method. This design tests main effects, not interactions.

4. Equivalency Testing

After a new method has been validated by the procedures listed above, it may be prudent to test the method for equivalency to standard methods, unless none exist. This requires analysis of a minimum of three concentrations by the alternate and by the standard method. If the range of concentration is very broad, test more concentrations. Once an initial set of analyses (five or more) has been made at each chosen concentration, apply the following statistical steps:²

1. Test the distribution of data for normality and transform the data if necessary (Section 1010B).
2. Select an appropriate sample size based on an estimate of the standard deviation.³

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3. Test the variances of the two methods using the F-ratio statistic.
4. Test the average values of the two methods using a Student-*t* statistic.

An explanation of each of these steps with additional techniques and examples has been published.⁴ Because the number of analyses can be very large, the calculations become complex and familiarity with basic statistics is necessary. A listing of standard, reference, and equivalent methods for water analysis is available.⁵

5. References

1. YOUNG, W.J. & E.H. STEINER. 1975. Statistical Manual of AOAC. Assoc. Official Analytical Chemists, Washington, D.C.
2. WILLIAMS, L.R. 1985. Harmonization of Biological Testing Methodology: A Performance Based Approach in Aquatic Toxicology and Hazard Assessment. 8th Symp. ASTM STP 891, R.C. Bahner & D.J. Hansen, eds. American Soc. Testing & Materials, Philadelphia, Pa.
3. NATRELLA, M.G. 1963. Experimental Statistics. National Bureau of Standards Handbook 91, Washington, D.C.
4. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1983. Guidelines for Establishing Method Equivalency to Standard Methods. Rep. 600/X-83-037, Environmental Monitoring Systems Lab., Las Vegas, Nev.
5. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1994. Guidelines establishing test procedures for the analysis of pollutants under the Clean Water Act. Final rule. 40 CFR Part 136; *Federal Register* 59:20:4504.

1040 C. Collaborative Testing

Once a new or modified method has been developed and validated it is appropriate to determine whether the method should be made a standard method. The procedure to convert a method to standard status is the collaborative test.¹ In this test, different laboratories use the standard operating procedure to analyze a select number of samples to determine the method's bias and precision as would occur in normal practice.

In planning for a collaborative test, consider the following factors: a precisely written standard operating procedure, the number of variables to be tested, the number of levels to be tested, and the number of replicates required. Because method precision is estimated by the standard deviation, which itself is the result of many sources of variation, the variables that affect it must be tested. These may include the laboratory, operator, apparatus, and concentration range.

1. Variables

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Test at least the following variables: Laboratory—Involve at least three different laboratories, although more are desirable to provide a better estimate of the standard deviation; Apparatus—Because model and manufacturer differences can be sources of error, analyze at least two replicates of each concentration per laboratory; Operators—To determine overall precision, involve at least six analysts with not more than two from each laboratory; Levels—If the method development has indicated that the relative standard deviation is constant, test three levels covering the range of the method. If it is not constant, use more levels spread uniformly over the operating range.

If matrix effects are suspected, conduct the test in each medium for which the method was developed. If this is not feasible, use appropriate grades of reagent water as long as this is stipulated in the resulting statement of method characteristics.

2. Number of Replicates

Calculate the number of replicates after the number of variables to be tested has been determined by using the formula:

$$r > 1 + (30/P)$$

where:

r = number of replicates and

P = the product of several variables.

The minimum number of replicates is two. As an example, if three levels of a substance are to be analyzed by single operators in six laboratories on a single apparatus, then P is calculated as follows:

$$P = 3 \times 1 \times 6 \times 1 = 18$$

and the number of replicates is

$$r > 1 + (30/18) > 2.7 \text{ or } r = 3.$$

3. Illustrative Collaborative Test

Send each of five laboratories four concentrations of a compound (4.3, 11.6, 23.4, and 32.7 mg/L) with instructions to analyze in triplicate using the procedure provided. Tabulate results as shown in Table 1040:IV below (the results for only one concentration are shown). Because there are no obviously aberrant values (use the method in Section 1010B to reject outliers), use all the data.

Calculate the average and standard deviation for each laboratory; use all 15 results to calculate a grand average and standard deviation. The difference between the average of each laboratory and the grand average reveals any significant bias, such as that shown for Laboratories 1 and 3. The difference between the grand average and the known value is the

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method bias, e.g., $33.0 - 32.7 = 0.3$ mg/L or 0.9%. The relative standard deviation of the grand average (1.5 mg/L) is 4.5%, which is the method precision, and the s for each laboratory is the single-operator precision.

As noted in Table 1040:IV, the sum of the deviations from the known value for the laboratories was 1.3, so the average deviation (bias) was $1.3/5 = 0.26$, rounded to 0.3, which is the same as the difference between the grand average and the known value.

For all four unknowns in this test, the percentage results indicated increasing bias and decreasing precision as the concentration decreased. Therefore, to describe the method in a formal statement, the precision would be given by a straight line with the formula $y = mx + b$; where y is the relative standard deviation, m is the slope of the line, x is the concentration, and b is the relative standard deviation at concentration = 0. The values found from the collaborative test are shown in Table 1040:V.

These results indicate that the method is acceptable. However, concentrations of less than about 10 mg/L require greater care in analysis.

4. Reference

1. YOUNDEN, W.J. & E.H. STEINER. 1975. Statistical Manual of the AOAC. Assoc. Official Analytical Chemists, Washington, D.C.

1050 EXPRESSION OF RESULTS

1050 A. Units

This text uses the International System of Units (SI) and chemical and physical results are expressed in milligrams per liter (mg/L). See Section 7020D for expression of radioactivity results. Record only the significant figures. If concentrations generally are less than 1 mg/L, it may be more convenient to express results in micrograms per liter ($\mu\text{g/L}$). Use $\mu\text{g/L}$ when concentrations are less than 0.1 mg/L.

Express concentrations greater than 10 000 mg/L in percent, 1% being equal to 10 000 mg/L when the specific gravity is 1.00. In solid samples and liquid wastes of high specific gravity, make a correction if the results are expressed as parts per million (ppm) or percent by weight:

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$$\text{ppm by weight} = \frac{\text{mg/L}}{\text{sp gr}}$$
$$\% \text{ by weight} = \frac{\text{mg/L}}{10\,000 \times \text{sp gr}}$$

In such cases, if the result is given as milligrams per liter, state specific gravity.

The unit equivalents per million (epm), or the identical and less ambiguous term milligram-equivalents per liter, or milliequivalents per liter (me/L), can be valuable for making water treatment calculations and checking analyses by anion-cation balance.

Table 1050:I presents factors for converting concentrations of common ions from milligrams per liter to milliequivalents per liter, and vice versa. The term milliequivalent used in this table represents 0.001 of an equivalent weight. The equivalent weight, in turn, is defined as the weight of the ion (sum of the atomic weights of the atoms making up the ion) divided by the number of charges normally associated with the particular ion. The factors for converting results from milligrams per liter to milliequivalents per liter were computed by dividing the ion charge by weight of the ion. Conversely, factors for converting results from milliequivalents per liter to milligrams per liter were calculated by dividing the weight of the ion by the ion charge.

1050 B. Significant Figures

1. Reporting Requirements

To avoid ambiguity in reporting results or in presenting directions for a procedure, it is the custom to use “significant figures.” All digits in a reported result are expected to be known definitely, except for the last digit, which may be in doubt. Such a number is said to contain only significant figures. If more than a single doubtful digit is carried, the extra digit or digits are not significant. If an analytical result is reported as “75.6 mg/L,” the analyst should be quite certain of the “75,” but may be uncertain as to whether the “.6” should be .5 or .7, or even .4 or .8, because of unavoidable uncertainty in the analytical procedure. If the standard deviation were known from previous work to be ± 2 mg/L, the analyst would have, or should have, rounded off the result to “76 mg/L” before reporting it. On the other hand, if the method were so good that a result of “75.61 mg/L” could have been conscientiously reported, then the analyst should not have rounded it off to 75.6.

Report only such figures as are justified by the accuracy of the work. Do not follow the all-too-common practice of requiring that quantities listed in a column have the same number of figures to the right of the decimal point.

2. Rounding Off

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Round off by dropping digits that are not significant. If the digit 6, 7, 8, or 9 is dropped, increase preceding digit by one unit; if the digit 0, 1, 2, 3, or 4 is dropped, do not alter preceding digit. If the digit 5 is dropped, round off preceding digit to the nearest even number: thus 2.25 becomes 2.2 and 2.35 becomes 2.4.

3. Ambiguous Zeros

The digit 0 may record a measured value of zero or it may serve merely as a spacer to locate the decimal point. If the result of a sulfate determination is reported as 420 mg/L, the report recipient may be in doubt whether the zero is significant or not, because the zero cannot be deleted. If an analyst calculates a total residue of 1146 mg/L, but realizes that the 4 is somewhat doubtful and that therefore the 6 has no significance, the answer should be rounded off to 1150 mg/L and so reported but here, too, the report recipient will not know whether the zero is significant. Although the number could be expressed as a power of 10 (e.g., 11.5×10^2 or 1.15×10^3), this form is not used generally because it would not be consistent with the normal expression of results and might be confusing. In most other cases, there will be no doubt as to the sense in which the digit 0 is used. It is obvious that the zeros are significant in such numbers as 104 and 40.08. In a number written as 5.000, it is understood that all the zeros are significant, or else the number could have been rounded off to 5.00, 5.0, or 5, whichever was appropriate. Whenever the zero is ambiguous, it is advisable to accompany the result with an estimate of its uncertainty.

Sometimes, significant zeros are dropped without good cause. If a buret is read as “23.60 mL,” it should be so recorded, and not as “23.6 mL.” The first number indicates that the analyst took the trouble to estimate the second decimal place; “23.6 mL” would indicate a rather careless reading of the buret.

4. Standard Deviation

If, for example, a calculation yields a result of 1449 mg/L or 1451 mg/L with a standard deviation of ± 100 mg/L, report as 1449 ± 100 mg/L or 1451 ± 100 mg/L, respectively. Ensure that the number of significant figures in the standard deviation is not reduced if the value is 100 ± 1 . This could cause incorrect rounding of data to 1400 or 1500 mg/L, respectively.

5. Calculations

As a practical operating rule, round off the result of a calculation in which several numbers are multiplied or divided to as few significant figures as are present in the factor with the fewest significant figures. Suppose that the following calculations must be made to obtain the result of an analysis:

$$\frac{56 \times 0.003462 \times 43.22}{1.684}$$

A ten-place calculator yields an answer of “4.975 740 998.” Round off this number to “5.0”

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because one of the measurements that entered into the calculation, 56, has only two significant figures. It was unnecessary to measure the other three factors to four significant figures because the “56” is the “weakest link in the chain” and limits accuracy of the answer. If the other factors were measured to only three, instead of four, significant figures, the answer would not suffer and the labor might be less.

When numbers are added or subtracted, the number that has the fewest decimal places, not necessarily the fewest significant figures, puts the limit on the number of places that justifiably may be carried in the sum or difference. Thus the sum

$$\begin{array}{r} 0.0072 \\ 12.02 \\ 4.0078 \\ 25.9 \\ \hline 4886 \\ 4927.9350 \end{array}$$

must be rounded off to “4928,” no decimals, because one of the addends, 4886, has no decimal places. Notice that another addend, 25.9, has only three significant figures and yet it does not set a limit to the number of significant figures in the answer. The preceding discussion is necessarily oversimplified. The reader is referred to mathematical texts for more detailed discussion.

1060 COLLECTION AND PRESERVATION OF SAMPLES

1060 A. Introduction

It is an old axiom that the result of any testing method can be no better than the sample on which it is performed. It is beyond the scope of this publication to specify detailed procedures for the collection of all samples because of varied purposes and analytical procedures. Detailed information is presented in specific methods. This section presents general considerations, applicable primarily to chemical analyses. See appropriate sections for samples to be used in toxicity testing and microbiological, biological, and radiological examinations.

The objective of sampling is to collect a portion of material small enough in volume to be transported conveniently and yet large enough for analytical purposes while still accurately representing the material being sampled. This objective implies that the relative proportions or concentrations of all pertinent components will be the same in the samples as in the material being sampled, and that the sample will be handled in such a way that no significant changes in composition occur before the tests are made.

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Frequently the objective of sampling and testing is to demonstrate whether continuing compliance with specific regulatory requirements has been achieved. Samples are presented to the laboratory for specific determinations with the sampler being responsible for collecting a valid and representative sample. Because of the increasing importance placed on verifying the accuracy and representativeness of data, greater emphasis is placed on proper sample collection, tracking, and preservation techniques. Often, laboratory personnel help in planning a sampling program, in consultation with the user of the test results. Such consultation is essential to ensure selecting samples and analytical methods that provide a sound and valid basis for answering the questions that prompted the sampling and that will meet regulatory and/or project-specific requirements.

This section addresses the collection and preservation of water and wastewater samples; the general principles also apply to the sampling of solid or semisolid matrices.

1. General Requirements

Obtain a sample that meets the requirements of the sampling program and handle it so that it does not deteriorate or become contaminated or compromised before it is analyzed.

Ensure that all sampling equipment is clean and quality-assured before use. Use sample containers that are clean and free of contaminants. Bake at 450°C all bottles to be used for organic-analysis sampling.

Fill sample containers without prerinsing with sample; prerinsing results in loss of any pre-added preservative and sometimes can bias results high when certain components adhere to the sides of the container. Depending on determinations to be performed, fill the container full (most organic compound determinations) or leave space for aeration, mixing, etc. (microbiological and inorganic analyses). If a bottle already contains preservative, take care not to overfill the bottle, as preservative may be lost or diluted. Except when sampling for analysis of volatile organic compounds, leave an air space equivalent to approximately 1% of the container volume to allow for thermal expansion during shipment.

Special precautions (discussed below) are necessary for samples containing organic compounds and trace metals. Because many constituents may be present at low concentrations (micrograms or nanograms per liter), they may be totally or partially lost or easily contaminated when proper sampling and preservation procedures are not followed.

Composite samples can be obtained by collecting over a period of time, depth, or at many different sampling points. The details of collection vary with local conditions, so specific recommendations are not universally applicable. Sometimes it is more informative to analyze numerous separate samples instead of one composite so that variability, maxima, and minima can be determined.

Because of the inherent instability of certain properties and compounds, composite sampling for some analytes is not recommended where quantitative values are desired (examples include oil and grease, acidity, alkalinity, carbon dioxide, chlorine residual, iodine, hexavalent chromium, nitrate, volatile organic compounds, radon-222, dissolved oxygen, ozone,

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temperature, and pH). In certain cases, such as for BOD, composite samples are routinely required by regulatory agencies. Refrigerate composite samples for BOD and nitrite.

Sample carefully to ensure that analytical results represent the actual sample composition. Important factors affecting results are the presence of suspended matter or turbidity, the method chosen for removing a sample from its container, and the physical and chemical changes brought about by storage or aeration. Detailed procedures are essential when processing (blending, sieving, filtering) samples to be analyzed for trace constituents, especially metals and organic compounds. Some determinations can be invalidated by contamination during processing. Treat each sample individually with regard to the substances to be determined, the amount and nature of turbidity present, and other conditions that may influence the results.

Carefully consider the technique for collecting a representative sample and define it in the sampling plan. For metals it often is appropriate to collect both a filtered and an unfiltered sample to differentiate between total and dissolved metals present in the matrix. Be aware that some metals may partially sorb to filters. Beforehand, determine the acid requirements to bring the pH to <2 on a separate sample. Add the same relative amount of acid to all samples; use ultrapure acid preservative to prevent contamination. Be sure that the dilution caused by acidifying is negligible or sufficiently reproducible for a dilution correction factor. When filtered samples are to be collected, filter them, if possible, in the field, or at the point of collection before preservation with acid. Filter samples in a laboratory-controlled environment if field conditions could cause error or contamination; in this case filter as soon as possible. Often slight turbidity can be tolerated if experience shows that it will cause no interference in gravimetric or volumetric tests and that its influence can be corrected in colorimetric tests, where it has potentially the greatest interfering effect. Sample collector must state whether or not the sample has been filtered.

Make a record of every sample collected and identify every bottle with a unique sample number, preferably by attaching an appropriately inscribed tag or label. Document sufficient information to provide positive sample identification at a later date, including the unique sample identification number, the name of the sample collector, the date, hour, exact location, and, if possible, sample type (e.g., grab or composite), and any other data that may be needed for correlation, such as water temperature, weather conditions, water level, stream flow, post-collection conditions, etc. If space for all pertinent information for label or attached tag is insufficient, record information in a bound sample log book at the sampling site at the time of sample collection. Use waterproof ink to record all information (preferably with black, non-solvent-based ink). Fix sampling points by detailed description in the sampling plan, by maps, or with the aid of stakes, buoys, or landmarks in a manner that will permit their identification by other persons without reliance on memory or personal guidance. Global positioning systems (GPS) also are used and supply accurate sampling position data. Particularly when sample results are expected to be involved in litigation, use formal “chain-of-custody” procedures (see ¶ B.2 below), which trace sample history from collection to final reporting.

Before collecting samples from distribution systems, flush lines with three to five pipe

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volumes (or until water is being drawn from the main source) to ensure that the sample is representative of the supply, taking into account the volume of pipe to be flushed and the flow velocity. If the distribution system volume is unavailable, flush with tap fully open for at least 2 to 3 min before sampling. An exception to these guidelines, i.e., collecting a first draw sample, is when information on areas of reduced or restricted flow is desired or when samples for lead in drinking water are being collected.

Although well pumping protocols depend on the objectives of an investigation and other factors such as well characteristics and available equipment, a general rule is to collect samples from wells only after the well has been purged sufficiently (usually with three to ten well volumes) to ensure that the sample represents the groundwater. Purging stagnant water is critical. Sometimes it will be necessary to pump at a specified rate to achieve a characteristic drawdown, if this determines the zones from which the well is supplied; record purging rate and drawdown, if necessary. By using methods with minimal drawdown, purging volumes can be reduced significantly.

When samples are collected from a river or stream, observed results may vary with depth, stream flow, and distance from each shore. Selection of the number and distribution of sites at which samples should be collected depends on study objectives, stream characteristics, available equipment, and other factors. If equipment is available, take an integrated sample from top to bottom in the middle of the main channel of the stream or from side to side at mid-depth. If only grab or catch samples can be collected, preferably take them at various points of equal distance across the stream; if only one sample can be collected, take it in the middle of the main channel of the stream and at mid-depth. Integrated samples are described further in ¶ B.1c below.

Rivers, streams, lakes, and reservoirs are subject to considerable variations from normal causes such as seasonal stratification, diurnal variations, rainfall, runoff, and wind. Choose location, depth, and frequency of sampling depending on local conditions and the purpose of the investigation.

Use the following examples for general guidance. Avoid areas of excessive turbulence because of potential loss of volatile constituents and of potential presence of denser-than-air toxic vapors. Avoid sampling at weirs if possible because such locations tend to favor retrieval of lighter-than-water, immiscible compounds. Generally, collect samples beneath the surface in quiescent areas and open sampling container below surface with the mouth directed toward the current to avoid collecting surface scum unless oil and grease is a constituent of interest; then collect water at the surface. If composite samples are required, ensure that sample constituents are not lost during compositing because of improper handling of portions being composited. If samples will be analyzed for organic constituents, refrigerate composited portions. Do not composite samples for VOC analysis because some of the components will be lost through volatilization.

2. Safety Considerations

Because sample constituents may be toxic, take adequate precautions during sampling and sample handling. Toxic substances can enter through the skin and eyes and, in the case of

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vapors, also through the lungs. Ingestion can occur via direct contact of toxic materials with foods or by adsorption of vapors onto foods. Precautions may be limited to wearing gloves or may include coveralls, aprons, or other protective apparel. Often, the degree of protection provided by chemical protective clothing (CPC) is specific for different manufacturers and their product models¹; ensure that the clothing chosen will offer adequate protection. Always wear eye protection (e.g., safety glasses with side shields or goggles). When toxic vapors may be present, sample only in well-ventilated areas, or use an appropriate respirator or self-contained breathing apparatus. In a laboratory, open sample containers in a fume hood. Never have food in the laboratory, near samples, or near sampling locations; always wash hands thoroughly before handling food.²

Always prohibit eating, drinking, or smoking near samples, sampling locations, and in the laboratory. Keep sparks, flames, and excessive heat sources away from samples and sampling locations. If flammable compounds are suspected or known to be present and samples are to be refrigerated, use only specially designed *explosion-proof* refrigerators.²

Collect samples safely, avoiding situations that may lead to accidents. When in doubt as to the level of safety precautions needed, consult a knowledgeable industrial hygienist or safety professional. Samples with radioactive contaminants may require other safety considerations; consult a health physicist.

Label adequately any sample known or suspected to be hazardous because of flammability, corrosivity, toxicity, oxidizing chemicals, or radioactivity, so that appropriate precautions can be taken during sample handling, storage, and disposal.

3. References

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1060 B. Collection of Samples

1. Types of Samples

a. *Grab samples*: Grab samples are single samples collected at a specific spot at a site over a short period of time (typically seconds or minutes). Thus, they represent a “snapshot” in both space and time of a sampling area. Discrete grab samples are taken at a selected location, depth, and time. Depth-integrated grab samples are collected over a predetermined part or the entire depth of a water column, at a selected location and time in a given body of water.

A sample can represent only the composition of its source at the time and place of

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collection. However, when a source is known to be relatively constant in composition over an extended time or over substantial distances in all directions, then the sample may represent a longer time period and/or a larger volume than the specific time and place at which it was collected. In such circumstances, a source may be represented adequately by single grab samples. Examples are protected groundwater supplies, water supplies receiving conventional treatment, some well-mixed surface waters, but rarely, wastewater streams, rivers, large lakes, shorelines, estuaries, and groundwater plumes.

When a source is known to vary with time, grab samples collected at suitable intervals and analyzed separately can document the extent, frequency, and duration of these variations. Choose sampling intervals on the basis of the expected frequency of changes, which may vary from as little as 5 min to as long as 1 h or more. Seasonal variations in natural systems may necessitate sampling over months. When the source composition varies in space (i.e., from location to location) rather than time, collect samples from appropriate locations that will meet the objectives of the study (for example, upstream and downstream from a point source, etc.).

The same principles apply to sampling wastewater sludges, sludge banks, and muds, although these matrices are not specifically addressed in this section. Take every possible precaution to obtain a representative sample or one conforming to a sampling program.

b. Composite samples: Composite samples should provide a more representative sampling of heterogeneous matrices in which the concentration of the analytes of interest may vary over short periods of time and/or space. Composite samples can be obtained by combining portions of multiple grab samples or by using specially designed automatic sampling devices. Sequential (time) composite samples are collected by using continuous, constant sample pumping or by mixing equal water volumes collected at regular time intervals. Flow-proportional composites are collected by continuous pumping at a rate proportional to the flow, by mixing equal volumes of water collected at time intervals that are inversely proportional to the volume of flow, or by mixing volumes of water proportional to the flow collected during or at regular time intervals.

Advantages of composite samples include reduced costs of analyzing a large number of samples, more representative samples of heterogeneous matrices, and larger sample sizes when amounts of test samples are limited. Disadvantages of composite samples include loss of analyte relationships in individual samples, potential dilution of analytes below detection levels, increased potential analytical interferences, and increased possibility of analyte interactions. In addition, use of composite samples may reduce the number of samples analyzed below the required statistical need for specified data quality objectives or project-specific objectives.

Do not use composite samples with components or characteristics subject to significant and unavoidable changes during storage. Analyze individual samples as soon as possible after collection and preferably at the sampling point. Examples are dissolved gases, residual chlorine, soluble sulfide, temperature, and pH. Changes in components such as dissolved oxygen or carbon dioxide, pH, or temperature may produce secondary changes in certain inorganic constituents such as iron, manganese, alkalinity, or hardness. Some organic analytes also may be changed by changes in the foregoing components. Use time-composite samples only for

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determining components that can be demonstrated to remain unchanged under the conditions of sample collection, preservation, and storage.

Collect individual portions in a wide-mouth bottle every hour (in some cases every half hour or even every 5 min) and mix at the end of the sampling period or combine in a single bottle as collected. If preservatives are used, add them to the sample bottle initially so that all portions of the composite are preserved as soon as collected.

Automatic sampling devices are available; however, do not use them unless the sample is preserved as described below. Composite samplers running for extended periods (weeks to months) should undergo routine cleaning of containers and sample lines to minimize sample growth and deposits.

c. Integrated (discharge-weighted) samples: For certain purposes, the information needed is best provided by analyzing mixtures of grab samples collected from different points simultaneously, or as nearly so as possible, using discharge-weighted methods such as equal-width increment (EWI) or equal discharge-increment (EDI) procedures and equipment. An example of the need for integrated sampling occurs in a river or stream that varies in composition across its width and depth. To evaluate average composition or total loading, use a mixture of samples representing various points in the cross-section, in proportion to their relative flows. The need for integrated samples also may exist if combined treatment is proposed for several separate wastewater streams, the interaction of which may have a significant effect on treatability or even on composition. Mathematical prediction of the interactions among chemical components may be inaccurate or impossible and testing a suitable integrated sample may provide more useful information.

Both lakes and reservoirs show spatial variations of composition (depth and horizontal location). However, there are conditions under which neither total nor average results are especially useful, but local variations are more important. In such cases, examine samples separately (i.e., do not integrate them).

Preparation of integrated samples usually requires equipment designed to collect a sample water uniformly across the depth profile. Knowledge of the volume, movement, and composition of the various parts of the water being sampled usually is required. Collecting integrated samples is a complicated and specialized process that must be described adequately in a sampling plan.

2. Chain-of-Custody Procedures

Properly designed and executed chain-of-custody forms will ensure sample integrity from collection to data reporting. This includes the ability to trace possession and handling of the sample from the time of collection through analysis and final disposition. This process is referred to as “chain-of-custody” and is required to demonstrate sample control when the data are to be used for regulation or litigation. Where litigation is not involved, chain-of-custody procedures are useful for routine control of samples.

A sample is considered to be under a person’s custody if it is in the individual’s physical possession, in the individual’s sight, secured and tamper-proofed by that individual, or secured in

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an area restricted to authorized personnel. The following procedures summarize the major aspects of chain of custody. More detailed discussions are available.^{1,2}

a. Sample labels (including bar-code labels): Use labels to prevent sample misidentification. Gummed paper labels or tags generally are adequate. Include at least the following information: a unique sample number, sample type, name of collector, date and time of collection, place of collection, and sample preservative. Also include date and time of preservation for comparison to date and time of collection. Affix tags or self-adhesive labels to sample containers before, or at the time of, sample collection.

b. Sample seals: Use sample seals to detect unauthorized tampering with samples up to the time of analysis. Use self-adhesive paper seals that include at least the following information: sample number (identical with number on sample label), collector's name, and date and time of sampling. Plastic shrink seals also may be used.

Attach seal in such a way that it is necessary to break it to open the sample container or the sample shipping container (e.g., a cooler). Affix seal to container before sample leaves custody of sampling personnel.

c. Field log book: Record all information pertinent to a field survey or sampling in a bound log book. As a minimum, include the following in the log book: purpose of sampling; location of sampling point; name and address of field contact; producer of material being sampled and address, if different from location; type of sample; and method, date, and time of preservation. If the sample is wastewater, identify process producing waste stream. Also provide suspected sample composition, including concentrations; number and volume of sample(s) taken; description of sampling point and sampling method; date and time of collection; collector's sample identification number(s); sample distribution and how transported; references such as maps or photographs of the sampling site; field observations and measurements; and signatures of personnel responsible for observations. Because sampling situations vary widely, it is essential to record sufficient information so that one could reconstruct the sampling event without reliance on the collector's memory. Protect log book and keep it in a safe place.

d. Chain-of-custody record: Fill out a chain-of-custody record to accompany each sample or group of samples. The record includes the following information: sample number; signature of collector; date, time, and address of collection; sample type; sample preservation requirements; signatures of persons involved in the chain of possession; and inclusive dates and times of possession.

e. Sample analysis request sheet: The sample analysis request sheet accompanies samples to the laboratory. The collector completes the field portion of such a form that includes most of the pertinent information noted in the log book. The laboratory portion of such a form is to be completed by laboratory personnel and includes: name of person receiving the sample, laboratory sample number, date of sample receipt, condition of each sample (i.e., if it is cold or warm, whether the container is full or not, color, if more than one phase is present, etc.), and determinations to be performed.

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f. Sample delivery to the laboratory: Deliver sample(s) to laboratory as soon as practicable after collection, typically within 2 d. Where shorter sample holding times are required, make special arrangements to insure timely delivery to the laboratory. Where samples are shipped by a commercial carrier, include the waybill number in the sample custody documentation. Insure that samples are accompanied by a completed chain-of-custody record and a sample analysis request sheet. Deliver sample to sample custodian.

g. Receipt and logging of sample: In the laboratory, the sample custodian inspects the condition and seal of the sample and reconciles label information and seal against the chain-of-custody record before the sample is accepted for analysis. After acceptance, the custodian assigns a laboratory number, logs sample in the laboratory log book and/or computerized laboratory information management system, and stores it in a secured storage room or cabinet or refrigerator at the specified temperature until it is assigned to an analyst.

h. Assignment of sample for analysis: The laboratory supervisor usually assigns the sample for analysis. Once the sample is in the laboratory, the supervisor or analyst is responsible for its care and custody.

i. Disposal: Hold samples for the prescribed amount of time for the project or until the data have been reviewed and accepted. Document the disposition of samples. Ensure that disposal is in accordance with local, state, and U.S. EPA approved methods.

3. Sampling Methods

a. Manual sampling: Manual sampling involves minimal equipment but may be unduly costly and time-consuming for routine or large-scale sampling programs. It requires trained field technicians and is often necessary for regulatory and research investigations for which critical appraisal of field conditions and complex sample collection techniques are essential. Manually collect certain samples, such as waters containing oil and grease.

b. Automatic sampling: Automatic samplers can eliminate human errors in manual sampling, can reduce labor costs, may provide the means for more frequent sampling,³ and are used increasingly. Be sure that the automatic sampler does not contaminate the sample. For example, plastic components may be incompatible with certain organic compounds that are soluble in the plastic parts or that can be contaminated (e.g., from phthalate esters) by contact with them. If sample constituents are generally known, contact the manufacturer of an automatic sampler regarding potential incompatibility of plastic components.

Program an automatic sampler in accordance with sampling needs. Carefully match pump speeds and tubing sizes to the type of sample to be taken.

c. Sorbent sampling: Use of solid sorbents, particularly membrane-type disks, is becoming more frequent. These methods offer advantages of rapid, inexpensive sampling if the analytes of interest can be adsorbed and desorbed efficiently and the water matrix is free of particulates that plug the sorbent.

4. Sample Containers

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The type of sample container used is of utmost importance. Test sample containers and document that they are free of analytes of interest, especially when sampling and analyzing for very low analyte levels. Containers typically are made of plastic or glass, but one material may be preferred over the other. For example, silica, sodium, and boron may be leached from soft glass but not plastic, and trace levels of some pesticides and metals may sorb onto the walls of glass containers.⁴ Thus, hard glass containers³ are preferred. For samples containing organic compounds, do not use plastic containers except those made of fluorinated polymers such as polytetrafluoroethylene (PTFE).³

Some sample analytes may dissolve (be absorbed) into the walls of plastic containers; similarly, contaminants from plastic containers may leach into samples. Avoid plastics wherever possible because of potential contamination from phthalate esters. Container failure due to breakdown of the plastic is possible. Therefore, use glass containers for all organics analyses such as volatile organics, semivolatile organics, pesticides, PCBs, and oil and grease. Some analytes (e.g., bromine-containing compounds and some pesticides, polynuclear aromatic compounds, etc.) are light-sensitive; collect them in amber-colored glass containers to minimize photodegradation. Container caps, typically plastic, also can be a problem. Do not use caps with paper liners. Use foil or PTFE liners but be aware that metal liners can contaminate samples collected for metals analysis and they may also react with the sample if it is acidic or alkaline. Serum vials with PTFE-lined rubber or plastic septa are useful.

In rare situations it may be necessary to use sample containers not specifically prepared for use, or otherwise unsuitable for the particular situation; thoroughly document these deviations. Documentation should include type and source of container, and the preparation technique, e.g., acid washed with reagent water rinse. For QA purposes the inclusion of a bottle blank may be necessary.

5. Number of Samples

Because of variability from analytical and sampling procedures (i.e., population variability), a single sample is insufficient to reach any reasonable desired level of confidence. If an overall standard deviation (i.e., the standard deviation of combined sampling and analysis) is known, the required number of samples for a mobile matrix such as water may be estimated as follows:⁴

$$N \geq \left(\frac{ts}{U} \right)^2$$

where:

N = number of samples,

t = Student- t statistic for a given confidence level,

s = overall standard deviation, and

U = acceptable level of uncertainty.

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To assist in calculations, use curves such as those in Figure 1060:1. As an example, if s is 0.5 mg/L, U is ± 0.2 mg/L, and a 95% confidence level is desired, approximately 25 to 30 samples must be taken.

The above equation assumes that total error (population variability) is known. Total variability consists of all sources of variability, including: the distribution of the analytes of interest within the sampling site, collection, preservation, preparation, and analysis of samples, and data handling and reporting. In simpler terms, error (variability) can be divided into sampling and analysis components. Sampling error due to population variability (including heterogeneous distribution of analytes in the environmental matrix) usually is much larger than analytical error components. Unfortunately, sampling error usually is not available and the analyst is left with only the published error of the measurement system (typically obtained by using a reagent water matrix under the best analytical conditions).

More accurate equations are available.⁵ These are based on the Z distribution for determining the number of samples needed to estimate a mean concentration when variability is estimated in absolute terms using the standard deviation. The coefficient of variation (relative standard deviation) is used when variability is estimated in relative terms.

The number of random samples to be collected at a site can be influenced partly by the method that will be used. The values for standard deviation (SD) or relative standard deviation (RSD) may be obtained from each of the methods or in the literature.⁶ However, calculations of estimated numbers of samples needed based only on this information will result in underestimated numbers of samples because only the analytical variances are considered, and the typically larger variances from the sampling operations are not included. Preferably, determine and use SDs or RSDs from overall sampling and analysis operations.

For estimates of numbers of samples needed for systematic sampling (e.g., drilling wells for sampling groundwater or for systematically sampling large water bodies such as lakes), equations are available⁷ that relate number of samples to shape of grid, area covered, and space between nodes of grid. The grid spacing is a complex calculation that depends on the size and shape of any contaminated spot (such as a groundwater plume) to be identified, in addition to the geometric shape of the sampling grid.

See individual methods for types and numbers of quality assurance (QA) and quality control (QC) samples, e.g., for normal-level (procedural) or low-level (contamination) bias or for precision, involving sampling or laboratory analysis (either overall or individually). Estimates of numbers of QC samples needed to achieve specified confidence levels also can be calculated. Rates of false positives (Type I error) and false negatives (Type II error) are useful parameters for estimating required numbers of QC samples. A false positive is the incorrect conclusion that an analyte is present when it is absent. A false negative is the incorrect conclusion that an analyte is absent when it is present. If the frequency of false positives or false negatives desired to be detected is less than 10%, then

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$$n = \frac{\ln \alpha}{\ln (1 - Y)}$$

where:

α = (1 – desired confidence level), and
 Y = frequency to detect (<10%).

If the frequency that is desirable to detect is more than 10%, iterative solution of a binomial equation is necessary.^{5,8}

Equations are available as a computer program†#(4) for computing sample number by the Z distribution, for estimating samples needed in systematic sampling, and for estimating required number of QC samples.

6. Sample Volumes

Collect a 1-L sample for most physical and chemical analyses. For certain determinations, larger samples may be necessary. Table 1060:I lists volumes ordinarily required for analyses, but it is strongly recommended that the laboratory that will conduct the analyses also be consulted to verify the analytical needs of sampling procedures as they pertain to the goals and data quality objective of an investigation.

Do not use samples from the same container for multiple testing requirements (e.g., organic, inorganic, radiological, bacteriological, and microscopic examinations) because methods of collecting and handling are different for each type of test. Always collect enough sample volume in the appropriate container in order to comply with sample handling, storage, and preservation requirements.

7. References

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1060 C. Sample Storage and Preservation

Complete and unequivocal preservation of samples, whether domestic wastewater, industrial wastes, or natural waters, is a practical impossibility because complete stability for every constituent never can be achieved. At best, preservation techniques only retard chemical and biological changes that inevitably continue after sample collection.

1. Sample Storage before Analysis

a. Nature of sample changes: Some determinations are more affected by sample storage than others. Certain cations are subject to loss by adsorption on, or ion exchange with, the walls of glass containers. These include aluminum, cadmium, chromium, copper, iron, lead, manganese, silver, and zinc, which are best collected in a separate clean bottle and acidified with nitric acid to a pH below 2.0 to minimize precipitation and adsorption on container walls. Also, some organics may be subject to loss by adsorption to the walls of glass containers.

Temperature changes quickly; pH may change significantly in a matter of minutes; dissolved gases (oxygen, carbon dioxide) may be lost. Because changes in such basic water quality properties may occur so quickly, determine temperature, reduction-oxidation potential, and dissolved gases *in situ* and pH, specific conductance, turbidity, and alkalinity immediately after sample collection. Many organic compounds are sensitive to changes in pH and/or temperature resulting in reduced concentrations during storage.

Changes in the pH-alkalinity-carbon dioxide balance may cause calcium carbonate to precipitate, decreasing the values for calcium and total hardness.

Iron and manganese are readily soluble in their lower oxidation states but relatively insoluble in their higher oxidation states; therefore, these cations may precipitate or they may dissolve from a sediment, depending on the redox potential of the sample. Microbiological activity may affect the nitrate-nitrite-ammonia content, phenol or BOD concentration, or the reduction of sulfate to sulfide. Residual chlorine is reduced to chloride. Sulfide, sulfite, ferrous iron, iodide,

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and cyanide may be lost through oxidation. Color, odor, and turbidity may increase, decrease, or change in quality. Sodium, silica, and boron may be leached from the glass container. Hexavalent chromium may be reduced to trivalent chromium.

Biological activity taking place in a sample may change the oxidation state of some constituents. Soluble constituents may be converted to organically bound materials in cell structures, or cell lysis may result in release of cellular material into solution. The well-known nitrogen and phosphorus cycles are examples of biological influences on sample composition.

Zero head-space is important in preservation of samples with volatile organic compounds and radon. Avoid loss of volatile materials by collecting sample in a completely filled container. Achieve this by carefully filling the bottle so that top of meniscus is above the top of the bottle rim. It is important to avoid spillage or air entrapment if preservatives such as HCl or ascorbic acid have already been added to the bottle. After capping or sealing bottle, check for air bubbles by inverting and gently tapping it; if one or more air bubbles are observed then, if practical, discard the sample and repeat refilling bottle with new sample until no air bubbles are observed (this cannot be done if bottle contained preservatives before it was filled).

Serum vials with septum caps are particularly useful in that a sample portion for analysis can be taken through the cap by using a syringe,¹ although the effect of pressure reduction in the head-space must be considered. Pulling a sample into a syringe under vacuum can result in low bias data for volatile compounds and the resulting headspace precludes taking further subsamples.

b. Time interval between collection and analysis: In general, the shorter the time that elapses between collection of a sample and its analysis, the more reliable will be the analytical results. For certain constituents and physical values, immediate analysis in the field is required. For composited samples it is common practice to use the time at the end of composite collection as the sample collection time.

Check with the analyzing laboratory to determine how much elapsed time may be allowed between sample collection and analysis; this depends on the character of the sample and the stability of the target analytes under the conditions of storage. Many regulatory methods limit the elapsed time between sample collection and analysis (see Table 1060:I). Changes caused by growth of microorganisms are greatly retarded by keeping the sample at a low temperature (<4°C but above freezing). When the interval between sample collection and analysis is long enough to produce changes in either the concentration or the physical state of the constituent to be measured, follow the preservation practices given in Table 1060:I. Record time elapsed between sampling and analysis, and which preservative, if any, was added.

2. Preservation Techniques

To minimize the potential for volatilization or biodegradation between sampling and analysis, keep samples as cool as possible without freezing. Preferably pack samples in crushed or cubed ice or commercial ice substitutes before shipment. Avoid using dry ice because it will freeze samples and may cause glass containers to break. Dry ice also may effect a pH change in

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samples. Keep composite samples cool with ice or a refrigeration system set at 4°C during compositing. Analyze samples as quickly as possible on arrival at the laboratory. If immediate analysis is not possible, preferably store at 4°C.¹

No single method of preservation is entirely satisfactory; choose the preservative with due regard to the determinations to be made. Use chemical preservatives only when they do not interfere with the analysis being made. When they are used, add them to the sample bottle initially so that all sample portions are preserved as soon as collected. Because a preservation method for one determination may interfere with another one, samples for multiple determinations may need to be split and preserved separately. All methods of preservation may be inadequate when applied to suspended matter. Do not use formaldehyde as a preservative for samples collected for chemical analysis because it affects many of the target analytes.

Methods of preservation are relatively limited and are intended generally to retard biological action, retard hydrolysis of chemical compounds and complexes, and reduce volatility of constituents.

Preservation methods are limited to pH control, chemical addition, the use of amber and opaque bottles, refrigeration, filtration, and freezing. Table 1060:I lists preservation methods by constituent. See Section 7010B for sample collection and preservation requirements for radionuclides.

The foregoing discussion is by no means exhaustive and comprehensive. Clearly it is impossible to prescribe absolute rules for preventing all possible changes. Additional advice will be found in the discussions under individual determinations, but to a large degree the dependability of an analytical determination rests on the experience and good judgment of the person collecting the sample. Numbers of samples required for confidence levels in data quality objectives, however, rely on statistical equations such as those discussed earlier.

3. Reference

1. WATER POLLUTION CONTROL FEDERATION. 1986. Removal of Hazardous Wastes in Wastewater Facilities—Halogenated Organics. Manual of Practice FD-11, Water Pollution Control Fed., Alexandria, Va.

4. Bibliography

KEITH, L.H., ed. 1996. Principles of Environmental Sampling, 2nd ed. ACS Professional Reference Book, American Chemical Soc., Washington, D.C.

1080 REAGENT WATER

1080 A. Introduction

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One of the most important aspects of analysis is the preparation of reagent water to be used for dilution of reagents and for blank analysis. Reagent water is water with no detectable concentration of the compound or element to be analyzed at the detection limit of the analytical method. Reagent water should be free of substances that interfere with analytical methods. The quality of water required is related directly to the analysis being made. Requirements for water quality may differ for organic, inorganic, and biological constituents depending on the use(s) for which the water is intended.

Any method of preparation of reagent water is acceptable provided that the requisite quality can be met. Improperly maintained systems may add contaminants. Reverse osmosis, distillation, and deionization in various combinations all can produce reagent water when used in the proper arrangement. Ultrafiltration and/or ultraviolet treatment also may be used as part of the process. Section 1080 provides general guidelines for the preparation of reagent water. Table 1080:I lists commonly available processes for water purification and major classes of contaminants removed by purification.

For details on preparing water for microbiological tests, see Section 9020B.3c.

1080 B. Methods for Preparation of Reagent Water

1. Distillation

Prepare laboratory-grade distilled water by distilling water from a still of all-borosilicate glass, fused quartz, tin, or titanium. To remove ammonia distill from an acid solution. Remove CO₂ by boiling the water for 15 min and cooling rapidly to room temperature; exclude atmospheric CO₂ by using a tube containing soda lime or a commercially available CO₂-removing agent.*#(5)

Boiling the water may add other impurities by leaching impurities from the container. Freshly replaced filters, cartridges and resins initially can release impurities. Pretreat feedwater and provide periodic maintenance to minimize scale formation within the still. Pretreatment may be required where the feedwater contains significant concentrations of calcium, magnesium, and bicarbonate ions; it may involve demineralization via reverse osmosis or ion exchange.

2. Reverse Osmosis

Reverse osmosis is a process in which water is forced under pressure through a semipermeable membrane removing a portion of dissolved constituents and suspended impurities. Product water quality depends on feedwater quality.

Select the reverse osmosis membrane module appropriate to the characteristics of the feedwater. Obtain rejection data for contaminants in the feedwater at the operating pressure to be used in preparing reagent water. Set overall water production to make the most economical use of water without compromising the final quality of the permeate. Selection of spiral-wound or hollow fiber configurations depends on fouling potential of the feedwater. Regardless of

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configuration used, pretreatment may be required to minimize membrane fouling with colloids or particulates and to minimize introduction of chlorine, iron, and other oxidizing compounds that may degrade reverse osmosis membranes. Periodic flushing of the membrane modules is necessary.

3. Ion Exchange

Prepare deionized water by passing feedwater through a mixed-bed ion exchanger, consisting of strong anion and strong cation resins mixed together. When the system does not run continuously, recirculate product water through ion-exchange bed.

Use separate anion and cation resin beds in applications where resin regeneration is economically attractive. In such instances, position the anion exchanger downstream of the cation exchanger to remove leachates from the cation resin. Proper bed sizing is critical to the performance of the resins. In particular, set the length-to-diameter ratio of the bed in accordance with the maximum process flow rate to ensure that optimal face velocities are not exceeded and that sufficient residence time is provided.

In applications where the feedwater has significant quantities of organic matter, remove organics to minimize potential fouling of the resins. Possible pretreatments include prefiltration, distillation, reverse osmosis, or adsorption.

4. Adsorption

Adsorption is generally used to remove chlorine and organic impurities. It is accomplished typically with granular activated carbon. Efficiency of organics removal depends on the nature of the organic contaminants, the physical characteristics of the activated carbon, and the operating conditions. In general, organics adsorption efficiency is inversely proportional to solubility and may be inadequate for the removal of low-molecular-weight, polar compounds. Performance differences among activated carbons are attributable to the use of different raw materials and activation procedures. Select the appropriate activated carbon with regard to these differences. Even with optimum activated carbon, proper performance will not be attained unless the column is sized to give required face velocity and residence time at the maximum process flow rate.

Use of activated carbon may adversely affect resistivity. This effect may be controlled by use of reverse osmosis, mixed resins, or special adsorbents. To achieve the lowest level of organic contamination, use mixtures of polishing resins with special carbons in conjunction with additional treatment steps, such as reverse osmosis, natural carbons, ultraviolet oxidation, or ultrafiltration.

1080 C. Reagent Water Quality

1. Quality Guidelines

Several guidelines for reagent water quality, based on contaminant levels, are available, but the final test is the appropriateness for the analysis. Table 1080:II lists some characteristics of

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various qualities of reagent water.

High-quality reagent water, having a minimum resistivity of 10 megohms-cm, 25°C (in line), typically is prepared by distillation, deionization, or reverse osmosis treatment of feedwater followed by polishing with a mixed-bed deionizer and passage through a 0.2- μ m-pore membrane filter. Alternatively treat by reverse osmosis followed by carbon adsorption and deionization. Determine quality at the time of production. Mixed-bed deionizers typically add small amounts of organic matter to water, especially if the beds are fresh. Resistivity should be >10 megohm-cm at 25°C, measured in-line. Resistivity measurements will not detect organics or nonionized contaminants, nor will they provide an accurate assessment of ionic contaminants at the microgram-per-liter level.

Medium-quality water typically is produced by distillation or deionization. Resistivity should be >1 megohm-cm at 25°C.

Low-quality water should have a minimum resistivity of 0.1 megohm-cm, and may be used for glassware washing, preliminary rinsing of glassware, and as feedwater for production of higher-grade waters.

The pH of high- or medium-quality water cannot be measured accurately without contaminating the water. Measure other constituents as required for individual tests.

High-quality water cannot be stored without significant degradation; produce it continuously and use it immediately after processing. Medium-quality water may be stored, but keep storage to a minimum and provide quality consistent with the intended use. Store only in materials that protect the water from contamination, such as TFE and glass for organics analysis or plastics for metals. Store low-quality water in materials that protect the water from contamination.

2. Bibliography

AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1991. Annual Book of ASTM Standards, Vol. 11-01, D 1193-91. American Soc. Testing & Materials, Philadelphia, Pa.

1090 LABORATORY OCCUPATIONAL HEALTH AND SAFETY

1090 A. Introduction

1. General Discussion

Achievement of a safe and healthful workplace is the responsibility of the organization, the laboratory manager, the supervisory personnel and, finally, the laboratory personnel themselves. All laboratory employees must make every effort to protect themselves and their fellow workers by conscientiously adhering to the health and safety program that has been developed and documented specifically for their laboratory.

2. Organizing for Safety

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a. Overall program: The responsibility for establishing and enforcing a laboratory health and safety (LH&S) program ultimately rests with the laboratory director. The LH&S program must, at the minimum, address how to protect oneself from the hazards of working with biological (1090H), chemical (1090J), and radiological (1090I) agents. Such a program is a necessary component of an overall laboratory quality system that provides for the health and safety of the entire laboratory staff. As a part of the quality system, all aspects of the LH&S program must be fully documented. Laboratory personnel must be trained. The LH&S program must be fully implemented and its application audited periodically. Appropriate records of all activities must be kept to document performance, meet appropriate regulatory requirements, and document the status of the LH&S program.

In the United States, the minimum standard of practice for health and safety activities is detailed in government documents.^{1,2} Each laboratory should appoint as needed a chemical hygiene officer (CHO), a biological hygiene officer (BHO), a radiological hygiene officer (RHO), and, where appropriate or desired, a LH&S committee. The CHO, the committee, and laboratory management must develop, document, and implement a “written” laboratory hygiene plan (LHP), or chemical hygiene plan (CHP).

b. Specific responsibilities: Specific responsibilities applicable at various levels within the organization are as follows:

1) The chief executive officer (CEO) has ultimate responsibility for LH&S within the organization and must, with other managers and supervisors, provide continuous support for the LH&S program.

2) The supervisor has primary responsibility for the LH&S program in his or her work group.

3) The biological hygiene officer (BHO) has the responsibility to work with managers, supervisors, and other employees to develop and implement appropriate biological hygiene policies and practices; monitor procurement, use, and disposal of biological agents used in the laboratory; see that appropriate audits are conducted and that records are maintained; know the current legal requirements concerning working with biological agents; and seek ways to improve the biological hygiene program.

4) The chemical hygiene officer (CHO) has the same responsibilities as the biological hygiene officer, but with respect to chemicals, and also is responsible for helping supervisors (project directors) develop precautions and adequate facilities and for keeping material safety data sheets (MSDSs) available for review.

5) The radiological hygiene officer (RHO), referred to as radiation safety officer in most regulatory language, has the same responsibilities as the chemical hygiene officer, but with respect to radiological chemicals and exposure.

6) The laboratory supervisor has overall responsibility for chemical hygiene in the laboratory, including responsibility to ensure that workers know and follow the chemical hygiene rules, that protective equipment is available and in working order, and that appropriate

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training has been provided; performs regular, formal chemical hygiene and housekeeping inspections, including routine inspections of emergency equipment, and maintenance of appropriate records; knows the current legal requirements concerning regulated substances; specifies the required levels of protective apparel and equipment needed to perform the work; and ensures that facilities and training for use of any material being ordered are adequate.

7) The project director (or a director of a specific operation) has primary responsibility for biological, chemical, and/or radiological hygiene procedures as appropriate for all operations under his or her control.

8) The laboratory worker has the responsibility for planning and conducting each operation in accordance with the institutional chemical hygiene, biological hygiene, and radiological hygiene procedures, and for developing good personal chemical, biological, and radiological hygiene habits.

3. Records

Maintain records of all accidents including “near-misses,” medical care audits, inspections, and training for specified time periods that depend on the nature of the requirement. Keep records on standardized report forms containing sufficient information to enable an investigator to determine who was involved, what happened, when and where it happened, and what injuries or exposures, if any, resulted. Most importantly, these records should enable the formulation of appropriate corrective actions where warranted. The standard of practice for LH&S activities requires that a log (record) be kept of those accidents causing major disability. Record not only all accidents, but also “near-misses,” to permit full evaluation of safety program effectiveness. Maintain a file detailing all of the recommendations for the LH&S program.

4. Information and Training²

The standard of practice for hazard communication or “right-to know” requires that employees be notified about hazards in the workplace.

Laboratory personnel must be under the direct supervision and regular observation of a technically qualified individual who must have knowledge of the hazards present, their health effects, and related emergency procedures. The supervisor must educate laboratory personnel in safe work practices at the time of initial assignment and when a new hazardous substance is introduced into the workplace. Personnel have a right to know what hazardous materials are present, the specific hazards created by those materials, and the required procedures to protect themselves against these hazards. The hazard communication standard ² requires information and training on material safety data sheets (MSDSs), labeling, chemical inventory of all hazardous substances in the workplace, and informing contractors of hazardous substances.

Training dealing with health and safety techniques and work practices requires a concerted effort by management, and must be conducted on a routine basis by competent and qualified individuals to be effective. Records of training must be maintained.

5. References

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1. OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION. Laboratory Standard. Occupational Exposure to Hazardous Chemicals in Laboratories. 29 CFR 1910.1450.
2. OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION. 1985. Hazard Communication. Final Rule. *Federal Register* 48:53280. 29 CFR 1910.1200.

6. Bibliography

- DUX, J.P. & R.F. STALZER. 1988. Managing Safety in the Chemical Laboratory. Van Nostrand Reinhold Co., Inc., New York, N.Y.
- FURR, A.K., ed. 1990. CRC Handbook of Laboratory Safety, 3rd ed. CRC Press, Inc., Boca Raton, Fla.

1090 B. Safe Laboratory Practices

Use the information, rules, work practices, and/or procedures discussed below for essentially all laboratory work with chemicals.

1. General Rules

a. Accidents and spills:

- 1) Eye contact—Promptly flush eyes with water for a prolonged period (minimum of 15 min) and seek immediate medical attention.
- 2) Ingestion—Encourage victim to drink large amounts of water.
- 3) Skin contact—Promptly flush affected area with water for approximately 15 min and remove any contaminated clothing. If symptoms persist after washing, seek medical attention.
- 4) Clean-up—Promptly clean up spills, using appropriate protective apparel and equipment and proper disposal procedures.
- 5) Working alone—Avoid working alone in a building; do not work alone in a laboratory if the procedures to be conducted are hazardous.

b. Vigilance: Be alert to unsafe conditions and see that they are corrected when detected.

2. Work Practices/Rules

- a. *Work habits:* Develop and encourage safe habits, avoid unnecessary exposure to chemicals by any route, and avoid working alone whenever possible.
- b. *Exhaust ventilation:* Do not smell or taste chemicals. Vent any apparatus that may discharge toxic chemicals (vacuum pumps, distillation columns, etc.) into local exhaust devices.
- c. *Glove boxes:* Inspect gloves and test glove boxes before use.
- d. *Cold and/or warm rooms:* Do not allow release of toxic substances in cold rooms and/or warm rooms, because these rooms usually have no provisions for exhausting contaminants.
- e. *Use/choice of chemicals:* Use only those chemicals for which the quality of the available

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ventilation system is appropriate.

f. Eating, smoking, and related activities: DO NOT eat, drink, smoke, chew gum, or apply cosmetics in areas where laboratory chemicals are present. Always wash hands before conducting these activities.

g. Food storage: DO NOT store, handle, or consume food or beverages in storage areas, refrigerators, or glassware and utensils that also are used for laboratory operations.

h. Equipment and glassware: Handle and store laboratory glassware with care to avoid damage. Do not use damaged glassware. Use extra care with Dewar flasks and other evacuated glass apparatus; shield or wrap them to contain chemicals and fragments should implosion occur. Use equipment for its designed purpose only.

i. Washing: Wash areas of exposed skin well before leaving the laboratory.

j. Horseplay: Avoid practical jokes or other behavior that might confuse, startle, or distract another worker.

k. Mouth suction: Do not use mouth suction for pipetting or starting a siphon.

l. Personal protective equipment: Do not wear personal protective clothing or equipment in nonlaboratory areas. Remove laboratory coats immediately on significant contamination with hazardous materials.

m. Personal apparel: Confine long hair and loose clothing. Wear shoes at all times in the laboratory but do not wear sandals or perforated shoes.

n. Personal housekeeping: Keep work area clean and uncluttered, with chemicals and equipment properly labeled and stored. Clean up work area on completion of an operation or at the end of each day.

o. Unattended operations: Leave lights on, place an appropriate sign on the door, and provide for containment of toxic substances in the event of failure of a utility service (such as cooling water) to an unattended operation.

3. Personal Protective Equipment

Carefully plan a program addressing the need for, use of, and the training with personal protective equipment. Such a program includes seeking information and advice about hazards, developing appropriate protective procedures, and proper positioning of equipment before beginning any new operations.

a. Eye protection: Wear appropriate eye protection (this applies to all persons, including visitors) where chemicals are stored or handled. Avoid use of contact lenses in the laboratory unless necessary; if contact lenses are used, inform supervisor so that special precautions can be taken.

b. Skin protection: Wear appropriate gloves when the potential for contact with toxic chemicals exists. Inspect gloves before each use, wash them before removal, and replace periodically.

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c. Respiratory protection: Use appropriate respiratory equipment when engineering controls are unable to maintain air contaminant concentrations below the action levels, i.e., one half the permissible exposure limit (PEL)¹ or threshold limit value (TLV⁷), i.e., levels below which no irreversible health effects are expected. When work practices are used that are expected to cause routine exposures that exceed the PEL or TLV, respiratory protection is required to prevent overexposure to hazardous chemicals. If respirators are used or provided in the laboratory then the LH&S standard of practice requires that a complete respiratory protection plan (RPP) be in place. The minimum requirements for an RPP meeting the LH&S standard of practice are published.¹ Inspect respirators before use and check for proper fit.

d. Other protective equipment: Provide and use any other protective equipment and/or apparel as appropriate.

4. Engineering Controls

Fume hoods: Use the hood for operations that might result in the release of toxic chemical vapors or dust. As a rule of thumb, use a hood or other local ventilation device when working with any appreciably volatile substance with a TLV of less than 50 ppm. Confirm that hood performance is adequate before use. Open hood minimally during work. Keep hood door closed at all other times except when adjustments within the hood are being made. Keep stored materials in hoods to a minimum, and do not block vents or air flow. Provide at least an 8-cm space under and around all items used in hoods, and ensure that they are at least 15 cm from the front of the hood.

5. Waste Disposal

Ensure that the plan for each laboratory operation includes plans and training for waste disposal. Deposit chemical waste in appropriately labeled receptacles and follow all other waste disposal procedures of the Chemical Hygiene Plan (see 1090J). Do not discharge any of the following contaminants to the sewer: concentrated acids or bases; highly toxic, malodorous, or lachrymatory substances; substances that might interfere with the biological activity of wastewater treatment plants; and substances that may create fire or explosion hazards, cause structural damage, or obstruct flow. For further information on waste disposal, see Section 1100.

6. Work with Chemicals of Moderate Chronic or High Acute Toxicity

Examples of chemicals in this category include diisopropylfluorophosphate, hydrofluoric acid, and hydrogen cyanide. The following rules are intended to supplement the rules listed previously for routine laboratory operations. Their purpose is to minimize exposure to these toxic substances by any exposure route using all reasonable precautions. The precautions are appropriate for substances with moderate chronic or high acute toxicity used in significant quantities.

a. Location: Use and store these substances only in areas of restricted access with special warning signs. Always use a hood (previously evaluated to confirm adequate performance with a face velocity of at least 24 m/min) or other containment device for procedures that may result in

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the generation of aerosols or vapors containing the substance; trap released vapors to prevent their discharge with the hood exhaust.

b. Personal protection: Always avoid skin contact by use of gloves and long sleeves, and other protective apparel as appropriate. Always wash hands and arms immediately after working with these materials.

c. Records: Maintain records of the amounts of these materials on hand, amounts used, and the names of the workers involved.

d. Prevention of spills and accidents: Be prepared for accidents and spills.

Ensure that at least two people are present at all times if a compound in use is highly toxic or of unknown toxicity.

Store breakable containers of these substances in chemically resistant trays; also work and mount apparatus above such trays or cover work and storage surfaces with removable, absorbent, plastic-backed paper. If a major spill occurs outside the hood, evacuate the area; ensure that cleanup personnel wear suitable protective apparel and equipment.

e. Waste: Thoroughly decontaminate or incinerate contaminated clothing or shoes. If possible, chemically decontaminate by chemical conversion. Store contaminated waste in closed, suitably labeled, impervious containers (for liquids, in glass or plastic bottles half-filled with vermiculite).

7. Work with Chemicals of High Chronic Toxicity

Examples of chemicals in this category include (where they are used in quantities above a few milligrams, or a few grams, depending on the substance) dimethyl mercury, nickel carbonyl, benzo(a)pyrene, *N*-nitrosodiethylamine, and other substances with high carcinogenic potency. The following rules are intended to supplement the rules listed previously for routine laboratory operations.

a. Access: Conduct all transfers and work with these substances in a controlled area, i.e., a restricted-access hood, glove box, or portion of a laboratory, designated for use of highly toxic substances, for which all people with access are aware of the substances being used and necessary precautions.

b. Approvals: Prepare a plan for use and disposal of these materials and obtain the approval of the laboratory supervisor.

c. Non-contamination/decontamination: Protect vacuum pumps against contamination by scrubbers or HEPA filters and vent them into the hood. Decontaminate vacuum pumps or other contaminated equipment, including glassware, in the hood before removing them from the controlled area. Decontaminate the controlled area before routine work is resumed.

d. Exiting: On leaving a controlled area, remove any protective apparel (place it in an appropriately labeled container), and thoroughly wash hands, forearms, face, and neck.

e. Housekeeping: Use a wet mop or a vacuum cleaner equipped with a HEPA filter. Do not dry sweep if the toxic substance is a dry powder.

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f. Medical surveillance: If using toxicologically significant quantities of such a substance on a regular basis (e.g., three times per week), consult a qualified physician about desirability of regular medical surveillance.

g. Records: Keep accurate records of the amounts of these substances stored and used, the dates of use, and names of users.

h. Signs and labels: Ensure that the controlled area is conspicuously marked with warning and restricted access signs and that all containers of these substances are appropriately labeled with identity and warning labels.

i. Spills: Ensure that contingency plans, equipment, and materials to minimize exposures of people and property are available in case of accident.

j. Storage: Store containers of these chemicals only in a ventilated, limited-access area.

k. Glove boxes: For a negative-pressure glove box, ensure that the ventilation rate is at least 2 volume changes/h and the pressure drop at least 1.3 cm of water. For a positive-pressure glove box, thoroughly check for leaks before each use. In either case, trap the exit gases or filter them through a HEPA filter and then release them into the hood.

l. Waste: Ensure that containers of contaminated waste (including washings from contaminated flasks) are transferred from the controlled area in a secondary container under the supervision of authorized personnel.

8. Physical Hazards

a. Electrical: Ensure that electrical wiring, connections, and apparatus conform to the requirement of the latest National Electrical Code. Fire, explosion, power outages, and electrical shocks are all serious hazards that may result from incorrect use of electrical devices. Ground all electrical equipment or use double-insulated equipment. Use ground fault interrupter circuit breakers to the maximum extent possible. Do not locate electrical receptacles inside fume hoods, and do not use equipment near volatile flammable solvents. Use approved safety refrigerators. Disconnect electrical equipment from the power supply before service or repair is attempted and never bypass safety interlocks. Attempting to repair equipment using employees not thoroughly acquainted with electrical principles may present particularly dangerous situations.

b. Non-ionizing radiation: Non-ionizing radiation, also called electromagnetic radiation, is generally considered to be the radio frequency region of the radiation spectrum. For the purposes of dealing with personal exposures in laboratories, it also includes the microwave frequency region. Typical laboratory exposures to non-ionizing radiation usually include ultraviolet, visible, infrared, and microwave radiation.

For normal environmental conditions and for incident electromagnetic energy of frequencies from 10 MHz to 100 GHz, the radiation protection guide is 10 mW/cm². The radiation protection applies whether the radiation is continuous or intermittent. This means a power density of 10 mW/cm² for periods of 0.1 h or more, or an energy density of 1 mW-h/cm² during any 0.1-h period. These recommendations apply to both whole-body irradiation and partial body

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irradiation.

Ultraviolet radiation (UV) and lasers are used frequently. With properly constructed and operated instruments, it is not a significant hazard but can be harmful when used for controlling microorganisms in laboratory rooms or for sterilizing objects.

When using devices that generate or use non-ionizing radiation, observe the following precautions: Wear safety glasses or goggles with solid side pieces whenever there is a possibility of exposure to harmful (UV) radiation. Provide proper shielding (shiny metal surfaces reflect this energy). Shut off all these devices (UV lamps) when not in use. Post warning signs and install indicator lights to serve as a constant reminder when these types of devices are in use (UV lamps).

c. Mechanical: Shield or guard drive belts, pulleys, chain drivers, rotating shafts, and other types of mechanical power transmission apparatus. Laboratory equipment requiring this guarding includes vacuum pumps, mixers, blenders, and grinders.

Shield portable power tools. Guard equipment such as centrifuges, which have high-speed revolving parts, against “fly-aways.” Securely fasten equipment that has a tendency to vibrate (e.g., centrifuges and air compressors) to prevent the tendency to “walk” and locate them away from bottles and other items that may fall from shelves or benches because of the vibration.

d. Compressed gases: Gas cylinders may explode or “rocket” if improperly handled. Leaking cylinders may present an explosion hazard if the contents are flammable; they are an obvious health hazard if the contents are toxic; and they may lead to death by suffocation if the contents are inert gases. The Compressed Gas Association has published procedures governing use and storage of compressed gases. Transfer gas cylinders only with carts, hand trucks, or dollies. Secure gas cylinders properly during storage, transport, and use, and leave valve safety covers on cylinders during storage and transport. Avoid the use of adapters or couplers with compressed gas. Properly identify cylinder contents.

9. Chemical Hazards

a. General precautions: Chemical injuries may be external or internal. External injuries may result from skin exposure to caustic or corrosive substances such as acids, bases, or reactive salts. Take care to prevent accidents, such as splashes and container spills. Internal injuries may result from the toxic or corrosive effects of substances absorbed by the body. These internal injuries may result from inhalation, skin contact, or ingestion.

Table 1090:I, Table 1090:II, and Table 1090:III list PELs, TLVs, and/or short-term exposure limits and ceilings for some chemical materials specified in *Standard Methods*, as given in various published sources.¹⁻⁸ The PEL values reported in these tables are in some instances higher than the levels that some nations believe to be appropriate. Because the health and safety program should be driven by meeting best industrial hygiene practice, always use the lowest recommended exposure values when protecting human health.

In addition, pay careful attention to equipment corrosion that ultimately may lead to safety hazards from equipment failure.

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b. Inorganic acids and bases: Many inorganic acids and bases have PELs and TLVs. Table 1090:I presents PELs (based on U.S. standards) and/or TLVs as well as short-term exposure limits and ceilings for some inorganic chemicals specified in *Standard Methods*. These PELs and TLVs indicate the maximum air concentration to which workers may be exposed. Fumes of these acids and bases are severe eye and respiratory system irritants. Liquid or solid acids and bases can quickly cause severe burns of the skin and eyes. When acids are heated to increase the rate of digestion of organic materials, they pose a significantly greater hazard because fumes are produced and the hot acid reacts very quickly with the skin.

Store acids and bases separately in well-ventilated areas and away from volatile organic and oxidizable materials. Use containers (rubber or plastic buckets) to transport acids and bases.

Work with strong acids and bases only in a properly functioning chemical fume hood. Slowly add acids and bases to water (with constant stirring) to avoid spattering. If skin contact is made, thoroughly flush the contaminated area with water and seek medical attention if irritation persists. Do not wear contaminated clothing until after it has been cleaned thoroughly. Leather items (e.g., belts and shoes) will retain acids even after rinsing with water and may cause severe burns if worn. If eye contact is made, immediately flush both eyes for at least 15 min with an eye wash and seek medical attention.

c. Perchloric acid and other highly reactive chemicals: Concentrated perchloric acid reacts violently or explosively on contact with organic material and may form explosive heavy metal perchlorates. Do not use laboratory fume hoods used with perchloric acid for organic reagents, particularly volatile solvents. In addition to these hazards, perchloric acid produces severe burns when contact is made with the skin, eyes, or respiratory tract. Preferably provide a dedicated perchloric acid hood. Follow the manufacturer's instructions for proper cleaning, because exhaust ducts become coated and must be washed down regularly.

Use extreme caution when storing and handling highly reactive chemicals, such as strong oxidizers. Improper storage can promote heat evolution and explosion. Do not store strong oxidizers and reducers in close proximity.

d. Organic solvents and reagents: Most solvents specified in *Standard Methods* have PELs and/or TLVs as well as short-term exposure limits or ceilings for workplace exposures (see Table 1090:II).

Many organic reagents, unlike most organic solvents, do not have PELs/TLVs or short-term exposure limits and ceilings, but this does not mean that they are less hazardous. Table 1090:III contains PELs/TLVs or short-term exposure limits and ceilings for some reagents specified in *Standard Methods*.

Some compounds are suspect carcinogens and should be treated with extreme caution. These compounds include solvents and reagents such as benzene, carbon tetrachloride, chloroform, dioxane, perchloroethylene, and benzhidine. Lists of chemicals with special hazardous characteristics are available from the Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health. In the U.S., the lists of "Regulated Carcinogens" and of "Chemicals Having Substantial Evidence of Carcinogenicity" are

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especially important. Developing and following laboratory handling procedures for compounds on such authoritative lists should significantly reduce the potential for exposures.

Solvents used in the laboratory usually fall into several major categories: alcohols, chlorinated compounds, and hydrocarbons. Exposure to each of these classes of compounds can have a variety of health effects. Alcohols, in general, are intoxicants, capable of causing irritation of the mucous membranes and drowsiness. Chlorinated hydrocarbons cause narcosis and damage to the central nervous system and liver. Hydrocarbons, like the other two groups, are skin irritants and may cause dermatitis after prolonged skin exposure. Because of the volatility of these compounds, hazardous vapor concentrations can occur (fire or explosion hazard). Proper ventilation is essential.

The majority of organic reagents used in this manual fall into four major categories: acids, halogenated compounds, dyes and indicators, and pesticides. Most organic acids have irritant properties. They are predominantly solids from which aerosols may be produced. Dyes and indicators also present an aerosol problem. Handle pesticides with caution because they are poisons, and avoid contact with the skin. Wear gloves and protective clothing. The chlorinated compounds present much the same hazards as the chlorinated solvents (narcosis and damage to the central nervous system and liver). Proper labeling for the compound, including a date for disposal based on the manufacturer's recommendations, permits tracking chemical usage and disposal of outdated chemicals.

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1090 C. Laboratory Facility/Fixed Equipment

1. Facility Design

The laboratory facility must have a general ventilation system with air intakes and exhausts located to avoid intake of contaminated air, well-ventilated stockrooms and/or storerooms, laboratory hoods and sinks, miscellaneous safety equipment including eyewash fountains and safety showers, and arrangements for the disposal of wastes and samples in accordance with applicable federal, state, and local regulations.

2. Facility and Fixed Equipment Maintenance

Maintain facilities and equipment with scheduled maintenance and continual surveillance to ensure proper operation. Give special attention to the adequacy of ventilation system.

a. Facility ventilation systems:

- 1) The general laboratory ventilation should provide a source of air for breathing and for input to local ventilation devices such as fume hoods. Do not rely on it for protection from exposure to toxic substances used during the working day. The system should direct air flow into the laboratory from nonlaboratory areas and then exhaust the air directly to the exterior of the building in a manner that will prevent its re-entry.

- 2) Laboratory fume hoods—As a minimum, provide at least 1 linear m of hood space per

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worker if workers spend most of their time working with chemicals or if they work with chemical substances with PELs or TLVs less than 100 ppm. Equip each hood with a continuous monitoring device to allow convenient confirmation of adequate hood performance before each use. If this is not possible, avoid work with substances with PELs or TLVs less than 100 ppm or with unknown toxicity, or provide other types of local ventilation devices.

3) Other local ventilation devices—Provide ventilated chemical/biological cabinets, canopy hoods and instrument/work station snorkels as needed. Many local ventilation devices require a separate exhaust duct, as do canopy hoods and snorkels.

4) Special ventilation areas/devices—It may be necessary to pass exhaust air from special ventilation areas or devices such as radiological hoods, glove boxes, and isolation rooms through HEPA filters, scrubbers, or other treatment before release into regular exhaust system. Ensure that cool rooms and warm rooms have provisions for rapid escape and for escape in the event of electrical failure.

5) Ventilation system modifications—Make alteration in the ventilation system only in consultation with an expert qualified in laboratory ventilation system design. Thoroughly test changes in the ventilation system to demonstrate adequate worker protection.

b. Facility ventilation system performance: A ventilation system rate of 4 to 12 room air changes per hour is considered adequate where local exhaust ventilation devices such as fume hoods are used as the primary method of control. General ventilation system air flow should not be turbulent and should be relatively uniform throughout the laboratory, with no high-velocity or static areas; air flow into and within laboratory fume hoods should not be excessively turbulent; fume hood face velocity should be adequate for the intended use (for general-purpose fume hoods this is typically 18 to 30 m/min). The effective protection provided by a fume hood depends on a number of factors including hood location and design, and cannot be determined solely on the basis of the face velocity.

c. Facility ventilation system evaluation: Evaluate performance characteristics (quality and quantity) of the ventilation system on installation, re-evaluate whenever a change in local ventilation devices is made, and monitor routinely. Schedule such monitoring with a frequency dictated by the type, age, condition, and any accessories associated with the device, but at least annually; monitor hoods at least quarterly. Document all ventilation system checks or actions such as flow checks, calibration, alterations, repairs, maintenance, or any other action that may determine or change flow efficiency or characteristics.

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1090 D. Hazard Evaluation

1. Hazard Evaluation

Hazard evaluation refers to the assessment of whether an employee has been overexposed to a hazardous substance or if such an exposure episode is likely to occur and to what extent.

The evaluation does not require monitoring airborne concentrations of the hazardous substances involved. Such an assessment may be informal and simply involve considering, among other factors, the chemical and physical properties of the substance and the quantity of

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substance used. In addition, the exposure assessment may be sufficient to estimate the probability of an overexposure.

Specify, document, and use hazard assessment criteria. Base such criteria on the toxicity of the substances to be used, the exposure potential of the chemical procedures to be performed, and the capacity of the available engineering control systems.

In cases where continuous monitoring devices are used, include resulting exposure data in the exposure evaluation. Air monitoring *only* provides information for inhalation exposure. Other means are required to determine whether overexposure could have occurred as a result of ingestion, or dermal or eye contact.

2. Spills of Toxic or Hazardous Substances

Spills are usually the result of loss of containment due to equipment failure or breakage (uncontrolled releases). The loss of containment can result in overexposure episodes. Calculations using data from material safety data sheets, the chemical and physical properties of the substance, known laboratory air changes, and work-station air volume will allow assessment of the possibility of an overexposure episode.

3. Work Practice Assignment

Use the information calculated from these exposure assessments to develop the written work practices needed to protect the health of the employee while conducting the procedure.

4. Documentation of Hazard Assessments

Document, validate, and authenticate all hazard assessments, preferably using a standard form.

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Also see references 2 through 8, Section 1090B.10.

1090 E. Personal Protective Equipment

1. Introduction

The employer must provide and maintain personal protective equipment (PPE) in condition that is sanitary and reliable against hazards in the workplace. All PPE also must be properly designed and constructed for the work to be performed. Several general references on PPE are available.¹⁻³

It is essential to select PPE based on an assessment of the hazards³ or potential hazards to which an employee is exposed, to insure that the correct PPE will be obtained. Use personal protective equipment only when it is not possible or feasible to provide engineering controls. Such personal protective equipment includes all clothing and other work accessories designed to create a barrier against workplace hazards.

The basic element of any personal protective equipment management program must be an in-depth evaluation of the equipment needed to protect against the hazards at the workplace. Management dedicated to the safety and health of employees must use that evaluation to set a standard operating procedure for personnel, then encourage those employees to use, maintain, and clean the equipment to protect themselves against those hazards.

Using personal protective equipment requires hazard awareness and training on the part of the user. Make employees aware that the equipment does not eliminate the hazard. If the equipment fails, exposure will occur. To reduce the possibility of failure, use equipment that is properly fitted and maintained in a clean and serviceable condition.

Selection of the proper piece of personal protective equipment for the job is important. Employers and employees must understand the equipment's purpose and its limitations. Do not alter or remove equipment even though an employee may find it uncomfortable (equipment may be uncomfortable simply because it does not fit properly).

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2. Eye Protection

The LH&S standard of practice requires the use of eye and face protective equipment³ where there is a reasonable probability of injury prevention through its use. Employers must provide a type of protector suitable for work to be performed, and employees must use the protectors. These requirements also apply to supervisors and management personnel, and to visitors while they are in hazardous areas.

Protectors must provide adequate protection against particular hazards for which they are designed, be reasonably comfortable when worn under the designated conditions, fit snugly without interfering with the movements or vision of the wearer, and be durable, easy to disinfect and clean, and kept in good repair.

In selecting the protector, consider the kind and degree of hazard. Where a choice of protectors is given, and the degree of protection required is not an important issue, worker comfort may be a deciding factor.

Persons using corrective glasses and those who are required to wear eye protection must wear glasses with protective lenses providing optical correction, goggles that can be worn over corrective glasses without disturbing the adjustment of the glasses, or goggles that incorporate corrective lenses mounted behind the protective lenses.

When limitations or precautions are indicated by the manufacturer, transmit them to the user and observe strictly. Safety glasses require special frames. Combinations of normal wire frames with safety lenses are not acceptable.

Design, construction, testing, and use of eye and face protection must be in accordance with national standards.⁴

3. Protective Work Gloves

Match glove material to the hazard: such materials as nitrile, neoprene, natural rubber, PVC, latex, and butyl rubber vary widely in chemical resistance. What may be safe with one chemical may prove harmful with another (see Table 1090:IV and Table 1090:V). Glove thickness may be as important as glove material in some cases. Many organic reagents, unlike most organic solvents, do not have PELs/TLVs but this does not mean that they are less hazardous.

Evaluate physical properties of the glove material: In addition to chemical resistance, glove materials vary in physical toughness. Select the glove that provides the abrasion, tear, flame, and puncture resistance required for the job.

Maximize comfort and dexterity. Lined gloves absorb perspiration and help insulate the hand. Unlined gloves conform to the hand. Lighter-gauge gloves improve touch sensitivity and flexibility, heavier-gauge gloves add protection and strength.

Ensure a safe grip. Nonslip grips allow for easier and safer handling. Embossed, pebbled, etched, and dotted coatings improve grip in wet or dry working conditions.

Measure proper size and length. Loose-fitting gloves affect dexterity and can be hazardous. Tight-fitting gloves may cause hand fatigue and tend to wear out faster. Gloves should fit

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comfortably without restricting motion and they should be long enough to protect the wrist, forearm, elbow, or the entire arm, depending on the application.

4. Head Protection

Water and wastewater laboratories seldom require this kind of personal protection, but field work may require such protection.

Head injuries are caused by falling or flying objects or by bumping the head against a fixed object. Head protection, in the form of protective hats, must both resist penetration and absorb the shock of a blow. Make the shell of the hat of a material hard enough to resist the blow, and utilize a shock-absorbing lining composed of head band and crown straps to keep the shell away from the wearer's skull. Protective materials used in helmets should be water-resistant and slow burning. Helmets consist essentially of a shell and suspension. Ventilation is provided by a space between the headband and the shell. Ensure that each helmet is accompanied by instructions explaining the proper method of adjusting and replacing the suspension and headband.

Visually inspect daily all components, shells, suspensions, headbands, sweatbands, and any accessories for signs of dents, cracks, penetration or any other damage that might reduce the degree of safety originally provided.

Do not store or carry helmets on the rear window deck of an automobile because sunlight and extreme heat may adversely affect the degree of protection.

Further information is available elsewhere.^{3,5}

5. Hearing Protection

Exposure to high noise levels can cause hearing loss or impairment, and it can create physical and psychological stress. There is no cure for noise-induced hearing loss, so prevention of excessive noise exposure is the only way to avoid hearing damage. Specifically designed protection is required, depending on the type of noise encountered.

Use preformed or molded ear plugs fitted individually by a professional. Waxed cotton, foam, or fiberglass wool earplugs are self-forming. When properly inserted, they work as well as most molded earplugs. Plain cotton is ineffective as protection against hazardous noise.

Some earplugs are disposable, to be used one time and then thrown away. Clean nondisposable types after each use for proper protection.

Earmuffs need to make a perfect seal around the ear to be effective. Glasses, long sideburns, long hair, and facial movements, such as chewing, can reduce protection. Special equipment is available for use with glasses or beards.

More specific information on hearing conservation is available.³

6. Foot and Leg Protection

According to accident reviews most workers who suffered impact injuries to the feet were not wearing protective footwear. Furthermore, most of their employers did not require them to wear safety shoes. The typical foot injury was caused by objects falling less than 1.2 m and the

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median weight was about 30 kg. Most workers were injured while performing their normal job activities at their worksites.

Safety shoes should be sturdy and have an impact-resistant toe. In some shoes, metal insoles protect against puncture wounds. Additional protection, such as metatarsal guards, may be found in some types of footwear. Safety shoes come in a variety of styles and materials, such as leather and rubber boots and oxfords.

Safety footwear is classified according to its ability to meet minimum requirements for both compression and impact test. Those requirements and testing procedures and further information may be found elsewhere.^{3,6}

7. References

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1090 F. Worker Protection Medical Program

1. Preventive Medicine Program

The preventive medicine program should include inoculations to provide protection from tetanus and other diseases that are associated with the types of samples received and analyzed by the laboratory. The scope of this program depends on the diseases prevalent in the area where the samples originate. The program also must comply with the appropriate regulations.

2. Medical Surveillance

Routine surveillance may be indicated for anyone whose work involves routine handling of

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hazardous chemical or biological substances. Consult a qualified occupational health physician and/or toxicologist to determine whether a regular schedule of medical surveillance is indicated.

3. Environmental Monitoring

a. *General:* The initiation of environmental monitoring (exposure monitoring) associated with laboratory uses of hazardous chemical substances is triggered by exposures exceeding the action level (usually defined as one-half the PEL or TLV), PEL, or TLV. The employer is responsible for ensuring that employees' exposures to such substances do not exceed the PELs specified in the regulations dealing with air contaminants.¹

b. *Employee exposure determination:* Determine a worker's exposure to any hazardous chemical substance if there is reason to believe that exposure levels for that substance routinely exceed the action level. Where there is no action level for a substance the worker exposure must not exceed the PEL or TLV. If the initial monitoring confirms that an employee exposure exceeds the action level, or in the absence of an action level, the PEL, the employer must immediately comply with the exposure monitoring provisions of the relevant national standard. Monitoring may be terminated in accordance with the relevant standard (if one exists) or when the exposures are found to be below the action level (one half the PEL or TLV) or in the absence of an action level, below the PEL or TLV. The workers are to be notified in accordance with national standard; if none exists, they should at least be notified within 15 working days after any monitoring results have become available to the employer, either by contacting the employee individually or by posting the results in an appropriate location accessible to employees.

4. Medical Consultation and Medical Examinations

All employees who work with hazardous chemicals should have an opportunity to receive medical attention (at no personal cost), including any follow up examinations that the examining physician determines to be necessary, under the following circumstances:

- Whenever an employee develops signs or symptoms associated with an exposure to a hazardous chemical that the employee may have been using.
- Where exposure monitoring reveals an exposure level routinely above the action level (or in the absence of an action level, the PEL or TLV). For a national regulated substance for which there are exposure monitoring and medical surveillance requirements, establish medical surveillance for the affected employee as prescribed by the particular standard.
- Whenever an uncontrolled event, such as a spill, leak, explosion, or other occurrence, takes place in the work area, resulting in the likelihood of a hazardous exposure. Provide the affected employee an opportunity for a medical consultation to determine the need for a medical examination.

All medical examinations and consultations should be performed by, or under the direct supervision of, a licensed physician, without cost to the employee or loss of pay, and at a

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reasonable time and place. Inform the physician of the identity of the hazardous chemical(s) to which the employee may have been exposed; the conditions under which the exposure occurred, including quantitative exposure data, if available; and the signs and symptoms of exposure that the employee is experiencing, if any. The employer must obtain from the examining physician a written opinion that includes any recommendation for further medical follow-up, the results of the medical examination and any associated tests, notice of any medical condition revealed during the examination that may place the employee at increased risk as a result of exposure to a hazardous chemical found in the workplace, and a statement that the employee has been informed by the physician of the results of the consultation or medical examination and any medical condition that may require further examination or treatment. The written opinion must not reveal specific findings or diagnoses unrelated to occupational exposure.

5. Reference

1. OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION. Air Contaminants. 29 CFR 1910.1000.

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1090 G. Provisions for Work with Particularly Hazardous Substances

The information outlined in the following paragraphs meets the LH&S standard of practice¹ and also represents good industrial hygiene practices.

1. Designated Area

Wherever appropriate, the employer must establish a "designated area," that is, an area that may be used for work with select carcinogens, reproductive toxins, or substances having a high degree of acute toxicity. A designated area may be the entire laboratory, an area of a laboratory, or a device such as a laboratory hood.

2. Select Carcinogen

In the U.S. a "select carcinogen" means any substance meeting at least one of the following criteria: the substance is regulated by OSHA as a carcinogen; it is listed under the category,

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“known to be carcinogenic,” by the U.S. National Toxicology Program (NTP);² it is listed under Group 1 (carcinogenic to humans) by the International Agency for Research on Cancer (IARC);³ or it is listed in either Group 2A or 2B by IARC³ or under the category, “reasonably anticipated to be carcinogenic” by NTP,² and causes statistically significant tumor incidence in experimental animals after inhalation exposure of 6 to 7 h/d, 5 d/week, for significant portion of a lifetime to dosages of less than 10 mg/m³, or after repeated skin application of less than 300 mg/kg of body weight/week, or after oral dosages of less than 50 mg/kg of body weight/d.

3. Use of Containment Devices

The work conducted and its scale must be appropriate to the physical facilities available and, especially, to the quality of ventilation.

The general laboratory ventilation system must be capable of providing air for breathing and for input to local ventilation devices. It should not be relied on for protection from toxic substances released into the laboratory, but should ensure that laboratory air is continually replaced, preventing increase of air concentrations of toxic substances during the working day, and that air flows into the laboratory from nonlaboratory areas and out to the exterior of the building.

4. References

1. OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION. Laboratory Standard. Occupational Exposure to Hazardous Chemicals in Laboratories. 29 CFR 1910.1450.
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5. Bibliography

NATIONAL INSTITUTES OF HEALTH. 1981. Guidelines for the Laboratory Use of Chemical Carcinogens. NIH Publ. No. 81-2385, U.S. Government Printing Off., Washington, D.C.

Also see Section 1090C.3.

1090 H. Biological Safety

1. Introduction

The analysis of environmental samples involves worker contact with samples that may be contaminated with agents that present microbiological hazards. The majority of these agents

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involve exposures to pathogenic microorganisms or viruses that may produce human disease by accidental ingestion, inoculation, injection, or other means of cutaneous penetration. The primary means of exposure to these microbiological hazards involves hand-mouth contact while handling the samples, contaminated laboratory materials and/or aerosols created by incubating, pipetting, centrifuging, or blending of samples or cultures. Use the following rules, work practices and/or procedures to control or minimize exposure to these agents.

2. General Rules

Do not mix dilutions by blowing air through a pipet into a microbiological culture.

When working with grossly polluted samples, such as wastewater or high-density microbial cultures, use a pipetting device attached to a pipetting bulb to prevent accidental ingestion (never pipet by mouth).

Because untreated waters may contain waterborne pathogens, place all used pipets in a jar containing disinfectant solution for decontamination before glassware washing. Do not place used pipets on table tops, on laboratory carts, or in sinks without adequate decontamination.

3. Work Practices

Good personal hygiene practices are essential to control contact exposures. Frequently disinfect hands and working surfaces. Encourage immunization of laboratory staff against tetanus and possibly typhoid and other infectious agents to minimize risk of exposure.

Provide drinking water outside the laboratory, preferably from a foot-operated drinking fountain.

Eliminate flies and other insects to prevent contamination of sterile equipment, media, samples, and bacterial cultures and to prevent spread of infectious organisms to personnel.

Observe appropriate precautions in use of laboratory equipment. Use a leakproof blender tightly covered during operation to minimize contamination. Use a centrifuge tightly covered to minimize exposure if culture-containing tubes should shatter during centrifuging. The tube breakage produces a cleanup problem and microbiological aerosols. Conduct activities such as inserting a hot loop into a flask of broth culture in a manner that eliminates or minimizes the hazards due to aerosolized microorganisms. Sterilize contaminated materials (cultures, samples, used glassware, serological discards, etc.) by autoclaving before discarding them or processing for reuse. Preferably use specially marked biohazard bags for disposal. Dispose of contaminated broken glass in a specially marked container.

4. Procedures

Quaternary ammonium compounds that include a compatible detergent, or solutions of sodium hypochlorite are satisfactory disinfectants for pipet discard jars. Use the highest concentrations recommended for these commercial products provided that this concentration does not cause a loss of markings or fogging of pipets.

Sterilize biological waste materials to eliminate all infectious substances, and sterilize all contaminated equipment or apparatus before washing, storage, or disposal, preferably by

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autoclaving. When decontaminating materials in the autoclave, heat them to at least 121°C under a pressure of 103 kPa for a minimum of 30 min. The contact time is measured from time the contact chamber reaches 121°C. If the waste is contained in bags, add water to the contents to insure wet heat. Dry heat and chemical treatment also may be used for sterilizing nonplastic items. After sterilization, the wastes can be handled safely and disposed of by conventional disposal systems in accordance with local regulations.

5. Waste Disposal

Sterilize contaminated materials by autoclaving (see ¶ 3 above) before discarding them.

If combustible materials cannot be decontaminated, burn them with special precautions; permits for burning may be required. Use temporary storage for decay or permanent storage for treating radioactive wastes when alternatives are not available. Collect contaminated combustible wastes and animal carcasses in impermeable containers for disposal by incineration.

6. Bibliography

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- INSTITUTE OF ENVIRONMENTAL SCIENCES. 1986. Recommended Practice for Laminar Flow Clean Devices. RP-CC-002-86. Inst. Environmental Sciences, Mount Prospect, Ill.
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1090 I. Radiological Safety

1. Introduction

This section discusses ionizing radiation safety related to gas chromatography detectors and specific analytical procedures (see Table 1090:VI). Ionizing radiation includes alpha particles, beta particles, gamma rays, and X-rays. Non-ionizing radiation safety is discussed elsewhere (Section 1090B.8).

All persons are exposed to ionizing radiation. The average annual radiation dose to the whole body from cosmic, terrestrial, and internal sources, medical and dental X-rays, etc., is about 185 mrem/year (1.85 mSieverts/year). It is essential to prevent unnecessary continuous or intermittent occupational exposures, and to take steps to eliminate accidents that may result in dangerous radiation exposure.

Personnel who work in laboratories may be exposed to ionizing radiation sources using both

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procedures and instruments. Evaluate potential exposures and control the associated equipment and procedures using work practices developed to minimize and/or eliminate exposures.

Users of radioactive materials are responsible for compliance with the requirements of their national nuclear regulatory body (in the United States, the Nuclear Regulatory Commission)¹ and/or related state regulations. In addition, administrative or local requirements may apply at specific facilities. The use of “exempt” quantities is regulated¹ even though the facility may be exempt from specific licensing requirements.

Radionuclides are used in laboratories to develop and evaluate analytical methods, to prepare counting standards, and to calibrate detectors and counting instruments (see Part 7000). Sealed sources, such as the nickel-63 detector cell used in electron capture gas chromatograph units, also are common.

2. Exposures

a. Exposure limits and control: The LH&S standard of practice² does not permit the use of personal protective equipment for allowing employee exposures above the limits specified by the NRC.¹ The NRC exposure limits are the maximum permissible exposures for 40 h in any workweek of 7 consecutive days.

The exposure limits may be adjusted proportionately (upward) for a period where the exposure is less than 40 h. However, the limit must be adjusted proportionately (downward) for periods where the exposure period is greater than 40 h.

Limiting exposure to ionizing radiation includes providing engineering (physical safeguards) and administrative (procedural) controls for using radioactive materials. Engineering controls include shields, barriers, and interlocks to limit external exposure, and exhaust ventilation systems and personal protective equipment to limit internal contamination. Administrative controls include conducting periodic surveys and reviews of activities, training in the use of radioactive materials, and documented procedures (see below).

Hazards associated with the use of devices, such as X-ray diffraction apparatus or an electron microscope, can be minimized or eliminated by following the manufacturer’s operating instructions and the laboratory safety procedures.

b. Monitoring procedures and equipment: Radiochemical exposure monitoring may be done by collecting and analyzing wipe samples, using portable survey instruments, and/or by collecting and analyzing air samples. More than one technique usually is required.

Survey equipment may either integrate the response over time (e.g., exposure, absorbed dose), or results may be presented as a response rate (e.g., count rate or exposure rate). Typical choices include ion chambers, G-M counters, and scintillation detectors.

Thin-windowed GM-counters are suitable for wipe samples and for monitoring skin and clothing. An alpha scintillation monitor is needed to detect alpha-emitters. An excellent discussion of monitoring techniques for radioisotopes is available.³

c. Facility surveys: Conduct periodic surveys to assess the effectiveness of physical and

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procedural controls. Survey procedures generally use wipe tests for removable contamination and/or portable measurement devices for locating or measuring fixed and removable radioactivity.

1) Sealed sources—Check these sources for integrity by wipe tests at least every 6 months. Electron capture detectors using ^{63}Ni or ^3H require counting wipes by liquid scintillation or windowless gas-flow proportional counters to measure low-energy beta radiation effectively.

2) Work and storage areas—Survey these areas periodically to assess possible contamination or external radiation fields using portable survey instruments. The frequency of the surveys is dictated by the documented contamination record for the laboratory. Usually the radioactivities presented using the methods of Part 7000 are not measurable with routine survey instruments. Therefore use blanks in the analytical process in determining the presence of low-level contamination.

3) Documentation and records—Completely document each survey, identifying the personnel involved, the location, the type, model, and serial numbers of survey instruments used, the type and energy of radiations measured, the date and time of the survey, the instrument response to a check source, the instrument background count or exposure rate, and the results of each measurement.⁴

d. Personnel surveys and monitoring: Conduct and document surveys after routine use of unsealed radionuclide sources to confirm that personnel and the work area have not been contaminated by the process. Wear monitoring devices if there is a reasonable probability of exceeding 25% of the occupational exposure dose equivalent limit. Personal monitoring devices include film badges, thermoluminescent dosimeters, and solid state electronic dosimeters. The length of time the personal monitoring badges are worn before evaluation depends on the ability of the device to integrate the exposure over long periods, the probability and magnitude of the exposure, and the need to assure that the device is available and used.

Personnel performing procedures in *Standard Methods* would not be expected to receive exposures approaching 1.25 rem/quarter and may wish to consider 3-month wear periods if dosimetry is required.

Personal (external radiation) exposure is evaluated by using a personal dosimeter, preferably the film dosimeter (badge). The dosimeter badge measures the accumulated radiation over a period of time. Pocket ionization chambers, thermoluminescent dosimeters, and thimble chambers also may be used to supplement the film dosimeter.

Whole body or gamma spectrometry radiation detectors may be used to determine the presence of radioactive substances in the body, but these instruments are expensive and require the operator to be specially trained. Evaluate equipment and supplies that have been, or are suspected to have been, in contact with radioactive substances to determine if contamination is present. Because body waste may contain radioactive contaminants evaluate it also for the presence of contamination where personal exposures have been confirmed.

3. Work Practices

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Each individual should be familiar with procedures for dealing with radiation emergencies from small spills to major accidents, depending on facility programs. Emergency procedures should include notifications required, containment methods, clean-up procedures, and survey techniques. Emergency supplies should be readily available for coping with major accidents.

Contamination is typically prevented through proper use of laboratory facilities and procedures. Procedures include the use of gloves, aprons, safety glasses, and other protective clothing to eliminate the possibility of skin contamination and transfer. Learn proper pipetting and weighing techniques before working with radioactive sources. Conduct work with unsealed radioactive sources in unobstructed work areas with adequate means of containing and absorbing potential spillage of liquids.

4. Procedures

Develop and implement a radiation safety plan and provide a copy to all persons working with radioactive materials or radiation-producing machines, and provide both lecture and practical training to all employees.

a. Safety plan elements: The recommended minimum plan should include procedures for obtaining authorization to use, order, handle, and store radionuclides; safe handling of unsealed radioactive material; safe response to radiation accidents; decontamination of personnel and facilities; personnel monitoring; laboratory monitoring; and disposal of radioactive materials.

b. Handling radioactive materials: Become knowledgeable about the hazards associated with the materials to be used. Plan work activities to minimize the time spent handling radioactive materials or in using radioactive sources. Work as far from radioactive sources as possible, use shielding appropriate for the materials to be used, and use radioactive materials only in defined work areas. Wear protective clothing and dosimeters as appropriate. Monitor work areas to ensure maximum contamination control. Minimize the accumulation of waste materials in the work area. Use appropriate personal hygiene and self-monitor after using radioactive materials and after each decontamination procedure.

c. Training of users: Train personnel working with radioactive materials in radiation safety as part of the overall occupational health program. Address at least the following topics: characteristics of ionizing radiation and radioactive contamination; radiation dose limits; environmental radiation background; acute and chronic effects; internal and external modes of exposure; basic protective measures; responsibilities of employer and employees; radiation protection program responsibilities; posting, warning signs, and alarms; radiation monitoring programs; and emergency procedures.⁵

5. Waste Disposal

Generalized disposal criteria for radioactive wastes have been developed by the U.S. National Committee on Radiation Protection and Measurements.⁴ Two general philosophies govern the disposal of radioactive wastes: dilution and dispersion to reduce the concentration of radionuclide by carrier dilution or dilution in a receiving medium, and concentration and

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confinement, usually involving reduction in waste volume with subsequent storage for decay purposes.

Airborne wastes can be treated by either method. Ventilation includes discharge from hooded operations to the atmosphere. Typical radioactive gases include iodine, krypton, and xenon. Iodine can be removed by scrubbing or by reaction with silver nitrate. Noble gases can be removed by absorption; standard techniques can be used for particulate. Dilution methods are suitable for liquids with low activity. Intermediate levels may be treated by various physical-chemical processes to separate the waste into a nonradioactive portion that can be disposed of by dilution and a high-activity portion to be stored. Solid wastes may consist of equipment, glassware, and other materials. When possible, decontaminate these materials and reuse. Decontamination usually results in a liquid waste.

Dispose of all waste in conformance with the requirements of the regulatory authority having jurisdiction.

Determine the laboratory's status and obtain approval before storing, treating and/or disposing of wastes.

6. References

1. NUCLEAR REGULATORY COMMISSION. Standards for Protection Against Radiation. 10 CFR Part 20.
2. OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION. Ionizing Radiation. 29 CFR 1910.96.
3. FURR, A.K., ed. 1990. CRC Handbook of Laboratory Safety, 3rd ed. CRC Press, Inc., Boca Raton, Fla.
4. NATIONAL COUNCIL ON RADIATION PROTECTION AND MEASUREMENTS. 1976. Environmental Radiation Measurements. Rep. No. 50, National Council. Radiation Protection & Measurements, Washington, D.C.

7. Bibliography

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1090 J. Chemical Hygiene Plan

1. Introduction

The information presented in this section describes the intent and lists the minimum requirements and critical elements of the OSHA laboratory standard.¹ This standard is performance-based and represents good industrial hygiene practice. Any organization that chooses not to follow or use the OSHA standards to meet that standard's requirements must demonstrate that the procedures it uses meet the minimum level of employee protection afforded by the OSHA standard.

Much of the information presented in the preceding subsections of 1090 was provided as guidance and should be used in developing and finalizing the laboratory chemical hygiene plan (CHP).

2. Requirements

Develop and implement a written CHP capable of protecting employees from health hazards associated with the chemicals used in the laboratory. This CHP must be capable of keeping exposures below the permissible exposure limits (PELs/TLVs) and also must be readily available to employees. The CHP must at minimum address the following elements, and must describe specific measures the employer will undertake to ensure laboratory employee protection.

a. Standard operating procedures or work practices: Include procedures and practices relevant to safety and health considerations. These are to be followed when laboratory work involves the use of hazardous chemicals. Include the information contained in MSDSs for hazardous chemicals when conducting a hazard assessment and developing work practices. Some of the guidance presented in an MSDS is intended for use in industrial settings where material is used in large quantities for a full work shift and not for the small-volume, short-duration exposures experienced in laboratories.

b. Exposure hazard criteria and procedures: These will be used to determine and implement control measures for reducing employee exposure to hazardous chemicals while conducting laboratory operations. They include engineering controls, the use of personal protective equipment, and hygiene practices. Pay particular attention to the selection of control measures for work activities that involve chemicals known to be extremely hazardous.

c. Protective equipment performance procedures: These include procedures for evaluating the performance of fume hoods and other protective equipment and specific measures to be taken to ensure proper and adequate performance of such equipment.

d. Employee information and training: This training must be timely, be refreshed periodically, evaluated for effectiveness, and documented.

e. Approval procedures: The plan must state the circumstances under which a specific laboratory operation, procedure, or activity requires prior approval before implementation.

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f. Employee medical consultation and medical examinations.

g. Safety personnel: The plan must designate personnel responsible for implementation of the CHP. This should include the formal assignment of a Chemical Hygiene Officer and, where appropriate, the establishment of a Chemical Hygiene Committee.

h. Additional employee protection: The plan should include provisions for working with particularly hazardous substances. These substances include “select carcinogens” (see Section 1090G.2), reproductive toxins, and substances that have a high degree of acute toxicity. Give specific consideration to establishment of a designated area, use of containment devices such as fume hoods or glove boxes, procedures for safe removal of containment waste, decontamination procedures, emergency plans and procedure (test annually as a minimum), and employer review and evaluation of the effectiveness of the CHP at least annually and updating as necessary.

3. Reference

1. OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION. Occupational Exposure to Hazardous Chemicals in Laboratories. 29 CFR 1910.1450.

1100 WASTE MINIMIZATION AND DISPOSAL

1100 A. Introduction

Waste minimization and disposal are part of integrated hazardous materials management. It is important to become familiar with federal regulations regarding the use and disposal of hazardous materials prior to their purchase, storage, and use for water and waste-water analysis. Proper management of hazardous materials will reduce the amount of hazardous waste and associated disposal costs.

1100 B. Waste Minimization

1. General Considerations

Waste minimization or pollution prevention in the laboratory is the preferred approach in managing laboratory waste. Minimizing waste makes good economic sense: it reduces both costs and liabilities associated with waste disposal. For certain hazardous-waste generators it also is a regulatory requirement.

2. Waste Minimization Methods

Waste minimization methods include source reduction, recycling, and reclamation.¹ Waste treatment, which also may be considered a form of waste minimization, is addressed in Section 1100C.

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Source reduction can be achieved through the purchase and use of smaller quantities of chemicals. While large-volume purchases may seem economical, the costs of disposing of expired-shelf-life materials also must be considered. Date chemical inventory and use oldest stock first, or if possible, use “just in time” material delivery. Commercial laboratories and chemical users in general can return samples or unopened chemicals to sender or supplier for recycling or disposal. Many suppliers will accept unopened containers of chemicals.

Substitute nonhazardous materials for hazardous chemicals where possible. Wherever possible use methods that do not require the use of hazardous chemicals or use micro-scale analytical methods.

Improving laboratory procedures, documentation, and training will increase awareness of waste minimization and proper disposal practices, and may allow different sections within a laboratory to share standards and stock chemicals. Evaluate hazardous materials storage and use areas for potential evaporation, spills, and leaks. Segregate waste streams where possible to keep nonhazardous waste from becoming hazardous waste through contact with hazardous waste. Segregation also facilitates treatment and disposal.

Transfer of unused stock chemicals to other areas of the laboratory where they may be used or to other institutions is a way to minimize waste.² Check with laboratory’s legal counsel before transferring chemicals.

Recycling/reclamation has limited potential in water and waste-water laboratories. Volumes generated are generally too small for economical reclamation and purity requirements are often too great. However, organic solvents often can be distilled and recovered for reuse and mercury and silver can be recovered.³

3. References

1. ASHBROOK, P.C. & P.A. REINHARDT. 1985. Hazardous wastes in academia. *Environ. Sci. Technol.* 19:1150.
2. PINE, S.H. 1984. Chemical management: A method for waste reduction. *J. Chem. Educ.* 61:A45.
3. HENDRICKSON, K.J., M.M. BENJAMIN, J.F. FERGUSON & L. GOEBEL. 1984. Removal of silver and mercury from spent COD test solutions. *J. Water Pollut. Control. Fed.* 56(5):468.

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AMERICAN CHEMICAL SOCIETY. 1985. Less is Better. Dep. Public Affairs, American Chemical Soc., Washington, D.C.

1100 C. Waste Treatment and Disposal

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1. General Considerations

Stringent penalties exist for the improper disposal of hazardous wastes. Potential criminal and civil liability exists for both organizations and individuals. Specific requirements vary by state and local jurisdiction and are subject to change. Federal requirements for hazardous waste generators and transporters and for treatment, storage, and disposal facilities (TSDFs) are found in regulations pursuant to the Resource Conservation and Recovery Act of 1976 (RCRA) as amended by the Hazardous and Solid Waste Amendments of 1984 (HSWA). Many activities, in particular treatment, storage, and disposal of hazardous wastes, require a permit or license.^{1,2}

Develop a plan for the safe and legal disposal of chemical and biological substances in conjunction with the laboratory supervisor and safety coordinator. Plan should address the proper transport, storage, treatment, and disposal of hazardous waste. Properly characterize composites and document wastes. Refer to Section 1090 on Safety with regard to protective equipment in the handling of hazardous materials.

2. Waste Treatment and Disposal Methods

Treatment can be used to reduce volume, mobility, and/or toxicity of hazardous waste where expertise and facilities are available. Treatment, even on a small scale, may require a permit. Consult with federal, state, and local regulatory officials.

Waste treatment methods include thermal, chemical, physical, and biological treatment, and combinations of these methods.¹

a. Thermal treatment: Thermal treatment methods include incineration and sterilization. They involve using high temperatures to change the chemical, physical, or biological character or composition of the waste. Incineration is often used to destroy organic solvents and is preferred for infectious wastes, although sterilization through autoclaving and/or ultraviolet light also may be allowed. Check with local health department officials.

b. Chemical treatment: Methods include chemical reaction (oxidation/reduction, neutralization, ion exchange, chemical fixation, photolysis, coagulation, precipitation) of the waste material. Neutralization of acidic or alkaline wastes is the most common form of chemical treatment. Elementary neutralization of corrosive wastes is exempt from federal RCRA permitting requirements. Before discharge of wastes to a publicly owned treatment works (POTW), ensure that they contain no pollutants (other than corrosivity) exceeding the limits set by the POTW. The oxidation of cyanide to cyanate with a strong chemical oxidant is an example of a toxicity-reducing chemical treatment.

c. Physical treatment: Methods include solidification, compaction, photo-induced reaction, distillation, flocculation, sedimentation, flotation, aeration, filtration, centrifugation, reverse osmosis, ultrafiltration, gravity thickening, and carbon or resin adsorption. Physical treatment generally reduces volume or mobility of waste materials.

d. Biological treatment: Methods include using biosolids to destroy organic compounds, composting organic-rich wastes, and using bioreactors to promote decomposition. Biological

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treatment usually is economical on a scale larger than is possible in most water and wastewater laboratories.

e. Ultimate disposal: After waste minimization and treatment, remaining waste streams require disposal. Nonhazardous wastes that cannot be treated further can be discharged as wastewater, emitted to the atmosphere, or placed on or in the ground.

With extreme caution, it may be permissible to dispose of limited quantities (at certain concentrations) of laboratory wastes to the sanitary sewer system or to evaporate volatile wastes in chemical ventilation hoods. Obtain written permission of local, state, and federal authorities to dispose of waste in this manner. With increasing regulatory constraints imposed by RCRA, the Clean Air Act, and Clean Water Act, these disposal options are becoming increasingly limited. Wastes disposed of in this manner may contact other substances in the sewer or ventilation systems and produce hazardous reactions.

Most hazardous wastes generated in laboratories must be sent off site for further treatment and disposal. Exercise extreme care in selecting a reputable waste hauler and disposal firm. Many firms will assist laboratories in packaging and manifesting “lab packs,” 19- to 208-L (5- to 55-gal) drums containing several smaller containers of wastes.¹ Liability does not disappear when the waste leaves the generator’s facility. Ensure that the laboratory receives a copy of the completed manifest and certificate of treatment and/or disposal. If possible, visit the disposal facility in advance to observe how it will manage a waste.

Certain wastes require special handling. As mentioned previously, incinerate infectious waste or sterilize it before disposal. Before reuse, sterilize all nondisposable equipment that has come into contact with infectious waste.

Although most water and wastewater laboratories do not work with radiochemical wastes, some do. Handle radiochemical wastes with extreme care. Generalized disposal criteria for radioactive wastes have been developed by the National Council on Radiation Protection and Measurements.³ Low-level radioactive waste must be in solid form for final disposal on land. Some firms will process liquid radioactive wastes into solids. Adding absorbent materials to liquid radioactive wastes is not permissible. Certain states allow low-level liquid radioactive waste to be discharged to a permitted publicly owned treatment works (POTW).

Other wastes that require special handling include polychlorinated biphenyls (PCBs), dioxin/furans and their precursors, petroleum products, and asbestos. Consult with federal and state officials before disposing of these wastes.

3. References

1. AMERICAN CHEMICAL SOCIETY. 1983. RCRA and Laboratories. Dep. Public Affairs, American Chemical Soc., Washington, D.C.
2. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1990. Standards for Owners and Operators of Hazardous Waste Treatment, Storage, and Disposal Facilities. 40 CFR Part 264.
3. U.S. ENVIRONMENTAL PROTECTION AGENCY. Standards for Protection Against

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Radiation. 10 CFR Part 20.

4. Bibliography

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- SNIDER, E.H. 1992. Waste minimization. *In* L.K. Wang & M.H.S. Wang, eds., Handbook of Industrial Waste Treatment, p.1. Marcel Dekker, Inc., New York, N.Y.
- DUFOUR, J.T. 1994. Hazardous Waste Management Guide for Laboratories. Dufour Group, Sacramento, Calif.

Figures

Figure 1010:1. Normal (left) and skewed (right) distributions.

Figure 1020:1. Control charts for means.

Figure 1020:2. Duplicate analyses of a standard.

Figure 1020:3. Range chart for variable concentrations.

Figure 1020:4. Range chart for variable ranges.

Figure 1020:5. Means control chart with out-of-control data (upper half).

Figure 1030:1. Detection limit relationship.

Figure 1060:1. Approximate number of samples required in estimating a mean concentration. Source: Methods for the Examination of Waters and Associated Materials: General Principles of Sampling and Accuracy of Results. 1980. Her Majesty's Stationery Off., London, England.

TABLES

TABLE 1010:I. CRITICAL VALUES FOR 5% AND 1% TESTS OF DISCORDANCY FOR A SINGLE OUTLIER IN A NORMAL SAMPLE

Number of Measurements <i>n</i>	Critical Value	
	5%	1%
3	1.15	1.15
4	1.46	1.49

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Number of Measurements <i>n</i>	Critical Value	
	5%	1%
5	1.67	1.75
6	1.82	1.94
7	1.94	2.10
8	2.03	2.22
9	2.11	2.32
10	2.18	2.41
12	2.29	2.55
14	2.37	2.66
15	2.41	2.71
16	2.44	2.75
18	2.50	2.82
20	2.56	2.88
30	2.74	3.10
40	2.87	3.24
50	2.96	3.34
60	3.03	3.41
100	3.21	3.60
120	3.27	3.66

Source: BARNETT, V. T. LEWIS. 1984. Outliers in Statistical Data. John Wiley Sons, New York, N.Y.

TABLE 1020:I. FACTORS FOR COMPUTING LINES ON RANGE CONTROL CHARTS

Number of Observations <i>n</i>	Factor for Central Line (<i>D</i> ₂)	Factor for Control Limits (<i>D</i> ₄)
2	1.128	3.267
3	1.693	2.575
4	2.059	2.282
5	2.326	2.115
6	2.534	2.004

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Source: ROSENSTEIN, M. A. S. GOLDEN. 1964. Statistical Techniques for Quality Control of Environmental Radioassays. AQCS Rep. Stat-1. Public Health Serv., Winchester, Mass.

TABLE 1020:II. EXAMPLE DATA QUALIFIERS

Symbol	Explanation
B	Analyte found in reagent blank. Indicates possible reagent or background contamination.
E	Reported value exceeded calibration range.
J	Reported value is an estimate because concentration is less than reporting limit or because certain QC criteria were not met.
N	Organic constituents tentatively identified. Confirmation is needed.
PND	Precision not determined.
R	Sample results rejected because of gross deficiencies in QC or method performance. Re-sampling and/or re-analysis is necessary.
RND	Recovery not determined.
U	Compound was analyzed for, but not detected.

* Based on U.S. Environmental Protection Agency guidelines.¹

TABLE 1020:III. AUDIT OF A SOIL ANALYSIS PROCEDURE

	Procedure	Comment	Remarks
1.a	Sample entered into logbook	yes	lab number assigned
2.a	Sample weighed	yes	dry weight
3.a	Drying procedure followed	no	maintenance of oven not done
4.a.	Balance calibrated	yes	once per year
b.	Cleaned and zero adjusted	yes	weekly
5.a	Sample ground	yes	to pass 50 mesh
6.a	Ball mill cleaned	yes	should be after each sample

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Procedure	Comment	Remarks
.		
.		

TABLE 1030:I. CONDUCTIVITY FACTORS OF IONS COMMONLY FOUND IN WATER

Ion	Conductivity (25°C) $\mu\text{mhos/cm}$	
	Per me/L	Per mg/L
Bicarbonate	43.6	0.715
Calcium	52.0	2.60
Carbonate	84.6	2.82
Chloride	75.9	2.14
Magnesium	46.6	3.82
Nitrate	71.0	1.15
Potassium	72.0	1.84
Sodium	48.9	2.13
Sulfate	73.9	1.54

TABLE 1040:I. PRECISION AND BIAS FOR A SINGLE CONCENTRATION IN A SINGLE MATRIX

Result mg/L	Difference (-1.30)	Squared Difference
1.23	-0.07	0.0049
1.21	-0.09	0.0081
1.30	0.0	0.0000
1.59	0.29	0.0841
1.57	0.27	0.0729
1.21	-0.09	0.0081
1.53	0.23	0.0529
1.25	-0.05	0.0025
Sum	0.49	0.2335

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Result <i>mg/L</i>	Difference (-1.30)	Squared Difference

TABLE 1040:II. VARIATIONS IN FACTORS FOR METHOD RUGGEDNESS DETERMINATION

Factor	Nominal	Variation
Mixing time	10 min	12 min
Portion size	5 g	10 g
Acid concentration	1 <i>M</i>	1.1 <i>M</i>
Heat to	100°C	95°C
Hold heat for	5 min	10 min
Stirring	yes	no
pH adjust	6.0	6.5

TABLE 1040:III. FACTOR MATRIX FOR METHOD RUGGEDNESS DETERMINATION

Factor value	Combinations							
	1	2	3	4	5	6	7	8
A or a	A	A	A	A	a	a	a	a
B or b	B	B	b	b	B	B	b	b
C or c	C	c	C	c	C	c	C	c
D or d	D	D	d	d	d	d	D	D
E or e	E	e	E	e	e	E	e	E
F or f	F	f	f	F	F	f	f	F
G or g	G	g	g	G	g	G	G	g
Result	s	t	u	v	w	x	y	z

Source: YODEN, W. J. E. H. STEINER. 1975. Statistical Manual of AOAC. Assoc. Official Analytical Chemists, Washington, D.C.

TABLE 1040:IV. SAMPLE COLLABORATIVE TEST RESULTS

Deviation

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TABLE 1040:IV. SAMPLE COLLABORATIVE TEST RESULTS

Laboratory	Result mg/L	Experimental x ± s	Deviation	
			From Known	From Grand Average
1	32.7			
	35.2	34.7 ± 1.8	−2.0	−1.7
	36.3			
2	32.6			
	33.7	33.3 ± 0.6	−0.6	−0.3
	33.6			
3	30.6			
	30.6	31.2 ± 1.0	−1.5	−1.8
	32.4			
4	32.6			
	32.5	33.0 ± 0.8	−0.3	−0
	33.9			
5	32.4			
	33.4	32.6 ± 0.8	−0.1	−0.4
	32.9			
(Σx)/n = 33			Σ = 1.3	Σ = −0.2
s = 1.5				

TABLE 1040:V. METHOD PRECISION AND BIAS

Known Amount mg/L	Amount Found mg/L	CV (% Standard Deviation)	Bias %
4.3	4.8	12.5	11.5
11.6	12.2	10.2	5.6
23.4	23.8	5.4	1.9
32.7	33.0	4.5	0.9

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TABLE 1050:I. CONVERSION FACTORS* (Milligrams per Liter—Milliequivalents per Liter)

Ion (Cation)			Ion (Anion)		
	me/L = mg/L ×	mg/L = me/L ×		me/L = mg/L ×	mg/L = me/L ×
Al ³⁺	0.111 2	8.994	BO ₂ ⁻	0.023 36	42.81
B ³⁺	0.277 5	3.604	Br ⁻	0.012 52	79.90
Ba ²⁺	0.014 56	68.66	Cl ⁻	0.028 21	35.45
Ca ²⁺	0.049 90	20.04	CO ₃ ²⁻	0.033 33	30.00
Cr ³⁺	0.057 70	17.33	CrO ₄ ²⁻	0.017 24	58.00
			F ⁻	0.052 64	19.00
Cu ²⁺	0.031 47	31.77	HCO ₃ ⁻	0.016 39	61.02
Fe ²⁺	0.035 81	27.92	HPO ₄ ²⁻	0.020 84	47.99
Fe ³⁺	0.053 72	18.62	H ₂ PO ₄ ⁻	0.010 31	96.99
H ⁺	0.992 1	1.008	HS ⁻	0.030 24	33.07
K ⁺	0.025 58	39.10	HSO ₃ ⁻	0.012 33	81.07
			HSO ₄ ⁻	0.010 30	97.07
Li ⁺	0.144 1	6.941	I ⁻	0.007 880	126.9
Mg ²⁺	0.082 29	12.15	NO ₂ ⁻	0.021 74	46.01
Mn ²⁺	0.036 40	27.47	NO ₃ ⁻	0.016 13	62.00
Mn ⁴⁺	0.072 81	13.73	OH ⁻	0.058 80	17.01
Na ⁺	0.043 50	22.99	PO ₄ ³⁻	0.031 59	31.66
NH ₄ ⁺	0.055 44	18.04	S ²⁻	0.062 37	16.03
Pb ²⁺	0.009 653	103.6	SiO ₃ ²⁻	0.026 29	38.04
Sr ²⁺	0.022 83	43.81	SO ₃ ²⁻	0.024 98	40.03
Zn ²⁺	0.030 59	32.70	SO ₄ ²⁻	0.020 82	48.03

* Factors are based on ion charge and not on redox reactions that may be possible for certain of these ions. Cations and anions are listed separately in alphabetical order.

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TABLE 1060:I. SUMMARY OF SPECIAL SAMPLING AND HANDLING REQUIREMENTS*

Determination	Container†	Minimum Sample Size mL	Sample Type‡	
Acidity	P, G(B)	100	g	Refrigerate
Alkalinity	P, G	200	g	Refrigerate
BOD	P, G	1000	g, c	Refrigerate
Boron	P (PTFE) or quartz	1000	g, c	HNO ₃ to pH < 2
Bromide	P, G	100	g, c	None required
Carbon, organic, total	G (B)	100	g, c	Analyze imme H ₃ PO ₄ , or H ₂ S
Carbon dioxide	P, G	100	g	Analyze imme
COD	P, G	100	g, c	Analyze as so refrigerate
Chloride	P, G	50	g, c	None required
Chlorine, total, residual	P, G	500	g	Analyze imme
Chlorine dioxide	P, G	500	g	Analyze imme
Chlorophyll	P, G	500	g	Unfiltered, dar Filtered, dark, (Do not store i
Color	P, G	500	g, c	Refrigerate
Specific conductance	P, G	500	g, c	Refrigerate
Cyanide				
Total	P, G	1000	g, c	Add NaOH to
Amenable to chlorination	P, G	1000	g, c	Add 0.6 g asc refrigerate
Fluoride	P	100	g, c	None required
Hardness	P, G	100	g, c	Add HNO ₃ or
Iodine	P, G	500	g	Analyze imme

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Determination	Container†	Minimum Sample Size mL	Sample Type‡	
Metals, general	P(A), G(A)	1000	g, c	For dissolved metals, pH <2
Chromium VI	P(A), G(A)	1000	g	Refrigerate
Copper by colorimetry*				
Mercury	P(A), G(A)	1000	g, c	Add HNO ₃ to 1
Nitrogen				
Ammonia	P, G	500	g, c	Analyze as soon as possible, refrigerate
Nitrate	P, G	100	g, c	Analyze as soon as possible
Nitrate + nitrite	P, G	200	g, c	Add H ₂ SO ₄ to
Nitrite	P, G	100	g, c	Analyze as soon as possible
Organic, Kjeldahl*	P, G	500	g, c	Refrigerate, at 4°C
Odor	G	500	g	Analyze as soon as possible
Oil and grease	G, wide-mouth calibrated	1000	g	Add HCl or H ₂ SO ₄
Organic compounds				
MBAs	P, G	250	g, c	Refrigerate
Pesticides*	G(S), PTFE-lined cap	1000	g, c	Refrigerate, at 4°C, no chlorine present
Phenols	P, G, PTFE-lined cap	500	g, c	Refrigerate, at 4°C
Purgeables* by purge and trap	G, PTFE-lined cap	2 × 40	g	Refrigerate; at 4°C, acid/L if residue
Base/neutral acids	G(S) amber	1000	g, c	Refrigerate
Oxygen, dissolved	G, BOD bottle	300		
Electrode				Analyze immediately
Winkler				Titration may be used
Ozone	G	1000	g	Analyze immediately

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Determination	Container†	Minimum Sample Size mL	Sample Type‡	
pH	P, G	50	g	Analyze imme
Phosphate	G(A)	100	g	For dissolved
Phosphorus, total	P, G	100	g, c	Add H ₂ SO ₄ to
Salinity	G, wax seal	240	g	Analyze imme
Silica	P (PTFE) or quartz	200	g, c	Refrigerate, dc
Sludge digester gas	G, gas bottle	—	g	—
Solids ⁹	P, G	200	g, c	Refrigerate
Sulfate	P, G	100	g, c	Refrigerate
Sulfide	P, G	100	g, c	Refrigerate; ac NaOH to pH >
Temperature	P, G	—	g	Analyze imme
Turbidity	P, G	100	g, c	Analyze same refrigerate

* For determinations not listed, use glass or plastic containers; preferably refrigerate during storage and analyze as soon as possible.

† P = plastic (polyethylene or equivalent); G = glass; G(A) or P(A) = rinsed with 1 + 1 HNO₃; G(B) = glass, borosilicate; G(S) = glass, rinsed with organic solvents or baked.

‡ g = grab; c = composite.

§ Refrigerate = storage at 4°C ± 2°C; in the dark; analyze immediately = analyze usually within 15 min of sample collection.

|| See citation¹⁰ for possible differences regarding container and preservation requirements. N.S. = not stated in cited reference; stat = no storage allowed; analyze immediately.

If sample is chlorinated, see text for pretreatment.

TABLE 1080:I. WATER PURIFICATION PROCESSES

Process	Major Classes of Contaminants*					P Er
	Dissolved Ionized Solids	Dissolved Ionized Gases	Dissolved Organics	Particulates	Bacteria	
Distillation	G-E†	P	G	E	E	

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Process	Major Classes of Contaminants*					P Er
	Dissolved Ionized Solids	Dissolved Ionized Gases	Dissolved Organics	Particulates	Bacteria	
Deionization	E	E	P	P	P	
Reverse osmosis	G†	P	G	E	E	
Carbon adsorption	P	p§	G-E	P	P	
Filtration	P	P	P	E	E	
Ultrafiltration	P	P	G#	E	E	
Ultraviolet oxidation	P	P	G-E**	P	G††	

Permission to use this table from C3-A2, Vol. 11, No. 13, Aug. 1991, "Preparation and Testing of Reagent Water in the Clinical Laboratory - Second Edition" has been granted by the National Committee for Clinical Laboratory Standards. The complete current standard may be obtained from National Committee for Clinical Laboratory Standards, 771 E. Lancaster Ave., Villanova, PA 19085.

* E = Excellent (capable of complete or near total removal), G = Good (capable of removing large percentages), P = Poor (little or no removal).

† Resistivity of water purified by distillation is an order of magnitude less than water produced by deionization, due mainly to the presence of CO₂ and sometimes H₂S, NH₃, and other ionized gases if present in the feedwater.‡ Resistivity of dissolved ionized solids in the product water depends on original feedwater resistivity.

§ Activated carbon removes chlorine by adsorption.

|| When used in combination with other purification processes, special grades of activated carbon and other synthetic adsorbents exhibit excellent capabilities for removing organic contaminants. Their use, however, is targeted toward specific compounds and applications.

Ultrafilters have demonstrated usefulness in reducing specific feedwater organic contaminants based on the rated molecular weight cut-off of the membrane.

** 185 nm ultraviolet oxidation (batch process systems) is effective in removing trace organic contaminants when used as post-treatment. Feedwater makeup plays a critical role in the performance of these batch processors.

†† 254 nm UV sterilizers, while not physically removing bacteria, may have bactericidal or bacteriostatic capabilities limited by intensity, contact time, and flow rate.

TABLE 1080:II. REAGENT WATER SPECIFICATIONS*

Quality Parameter	High	Medium	Low
Resistivity, megohm-cm at 25°C	>10	>1	0.1

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Quality Parameter	High	Medium	Low
Conductivity, $\mu\text{mho/cm}$ at 25°C	<0.1	<1	10
SiO ₂ , mg/L	<0.05	<0.1	<1

TABLE 1090:I. PERMISSIBLE EXPOSURE LIMITS, THRESHOLD LIMIT VALUES, SHORT-TERM EXPOSURE LIMITS, AND/OR CEILINGS FOR SOME INORGANIC CHEMICALS SPECIFIED IN *STANDARD METHODS*

Compound	Chemical Abstract No. CAS No.	PEL/TLV STEL mg/m ³
Chromic acid and chromates†‡ (as CrO ₃)	7440-47-3	0.1/0.05
Chromium, soluble chromic, chromous salts (as Cr)	7440-47-3	0.5/0.5
Chromium metal and insoluble salts	7440-47-3	1/0.5
Hydrogen chloride	7647-01-0	7.5(C)/7.5(C)
Hydrogen peroxide	7722-84-1	1.4/1.4
Lead‡	7439-92-1	–/0.15
Mercury†§	7439-97-6	0.1/0.05
Nitric acid	7697-37-2	5/5.2, 10(S)
Phosphoric acid	7664-38-2	1/1, 3(S)
Potassium hydroxide	1310-58-3	–/2(C)
Silver (metal and soluble compounds, as Ag)	7440-22-4	0.01/0.1 met
Sodium azide	26628-22-8	–/0.29(C)
Sodium hydroxide	1310-73-2	2(C)/2(C)
Sulfuric acid	7664-93-9	1/1, 3(S)

* Short-term exposure limit. See 29 CFR 1910.1028.

† (Suspect) carcinogen.

‡ Substance has a Biological Exposure Index (BEI).

§ Skin hazard.

TABLE 1090:II. PERMISSIBLE EXPOSURE LIMITS, THRESHOLD LIMIT VALUES, SHORT-TERM EXPOSURE LIMITS, AND/OR CEILINGS FOR ORGANIC SOLVENTS SPECIFIED IN *STANDARD METHODS*

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EXPOSURE LIMITS, AND/OR CEILINGS FOR ORGANIC SOLVENTS SPECIFIED IN *STANDARD METHODS*

Compound	Chemical Abstract No. CAS No.	PEL/TLV STEL(S)* c ppm (v/v)
Acetic acid	64-19-7	10/10, 15(S)
Acetone	67-64-1	1000/750, 1000(S)
Acetonitrile	75-05-8	40/40, 60(S)
Benzene†‡	71-43-2	10, 25(C), 50 peak 10 mi
<i>n</i> -Butyl alcohol [§]	71-36-3	100/50(C)
<i>tert</i> -Butyl alcohol	75-65-0	100/100, 150(S)
Carbon disulfide‡	75-15-0	20, 30(C), 100 peak 30 n
Carbon tetrachloride† [§]	56-23-5	10, 25, 200 peak 5 min/4
Chloroform†	67-66-3	50(C)/10
Cyclohexanone [§]	108-94-1	50/50
Dioxane [§] (diethylene dioxide)	123-91-1	100/25
Ethyl acetate	141-78-6	400/400
Ethyl alcohol	64-17-5	1000/1000
Ethyl ether (diethyl ether)	60-29-7	400/400, 500(S)
Ethylene glycol	107-21-1	–/50(C)
<i>n</i> -Hexane‡	110-54-3	100/50
Isoamyl alcohol (primary and secondary)	123-51-3	100/100, 125(S)
Isobutyl alcohol	78-83-1	100/50
Isopropyl alcohol	67-63-0	400/400, 500(S)
Isopropyl ether	108-20-3	500/250, 310(S)
Methyl alcohol [§]	67-56-1	200/200, 250(S)
2-Methoxyethanol [§] (methyl cellosolve)	109-86-4	25/5
Methylene chloride†	75-09-2	500, 1000(C), 2000 peak
Pentane	109-66-0	1000/600, 750(S)
Perchloroethylene†‡ (tetrachloroethylene)	127-18-4	100, 200(C), 300 peak 5 200(S)
<i>n</i> -Propyl alcohol [§]	71-23-8	200/200, 250(S)
Pyridine	110-86-1	5/5
Toluene‡ [§]	108-88-3	200, 300(C), 500 peak 10

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Compound	Chemical Abstract No. CAS No.	PEL/TLV STEL(S)* or ppm (v/v)
Xylenes‡ (o-, m-, p-isomers)	1330-20-7 (95-47-6, 108-38-3, 106-42-3)	100/100, 150(S)

* Short-term exposure limit. See 29 CFR 1910.1028.

† (Suspect) carcinogen.

‡ Substance has a Biological Exposure Index (BEI).

§ Skin hazard.

TABLE 1090:III. PERMISSIBLE EXPOSURE LIMITS, THRESHOLD LIMIT VALUES, SHORT-TERM EXPOSURE LIMITS, AND/OR CEILINGS FOR SOME OF THE REAGENTS SPECIFIED IN *STANDARD METHODS*

Compound	Chemical Abstract No. CAS No.	PEL/TLV STEL(S)* or ppm (v/v)
2-Aminoethanol (ethanolamine)	141-43-5	3/3, 6(S)
Benzidine†‡	92-87-5	Confirmed human ca
Benzyl chloride	100-44-7	1/1
Chlorobenzene	108-90-7	75/10
Diethanolamine	111-42-2	–/3
Naphthalene	91-20-3	10/10, 15(S)
Oxalic acid	144-62-7	1/1 mg/m ³
Phenol‡	108-95-2	5/5
2-Chloro-6-(trichloromethyl) pyridine (nitrapyrin)	1929-82-4	
Total dust		15/10 mg/m ³
Respirable fraction		5/– mg/m ³

* Short-term exposure limit. See 29 CFR 1910.1028.

† (Suspect) carcinogen.

‡ Skin hazard.

TABLE 1090:IV. GLOVE SELECTION FOR ORGANIC CHEMICAL HANDLING

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TABLE 1090:IV. GLOVE SELECTION FOR ORGANIC CHEMICAL HANDLING

Compound	Chemical Abstract No. CAS No.	Chemical Class	Butyl	
			Rubber	Neoprene
Ethers:		241		
Ethyl ether (diethyl ether)	60-29-7	241		
Isopropyl ether	108-20-3	241		
2-Methoxyethanol† (methyl cellosolve)	109-86-4	245	X	
Halogen compounds:		261		
Carbon tetrachloride*	56-23-5	261		
Chloroform*	67-66-3	261		
Methylene chloride	75-09-2	261		
Perchloroethylene* (tetrachloroethylene)	127-18-4	261		
Hydrocarbons:		291		
<i>n</i> -Hexane	110-54-3	291		
Pentane	109-66-0	291		
Benzene*	71-43-2	292		
Toluene	108-88-3	292		
Xylenes (<i>o</i> -, <i>m</i> -, <i>p</i> -isomers)	1330-20-7	292		
Hydroxyl compounds:		311		
<i>n</i> -Butyl alcohol	71-36-3	311	X	X
Ethyl alcohol	64-17-5	311	X	
Methyl alcohol	67-56-1	311	X	
<i>n</i> -Propyl alcohol	71-23-8	311		
Isoamyl alcohol	123-51-3	311		
Isobutyl alcohol	78-83-1	311	X	
Isopropyl alcohol	67-63-0	312		X
<i>tert</i> -Butyl alcohol (2,2-methylpropanol)	75-65-0	313	X	
Ethylene glycol	107-21-1	314	X	X
Ketones:		391		
Acetone	67-64-1	391	X	

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Compound	Chemical Abstract No. CAS No.	Chemical Class	Suit	
			Butyl Rubber	Neoprene
Cyclohexanone	108-94-1	391	X	
Heterocyclic compounds:		271		
Dioxane† (diethylene dioxide)	123-91-1	278	X	
Pyridine	110-86-1	271	X	
Miscellaneous organic compounds:				
Acetic acid	75-07-0	102	X	X
Ethyl acetate	141-78-6	222	X	
Acetonitrile	75-05-8	431	X	
Carbon disulfide	75-15-0	502		

* (Suspect) carcinogen.

† Skin hazard.

TABLE 1090:V. GLOVE SELECTION FOR INORGANIC CHEMICAL HANDLING

Compound	Chemical Abstract No. (CAS No.)	Chemical Class	Suit	
			Butyl Rubber	Natural Rubber
Inorganic acids:		370		
Chromic acid,* (Cr ⁺⁶)	7440-47-3	370	X	
Hydrochloric acid, 30–70% solutions	10035-10-6	370	X	X
Hydrochloric acid, <30% solutions	10035-10-6	370	X	X
Nitric acid, 30–70% solutions	7697-37-2	370	X	
Nitric acid, <30% solutions	7697-37-2	370	X	X
Phosphoric acid, >70% solutions	7664-38-2	370		X
Phosphoric acid, 30–70% solutions	7664-38-2	370		X
Sulfuric acid, >70% solutions	7664-93-9	370	X	
Sulfuric acid, 30–70% solutions	7664-93-9	370	X	X
Sulfuric acid, <30% solutions	7664-93-9	370	X	X
Inorganic bases:		380		

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Compound	Chemical Abstract No. (CAS No.)	Chemical Class	Suit	
			Butyl Rubber	Natural Rubber
Ammonium hydroxide, 30–70% solutions	7664-41-7	380	X	
Ammonium hydroxide, <30% solutions	7664-41-7	380	X	
Potassium hydroxide, 30–70% solutions	1310-58-3	380	X	X
Sodium hydroxide, >70% solutions	1310-73-2	380		
Sodium hydroxide, 30–70% solutions	1310-73-2	380	X	X
Inorganic salt solutions:		340		
Dichromate solutions, <30%,* (Cr ⁺⁶)	7440-47-3	340		
Inorganic miscellaneous:				
Hydrogen peroxide, 30–70% solutions	7722-39-3	300		X
Mercury†	7439-97-6	560		

* (Suspect) carcinogen.

† Skin hazard.

TABLE 1090:VI. PROCEDURES INVOLVING POTENTIAL EXPOSURE TO IONIZING RADIATION

Standard Methods Section	Radionuclide	Type of Radiation
Part 6000 methods: GC with electron capture detectors	⁶³ Ni or ³ H	Low-energy beta (⁶³ Ni, 17 keV)
7110 Gross Alpha and Gross Beta Radioactivity	Alpha: Uranium, ²³⁰ Th, ²³⁹ Pu, ²⁴¹ Am Beta: ¹³⁷ Cs, ⁹⁰ Sr	Alpha; beta and gamma; or both
7500-Cs Radioactive Cesium	¹³⁴ Cs, ¹³⁷ Cs	Beta, gamma
7500-I Radioactive Iodine	¹³¹ I	Beta, gamma
7500-Ra Radium	²²⁶ Ra	Alpha, beta, gamma
7500-Sr A Total Radioactive Strontium and Strontium-90	⁸⁹ Sr, ⁹⁰ Sr	Beta-emitters
7500- ³ H Tritium	³ H	Low-energy beta (6 keV avg)

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<i>Standard Methods</i> Section	Radionuclide	Type of Radiation
7500-U Uranium	U series	Alpha, beta, gamma

Part 2000 PHYSICAL & AGGREGATE PROPERTIES

2010 INTRODUCTION

This part deals primarily with measurement of the physical properties of a sample, as distinguished from the concentrations of chemical or biological components. Many of the determinations included here, such as color, electrical conductivity, and turbidity, fit this category unequivocally. However, physical properties cannot be divorced entirely from chemical composition, and some of the techniques of this part measure aggregate properties resulting from the presence of a number of constituents. Others, for example, calcium carbonate saturation, are related to, or depend on, chemical tests. Also included here are tests for appearance, odor, and taste, which have been classified traditionally among physical properties, although the point could be argued. Finally, Section 2710, Tests on Sludges, includes certain biochemical tests. However, for convenience they are grouped with the other tests used for sludge.

With these minor exceptions, the contents of this part have been kept reasonably faithful to its name. Most of the methods included are either inherently or at least traditionally physical, as distinguished from the explicitly chemical, radiological, biological, or bacteriological methods of other parts.

2020 QUALITY ASSURANCE/QUALITY CONTROL

Part 2000 contains a variety of analytical methods, many of which are not amenable to standard quality-control techniques. General information on quality control is provided in Part 1000 and specific quality-control techniques are outlined in the individual methods. The following general guidelines may be applied to many of the methods in this part:

Evaluate analyst performance for each method. Determine competence by analyses of samples containing known concentrations.

Calibrate instruments and ensure that instrument measurements do not drift.

Assess the precision of analytical procedures by analyzing at least 10% of samples in duplicate. Analyze a minimum of one duplicate with each set of samples.

Determine bias of an analytical procedure in each sample batch by analysis of blanks, known additions with a frequency of at least 5% of samples, and, if possible, an externally provided

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standard.

2110 APPEARANCE*(6)

To record the general physical appearance of a sample, use any terms that briefly describe its visible characteristics. These terms may state the presence of color, turbidity, suspended solids, organisms and their immature forms, sediment, floating material, and similar particulate matter detectable by the unaided eye. Use numerical values when they are available, as for color, turbidity, and suspended solids.

2120 COLOR*(7)

2120 A. Introduction

Color in water may result from the presence of natural metallic ions (iron and manganese), humus and peat materials, plankton, weeds, and industrial wastes. Color is removed to make a water suitable for general and industrial applications. Colored industrial wastewaters may require color removal before discharge into watercourses.

1. Definitions

The term “color” is used here to mean true color, that is, the color of water from which turbidity has been removed. The term “apparent color” includes not only color due to substances in solution, but also that due to suspended matter. Apparent color is determined on the original sample without filtration or centrifugation. In some highly colored industrial wastewaters color is contributed principally by colloidal or suspended material. In such cases both true color and apparent color should be determined.

2. Pretreatment for Turbidity Removal

To determine color by currently accepted methods, turbidity must be removed before analysis. Methods for removing turbidity without removing color vary. Filtration yields results that are reproducible from day to day and among laboratories. However, some filtration procedures also may remove some true color. Centrifugation avoids interaction of color with filter materials, but results vary with the sample nature and size and speed of the centrifuge. When sample dilution is necessary, whether it precedes or follows turbidity removal, it can alter the measured color.

Acceptable pretreatment procedures are included with each method. State the pretreatment method when reporting results.

3. Selection of Method

The visual comparison method is applicable to nearly all samples of potable water. Pollution

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by certain industrial wastes may produce unusual colors that cannot be matched. In this case use an instrumental method. A modification of the tristimulus and the spectrophotometric methods allows calculation of a single color value representing uniform chromaticity differences even when the sample exhibits color significantly different from that of platinum cobalt standards. For comparison of color values among laboratories, calibrate the visual method by the instrumental procedures.

4. Bibliography

- OPTICAL SOCIETY OF AMERICA. 1943. Committee Report. The concept of color. *J. Opt. Soc. Amer.* 33:544.
- JONES, H. et al. 1952. The Science of Color. Thomas Y. Crowell Co., New York, N.Y.

2120 B. Visual Comparison Method

1. General Discussion

a. Principle: Color is determined by visual comparison of the sample with known concentrations of colored solutions. Comparison also may be made with special, properly calibrated glass color disks. The platinum-cobalt method of measuring color is the standard method, the unit of color being that produced by 1 mg platinum/L in the form of the chloroplatinate ion. The ratio of cobalt to platinum may be varied to match the hue in special cases; the proportion given below is usually satisfactory to match the color of natural waters.

b. Application: The platinum-cobalt method is useful for measuring color of potable water and of water in which color is due to naturally occurring materials. It is not applicable to most highly colored industrial wastewaters.

c. Interference: Even a slight turbidity causes the apparent color to be noticeably higher than the true color; therefore remove turbidity before approximating true color by differential reading with different color filters¹ or by differential scattering measurements.² Neither technique, however, has reached the status of a standard method. Remove turbidity by centrifugation or by the filtration procedure described under Method C. Centrifuge for 1 h unless it has been demonstrated that centrifugation under other conditions accomplishes satisfactory turbidity removal.

The color value of water is extremely pH-dependent and invariably increases as the pH of the water is raised. When reporting a color value, specify the pH at which color is determined. For research purposes or when color values are to be compared among laboratories, determine the color response of a given water over a wide range of pH values.³

d. Field method: Because the platinum-cobalt standard method is not convenient for field use, compare water color with that of glass disks held at the end of metallic tubes containing glass comparator tubes filled with sample and colorless distilled water. Match sample color with the color of the tube of clear water plus the calibrated colored glass when viewed by looking

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toward a white surface. Calibrate each disk to correspond with the colors on the platinum-cobalt scale. The glass disks give results in substantial agreement with those obtained by the platinum-cobalt method and their use is recognized as a standard field procedure.

e. Nonstandard laboratory methods: Using glass disks or liquids other than water as standards for laboratory work is permissible only if these have been individually calibrated against platinum-cobalt standards. Waters of highly unusual color, such as those that may occur by mixture with certain industrial wastes, may have hues so far removed from those of the platinum-cobalt standards that comparison by the standard method is difficult or impossible. For such waters, use the methods in Section 2120C and Section 2120D. However, results so obtained are not directly comparable to those obtained with platinum-cobalt standards.

f. Sampling: Collect representative samples in clean glassware. Make the color determination within a reasonable period because biological or physical changes occurring in storage may affect color. With naturally colored waters these changes invariably lead to poor results.

2. Apparatus

- a. Nessler tubes*, matched, 50-mL, tall form.
- b. pH meter*, for determining sample pH (see Section 4500-H).

3. Preparation of Standards

a. If a reliable supply of potassium chloroplatinate cannot be purchased, use chloroplatinic acid prepared from metallic platinum. Do not use commercial chloroplatinic acid because it is very hygroscopic and may vary in platinum content. Potassium chloroplatinate is not hygroscopic.

b. Dissolve 1.246 g potassium chloroplatinate, K_2PtCl_6 (equivalent to 500 mg metallic Pt) and 1.00 g crystallized cobaltous chloride, $CoCl_2 \cdot 6H_2O$ (equivalent to about 250 mg metallic Co) in distilled water with 100 mL conc HCl and dilute to 1000 mL with distilled water. This stock standard has a color of 500 units.

c. If K_2PtCl_6 is not available, dissolve 500 mg pure metallic Pt in aqua regia with the aid of heat; remove HNO_3 by repeated evaporation with fresh portions of conc HCl. Dissolve this product, together with 1.00 g crystallized $CoCl_2 \cdot 6H_2O$, as directed above.

d. Prepare standards having colors of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, and 70 by diluting 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, and 7.0 mL stock color standard with distilled water to 50 mL in nessler tubes. Protect these standards against evaporation and contamination when not in use.

4. Procedure

a. Estimation of intact sample: Observe sample color by filling a matched nessler tube to the 50-mL mark with sample and comparing it with standards. Look vertically downward through tubes toward a white or specular surface placed at such an angle that light is reflected upward

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through the columns of liquid. If turbidity is present and has not been removed, report as “apparent color.” If the color exceeds 70 units, dilute sample with distilled water in known proportions until the color is within the range of the standards.

b. Measure pH of each sample.

5. Calculation

a. Calculate color units by the following equation:

$$\text{Color units} = \frac{A \times 50}{B}$$

where:

A = estimated color of a diluted sample and

B = mL sample taken for dilution.

b. Report color results in whole numbers and record as follows:

Color Units	Record to Nearest
1–50	1
51–100	5
101–250	10
251–500	20

c. Report sample pH.

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2120 C. Spectrophotometric Method

1. General Discussion

a. Principle: The color of a filtered sample is expressed in terms that describe the sensation realized when viewing the sample. The hue (red, green, yellow, etc.) is designated by the term “dominant wavelength,” the degree of brightness by “luminance,” and the saturation (pale, pastel, etc.) by “purity.” These values are best determined from the light transmission characteristics of the filtered sample by means of a spectrophotometer.

b. Application: This method is applicable to potable and surface waters and to wastewaters, both domestic and industrial.

c. Interference: Turbidity interferes. Remove by the filtration method described below.

2. Apparatus

a. Spectrophotometer, having 10-mm absorption cells, a narrow (10-nm or less) spectral band, and an effective operating range from 400 to 700 nm.

b. Filtration system, consisting of the following (see Figure 2120:1):

- 1) *Filtration flasks,* 250-mL, with side tubes.
- 2) *Walter crucible holder.*
- 3) *Glass Gooch filtering crucible with fritted disk,* pore size 40 to 60 μm .
- 4) *Calcined filter aid.*#(8)*
- 5) *Vacuum system.*

3. Procedure

a. Preparation of sample: Bring two 50-mL samples to room temperature. Use one sample at the original pH; adjust pH of the other to 7.6 by using sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH) of such concentrations that the resulting volume change does not exceed 3%. A standard pH is necessary because of the variation of color with pH. Remove excessive quantities of suspended materials by centrifuging. Treat each sample separately, as follows:

Thoroughly mix 0.1 g filter aid in a 10-mL portion of centrifuged sample and filter to form a

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precoat in the filter crucible. Direct filtrate to waste flask as indicated in Figure 2120:1. Mix 40 mg filter aid in a 35-mL portion of centrifuged sample. With vacuum still on, filter through the precoat and pass filtrate to waste flask until clear; then direct clear-filtrate flow to clean flask by means of the three-way stopcock and collect 25 mL for the transmittance determination.

b. Determination of light transmission characteristics: Thoroughly clean 1-cm absorption cells with detergent and rinse with distilled water. Rinse twice with filtered sample, clean external surfaces with lens paper, and fill cell with filtered sample.

Determine transmittance values (in percent) at each visible wavelength value presented in Table 2120:I, using the 10 ordinates marked with an asterisk for fairly accurate work and all 30 ordinates for increased accuracy. Set instrument to read 100% transmittance on the distilled water blank and make all determinations with a narrow spectral band.

4. Calculation

a. Tabulate transmittance values corresponding to wavelengths shown in Columns X, Y, and Z in Table 2120:I. Total each transmittance column and multiply totals by the appropriate factors (for 10 or 30 ordinates) shown at the bottom of the table, to obtain tristimulus values X, Y, and Z. The tristimulus value Y is *percent luminance*.

b. Calculate the trichromatic coefficients x and y from the tristimulus values X, Y, and Z by the following equations:

$$x = \frac{X}{X + Y + Z}$$

$$y = \frac{Y}{X + Y + Z}$$

Locate point (x, y) on one of the chromaticity diagrams in Figure 2120:2 and determine the dominant wavelength (in nanometers) and the purity (in percent) directly from the diagram.

Determine hue from the dominant-wavelength value, according to the ranges in Table 2120:II.

5. Expression of Results

Express color characteristics (at pH 7.6 and at the original pH) in terms of *dominant wavelength* (nanometers, to the nearest unit), *hue* (e.g., blue, blue-green, etc.), *luminance* (percent, to the nearest tenth), and *purity* (percent, to the nearest unit). Report type of instrument (i.e., spectrophotometer), number of selected ordinates (10 or 30), and the spectral band width (nanometers) used.

6. Bibliography

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2120 D. Tristimulus Filter Method

1. General Discussion

a. Principle: Three special tristimulus light filters, combined with a specific light source and photoelectric cell in a filter photometer, may be used to obtain color data suitable for routine control purposes.

The percentage of tristimulus light transmitted by the solution is determined for each of the three filters. The transmittance values then are converted to trichromatic coefficients and color characteristic values.

b. Application: This method is applicable to potable and surface waters and to wastewaters, both domestic and industrial. Except for most exacting work, this method gives results very similar to the more accurate Method C.

c. Interference: Turbidity must be removed.

2. Apparatus

*a. Filter photometer.**(9)

b. Filter photometer light source: Tungsten lamp at a color temperature of 3000°C.†(10)

c. Filter photometer photoelectric cells, 1 cm.‡(11)

d. Tristimulus filters.§(12)

e. Filtration system: See Section 2120C.2b and Figure 2120:1.

3. Procedure

a. Preparation of sample: See Section 2120C.3a.

b. Determination of light transmission characteristics: Thoroughly clean (with detergent) and rinse 1-cm absorption cells with distilled water. Rinse each absorption cell twice with filtered sample, clean external surfaces with lens paper, and fill cell with filtered sample.

Place a distilled water blank in another cell and use it to set the instrument at 100% transmittance. Determine percentage of light transmission through sample for each of the three tristimulus light filters, with the filter photometer lamp intensity switch in a position equivalent to 4 V on the lamp.

4. Calculation

a. Determine luminance value directly as the percentage transmittance value obtained with the No. 2 tristimulus filter.

b. Calculate tristimulus values X , Y , and Z from the percentage transmittance (T_1 , T_2 , T_3) for filters No. 1, 2, 3, as follows:

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$$X = T_3 \times 0.06 + T_1 \times 0.25$$

$$Y = T_2 \times 0.316$$

$$Z = T_3 \times 0.374$$

Calculate and determine trichromatic coefficients x and y , dominant wavelength, hue, and purity as in Section 2120C.4b above.

5. Expression of Results

Express results as prescribed in Section 2120C.5.

2120 E. ADMI Tristimulus Filter Method

1. General Discussion

a. Principle: This method is an extension of Tristimulus Method 2120D. By this method a measure of the sample color, independent of hue, may be obtained. It is based on use of the Adams-Nickerson chromatic value formula¹ for calculating single number color difference values, i.e., uniform color differences. For example, if two colors, A and B, are judged visually to differ from colorless to the same degree, their ADMI color values will be the same. The modification was developed by members of the American Dye Manufacturers Institute (ADMI).²

b. Application: This method is applicable to colored waters and wastewaters having color characteristics significantly different from platinum-cobalt standards, as well as to waters and wastewaters similar in hue to the standards.

c. Interference: Turbidity must be removed.

2. Apparatus

*a. Filter photometer**(13) equipped with CIE tristimulus filters (see Section 2120D.2d).

b. Filter photometer light source: Tungsten lamp at a color temperature of 3000°C (see Section 2120D.2b).

c. Absorption cells and appropriate cell holders: For color values less than 250 ADMI units, use cells with a 5.0-cm light path; for color values greater than 250, use cells with 1.0-cm light path.

d. Filtration system: See Section 2120C.2b and Figure 2120:1; or a centrifuge capable of achieving $1000 \times g$. (See Section 2120B.)

3. Procedure

a. Instrument calibration: Establish curves for each photometer; calibration data for one instrument cannot be applied to another one. Prepare a separate calibration curve for each absorption cell path length.

1) Prepare standards as described in Section 2120B.3. For a 5-cm cell length prepare

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standards having color values of 25, 50, 100, 200, and 250 by diluting 5.0, 10.0, 20.0, 40.0, and 50.0 mL stock color standard with distilled water to 100 mL in volumetric flasks. For the shorter pathlength, prepare appropriate standards with higher color values.

- 2) Determine light transmittance (see ¶ 3c, below) for each standard with each filter.
- 3) Using the calculations described in ¶ 3d below, calculate the tristimulus values (X_s , Y_s , Z_s) for each standard, determine the Munsell values, and calculate the intermediate value (DE).
- 4) Using the DE values for each standard, calculate a calibration factor F_n for each standard from the following equation:

$$F_n = \frac{(APHA)_n (b)}{(DE)_n}$$

where:

$(APHA)_n$ = APHA color value for standard n ,

$(DE)_n$ = intermediate value calculated for standard n , and

b = cell light path, cm.

Placing $(DE)_n$ on the X axis and F_n on the Y axis, plot a curve for the standard solutions. Use calibration curve to derive the F value from DE values obtained with samples.

b. Sample preparation: Prepare two 100-mL sample portions (one at the original pH, one at pH 7.6) as described in Section 2120C.3a, or by centrifugation. (NOTE: Centrifugation is acceptable only if turbidity removal equivalent to filtration is achieved.)

c. Determination of light transmission characteristics: Thoroughly clean absorption cells with detergent and rinse with distilled water. Rinse each absorption cell twice with filtered sample. Clean external surfaces with lens paper and fill cell with sample. Determine sample light transmittance with the three filters to obtain the transmittance values: T_1 from Filter 1, T_2 from Filter 2, and T_3 from Filter 3. Standardize the instrument with each filter at 100% transmittance with distilled water.

d. Calculation of color values: Tristimulus values for samples are X_s , Y_s , and Z_s ; for standards X_r , Y_r , and Z_r ; and for distilled water X_c , Y_c , and Z_c . Munsell values for samples are V_{xs} , V_{ys} , and V_{zs} ; for standards V_{xr} , V_{yr} , and V_{zr} ; and for distilled water V_{xc} , Y_{yc} , and V_{zc} .

For each standard or sample calculate the tristimulus values from the following equations:

$$X = (T_3 \times 0.1899) + (T_1 \times 0.791)$$

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$$Y = T_2$$

$$Z = T_3 \times 1.1835$$

Tristimulus values for the distilled water blank used to standardize the instrument are always:

$$X_c = 98.09$$

$$Y_c = 100.0$$

$$Z_c = 118.35$$

Convert the six tristimulus values (X_s , Y_s , Z_s , X_c , Y_c , Z_c) to the corresponding Munsell values using published tables 2, 3, 4†#(14) or by the equation given by Bridgeman.³

Calculate the intermediate value of DE from the equation:

$$DE = \{(0.23 \Delta V_y)^2 + [\Delta(V_x - V_y)]^2 + [0.4 \Delta(V_y - V_z)]^2\}^{1/2}$$

where:

$$V_y = V_{ys} - V_{yc}$$

$$\Delta(V_x - V_y) = (V_{xs} - V_{ys}) - (V_{xc} - V_{yc})$$

$$\Delta(V_y - V_z) = (V_{ys} - V_{zs}) - (V_{yc} - V_{zc})$$

when the sample is compared to distilled water.

With the standard calibration curve, use the DE value to determine the calibration factor F .

Calculate the final ADMI color value as follows:

$$\text{ADMI value} = \frac{(F)(DE)}{b}$$

where:

b = absorption cell light path, cm.

Report ADMI color values at pH 7.6 and at the original pH.

4. Alternate Method

The ADMI color value also may be determined spectrophotometrically, using a spectrophotometer with a narrow (10-nm or less) spectral band and an effective operating range

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of 400 to 700 nm. This method is an extension of 2120C. Tristimulus values may be calculated from transmittance measurements, preferably by using the weighted ordinate method or by the selected ordinate method. The method has been described by Allen et al.,² who include work sheets and worked examples.

5. References

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2130 TURBIDITY*(15)

2130 A. Introduction

1. Sources and Significance

Clarity of water is important in producing products destined for human consumption and in many manufacturing operations. Beverage producers, food processors, and potable water treatment plants drawing from a surface water source commonly rely on fluid-particle separation processes such as sedimentation and filtration to increase clarity and insure an acceptable product. The clarity of a natural body of water is an important determinant of its condition and productivity.

Turbidity in water is caused by suspended and colloidal matter such as clay, silt, finely divided organic and inorganic matter, and plankton and other microscopic organisms. Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted with no change in direction or flux level through the sample. Correlation of turbidity with the weight or particle number concentration of suspended matter is difficult because the size, shape, and refractive index of the particles affect the light-scattering properties of the suspension. When present in significant concentrations, particles consisting of light-absorbing

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materials such as activated carbon cause a negative interference. In low concentrations these particles tend to have a positive influence because they contribute to turbidity. The presence of dissolved, color-causing substances that absorb light may cause a negative interference. Some commercial instruments may have the capability of either correcting for a slight color interference or optically blanking out the color effect.

2. Selection of Method

Historically, the standard method for determination of turbidity has been based on the Jackson candle turbidimeter; however, the lowest turbidity value that can be measured directly on this device is 25 Jackson Turbidity Units (JTU). Because turbidities of water treated by conventional fluid-particle separation processes usually fall within the range of 0 to 1 unit, indirect secondary methods were developed to estimate turbidity. Electronic nephelometers are the preferred instruments for turbidity measurement.

Most commercial turbidimeters designed for measuring low turbidities give comparatively good indications of the intensity of light scattered in one particular direction, predominantly at right angles to the incident light. Turbidimeters with scattered-light detectors located at 90° to the incident beam are called nephelometers. Nephelometers are relatively unaffected by small differences in design parameters and therefore are specified as the standard instrument for measurement of low turbidities. Instruments of different make and model may vary in response.†#(16) However, interinstrument variation may be effectively negligible if good measurement techniques are used and the characteristics of the particles in the measured suspensions are similar. Poor measurement technique can have a greater effect on measurement error than small differences in instrument design. Turbidimeters of nonstandard design, such as forward-scattering devices, may be more sensitive than nephelometers to the presence of larger particles. While it may not be appropriate to compare their output with that of instruments of standard design, they still may be useful for process monitoring.

An additional cause of discrepancies in turbidity analysis is the use of suspensions of different types of particulate matter for instrument calibration. Like water samples, prepared suspensions have different optical properties depending on the particle size distributions, shapes, and refractive indices. A standard reference suspension having reproducible light-scattering properties is specified for nephelometer calibration.

Its precision, sensitivity, and applicability over a wide turbidity range make the nephelometric method preferable to visual methods. Report nephelometric measurement results as nephelometric turbidity units (NTU).

3. Storage of Sample

Determine turbidity as soon as possible after the sample is taken. Gently agitate all samples before examination to ensure a representative measurement. Sample preservation is not practical; begin analysis promptly. Refrigerate or cool to 4°C, to minimize microbiological decomposition of solids, if storage is required. For best results, measure turbidity immediately without altering the original sample conditions such as temperature or pH.

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2130 B. Nephelometric Method

1. General Discussion

a. Principle: This method is based on a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. The higher the intensity of scattered light, the higher the turbidity. Formazin polymer is used as the primary standard reference suspension. The turbidity of a specified concentration of formazin suspension is defined as 4000 NTU.

b. Interference: Turbidity can be determined for any water sample that is free of debris and rapidly settling coarse sediment. Dirty glassware and the presence of air bubbles give false results. “True color,” i.e., water color due to dissolved substances that absorb light, causes measured turbidities to be low. This effect usually is not significant in treated water.

2. Apparatus

a. Laboratory or process nephelometer consisting of a light source for illuminating the sample and one or more photoelectric detectors with a readout device to indicate intensity of light scattered at 90° to the path of incident light. Use an instrument designed to minimize stray light reaching the detector in the absence of turbidity and to be free from significant drift after a short warmup period. The sensitivity of the instrument should permit detecting turbidity differences of 0.02 NTU or less in the lowest range in waters having a turbidity of less than 1 NTU. Several ranges may be necessary to obtain both adequate coverage and sufficient sensitivity for low turbidities. Differences in instrument design will cause differences in measured values for turbidity even though the same suspension is used for calibration. To minimize such differences, observe the following design criteria:

1) Light source—Tungsten-filament lamp operated at a color temperature between 2200 and 3000°K.

2) Distance traversed by incident light and scattered light within the sample tube—Total not to exceed 10 cm.

3) Angle of light acceptance by detector—Centered at 90° to the incident light path and not to exceed $\pm 30^\circ$ from 90°. The detector and filter system, if used, shall have a spectral peak response between 400 and 600 nm.

b. Sample cells: Use sample cells or tubes of clear, colorless glass or plastic. Keep cells scrupulously clean, both inside and out, and discard if scratched or etched. Never handle them where the instrument’s light beam will strike them. Use tubes with sufficient extra length, or with a protective case, so that they may be handled properly. Fill cells with samples and standards that have been agitated thoroughly and allow sufficient time for bubbles to escape.

Clean sample cells by thorough washing with laboratory soap inside and out followed by multiple rinses with distilled or deionized water; let cells air-dry. Handle sample cells only by

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the top to avoid dirt and fingerprints within the light path.

Cells may be coated on the outside with a thin layer of silicone oil to mask minor imperfections and scratches that may contribute to stray light. Use silicone oil with the same refractive index as glass. Avoid excess oil because it may attract dirt and contaminate the sample compartment of the instrument. Using a soft, lint-free cloth, spread the oil uniformly and wipe off excess. The cell should appear to be nearly dry with little or no visible oil.

Because small differences between sample cells significantly impact measurement, use either matched pairs of cells or the same cell for both standardization and sample measurement.

3. Reagents

a. Dilution water: High-purity water will cause some light scattering, which is detected by nephelometers as turbidity. To obtain low-turbidity water for dilutions, nominal value 0.02 NTU, pass laboratory reagent-grade water through a filter with pore size sufficiently small to remove essentially all particles larger than $0.1\ \mu\text{m}$; [†](17) the usual membrane filter used for bacteriological examinations is not satisfactory. Rinse collecting flask at least twice with filtered water and discard the next 200 mL.

Some commercial bottled demineralized waters have a low turbidity. These may be used when filtration is impractical or a good grade of water is not available to filter in the laboratory. Check turbidity of bottled water to make sure it is lower than the level that can be achieved in the laboratory.

b. Stock primary standard formazin suspension:

1) Solution I—Dissolve 1.000 g hydrazine sulfate, $(\text{NH}_2)_2\cdot\text{H}_2\text{SO}_4$, in distilled water and dilute to 100 mL in a volumetric flask. CAUTION: *Hydrazine sulfate is a carcinogen; avoid inhalation, ingestion, and skin contact. Formazin suspensions can contain residual hydrazine sulfate.*

2) Solution II—Dissolve 10.00 g hexamethylenetetramine, $(\text{CH}_2)_6\text{N}_4$, in distilled water and dilute to 100 mL in a volumetric flask.

3) In a flask, mix 5.0 mL Solution I and 5.0 mL Solution II. Let stand for 24 h at $25 \pm 3^\circ\text{C}$. This results in a 4000-NTU suspension. Transfer stock suspension to an amber glass or other UV-light-blocking bottle for storage. Make dilutions from this stock suspension. The stock suspension is stable for up to 1 year when properly stored.

c. Dilute turbidity suspensions: Dilute 4000 NTU primary standard suspension with high-quality dilution water. Prepare immediately before use and discard after use.

d. Secondary standards: Secondary standards are standards that the manufacturer (or an independent testing organization) has certified will give instrument calibration results equivalent (within certain limits) to the results obtained when the instrument is calibrated with the primary standard, i.e., user-prepared formazin. Various secondary standards are available including: commercial stock suspensions of 4000 NTU formazin, commercial suspensions of microspheres of styrene-divinylbenzene copolymer, [†](18) and items supplied by instrument manufacturers,

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such as sealed sample cells filled with latex suspension or with metal oxide particles in a polymer gel. The U.S. Environmental Protection Agency¹ designates user-prepared formazin, commercial stock formazin suspensions, and commercial styrene-divinylbenzene suspensions as “primary standards,” and reserves the term “secondary standard” for the sealed standards mentioned above.

Secondary standards made with suspensions of microspheres of styrene-divinylbenzene copolymer typically are as stable as concentrated formazin and are much more stable than diluted formazin. These suspensions can be instrument-specific; therefore, use only suspensions formulated for the type of nephelometer being used. Secondary standards provided by the instrument manufacturer (sometimes called “permanent” standards) may be necessary to standardize some instruments before each reading and in other instruments only as a calibration check to determine when calibration with the primary standard is necessary.

All secondary standards, even so-called “permanent” standards, change with time. Replace them when their age exceeds the shelf life. Deterioration can be detected by measuring the turbidity of the standard after calibrating the instrument with a fresh formazin or microsphere suspension. If there is any doubt about the integrity or turbidity value of any secondary standard, check instrument calibration first with another secondary standard and then, if necessary, with user-prepared formazin. Most secondary standards have been carefully prepared by their manufacturer and should, if properly used, give good agreement with formazin. Prepare formazin primary standard only as a last resort. Proper application of secondary standards is specific for each make and model of nephelometer. Not all secondary standards have to be discarded when comparison with a primary standard shows that their turbidity value has changed. In some cases, the secondary standard should be simply relabeled with the new turbidity value. Always follow the manufacturer’s directions.

4. Procedure

a. General measurement techniques: Proper measurement techniques are important in minimizing the effects of instrument variables as well as stray light and air bubbles. Regardless of the instrument used, the measurement will be more accurate, precise, and repeatable if close attention is paid to proper measurement techniques.

Measure turbidity immediately to prevent temperature changes and particle flocculation and sedimentation from changing sample characteristics. If flocculation is apparent, break up aggregates by agitation. Avoid dilution whenever possible. Particles suspended in the original sample may dissolve or otherwise change characteristics when the temperature changes or when the sample is diluted.

Remove air or other entrained gases in the sample before measurement. Preferably degas even if no bubbles are visible. Degas by applying a partial vacuum, adding a nonfoaming-type surfactant, using an ultrasonic bath, or applying heat. In some cases, two or more of these techniques may be combined for more effective bubble removal. For example, it may be necessary to combine addition of a surfactant with use of an ultrasonic bath for some severe

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conditions. Any of these techniques, if misapplied, can alter sample turbidity; *use with care*. If degassing cannot be applied, bubble formation will be minimized if the samples are maintained at the temperature and pressure of the water before sampling.

Do not remove air bubbles by letting sample stand for a period of time because during standing, turbidity-causing particulates may settle and sample temperature may change. Both of these conditions alter sample turbidity, resulting in a nonrepresentative measurement.

Condensation may occur on the outside surface of a sample cell when a cold sample is being measured in a warm, humid environment. This interferes with turbidity measurement. Remove all moisture from the outside of the sample cell before placing the cell in the instrument. If fogging recurs, let sample warm slightly by letting it stand at room temperature or by partially immersing it in a warm water bath for a short time. Make sure samples are again well mixed.

b. Nephelometer calibration: Follow the manufacturer's operating instructions. Run at least one standard in each instrument range to be used. Make certain the nephelometer gives stable readings in all sensitivity ranges used. Follow techniques outlined in ¶s 2*b* and 4*a* for care and handling of sample cells, degassing, and dealing with condensation.

c. Measurement of turbidity: Gently agitate sample. Wait until air bubbles disappear and pour sample into cell. When possible, pour well-mixed sample into cell and immerse it in an ultrasonic bath for 1 to 2 s or apply vacuum degassing, causing complete bubble release. Read turbidity directly from instrument display.

d. Calibration of continuous turbidity monitors: Calibrate continuous turbidity monitors for low turbidities by determining turbidity of the water flowing out of them, using a laboratory-model nephelometer, or calibrate the instruments according to manufacturer's instructions with formazin primary standard or appropriate secondary standard.

5. Interpretation of Results

Report turbidity readings as follows:

Turbidity Range <i>NTU</i>	Report to the Nearest <i>NTU</i>
0–1.0	0.05
1–10	0.1
10–40	1
40–100	5
100–400	10
400–1000	50
>1000	100

When comparing water treatment efficiencies, do not estimate turbidity more closely than specified above. Uncertainties and discrepancies in turbidity measurements make it unlikely that

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results can be duplicated to greater precision than specified.

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2150 ODOR*#(19)

2150 A. Introduction

1. Discussion

Odor, like taste, depends on contact of a stimulating substance with the appropriate human receptor cell. The stimuli are chemical in nature and the term “chemical senses” often is applied to odor and taste. Water is a neutral medium, always present on or at the receptors that perceive sensory response. In its pure form, water is odor-free. Man and other animals can avoid many potentially toxic foods and waters because of adverse sensory response. These senses often provide the first warning of potential hazards in the environment.

Odor is recognized¹ as a quality factor affecting acceptability of drinking water (and foods prepared with it), tainting of fish and other aquatic organisms, and esthetics of recreational waters. Most organic and some inorganic chemicals contribute taste or odor. These chemicals

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may originate from municipal and industrial waste discharges, from natural sources such as decomposition of vegetable matter, or from associated microbial activity, and from disinfectants or their products.

The potential for impairment of the sensory quality of water has increased as a result of expansion in the variety and quantity of waste materials, demand for water disposal of captured air pollutants, and increased reuse of available water supplies by a growing population. Domestic consumers and process industries such as food, beverage, and pharmaceutical manufacturers require water essentially free of tastes and odors.

Some substances, such as certain inorganic salts, produce taste without odor and are evaluated by taste testing (Section 2160). Many other sensations ascribed to the sense of taste actually are odors, even though the sensation is not noticed until the material is taken into the mouth. Because some odorous materials are detectable when present in only a few nanograms per liter, it is usually impractical and often impossible to isolate and identify the odor-producing chemical. The human nose is the practical odor-testing device used in this method. Odor tests are performed to provide qualitative descriptions and approximate quantitative measurements of odor intensity. The method for intensity measurement presented here is the *threshold odor* test, based on a method of limits.² This procedure, while not universally preferred,³ has definite strengths.⁴

Sensory tests are useful as a check on the quality of raw and finished water and for control of odor through the treatment process.^{2,3,5} They can assess the effectiveness of different treatments and provide a means of tracing the source of contamination.

Section 6040B provides an analytical procedure for quantifying several organic odor-producing compounds including geosmin and methylisoborneol.

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2150 B. Threshold Odor Test

1. General Discussion

a. Principle: Determine the threshold odor by diluting a sample with odor-free water until the least definitely perceptible odor is achieved. There is no absolute threshold odor concentration, because of inherent variation in individual olfactory capability. A given person varies in sensitivity over time. Day-to-day and within-day differences occur. Furthermore, responses vary as a result of the characteristic, as well as concentration, of odorant. The number of persons selected to measure threshold odor will depend on the objective of the tests, economics, and available personnel. Larger-sized panels are needed for sensory testing when the results must represent the population as a whole or when great precision is desired. Under such circumstances, panels of no fewer than five persons, and preferably ten or more, are recommended.¹ Measurement of threshold levels by one person is often a necessity at water treatment plants. Interpretation of the single tester result requires knowledge of the relative acuity of that person. Some investigators have used specific odorants, such as *m*-cresol or *n*-butanol, to calibrate a tester's response.² Others have used the University of Pennsylvania Smell Identification Test to assess an individual's ability to identify odors correctly.³

b. Application: This threshold method is applicable to samples ranging from nearly odorless natural waters to industrial wastes with threshold numbers in the thousands. There are no intrinsic difficulties with the highly odorous samples because they are reduced in concentration proportionately before being presented to the test observers.

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c. Qualitative descriptions: A fully acceptable system for characterizing odor has not been developed despite efforts over more than a century. Nevertheless, Section 2170 (Flavor Profile Analysis) specifies a set of 23 odor reference standards that may be used if qualitative descriptions are important. These descriptors can be used with the Threshold Odor Test to standardize methods for sensory analysis.

d. Sampling and storage: Collect samples for odor testing in glass bottles with glass or TFE-lined closures. Complete tests as soon as possible after sample collection. If storage is necessary, collect at least 500 mL of sample in a bottle filled to the top; refrigerate, making sure that no extraneous odors can be drawn into the sample as it cools. Do not use plastic containers.

e. Dechlorination: Most tap waters and some wastewaters are chlorinated. Often it is desirable to determine the odor of the chlorinated sample as well as that of the same sample after dechlorination. Dechlorinate with thiosulfate in exact stoichiometric quantity as described under Nitrogen (Ammonia), Section 4500-NH₃.

f. Temperature: Threshold odor values vary with temperature. For most tap waters and raw water sources, a sample temperature of 60°C will permit detection of odors that otherwise might be missed; 60°C is the standard temperature for hot threshold odor tests. For some purposes—because the odor is too fleeting or there is excessive heat sensation—the hot odor test may not be applicable; where experience shows that a lower temperature is needed, use a standard test temperature of 40°C. For special purposes, other temperatures may be used. *Report temperature at which observations are made.*

2. Apparatus

To assure reliable threshold measurements, use odor-free glassware. Clean glassware shortly before use with nonodorous soap and acid cleaning solution and rinse with odor-free water. Reserve this glassware exclusively for threshold testing. Do not use rubber, cork, or plastic stoppers. Do not use narrow-mouth vessels.

a. Sample bottles, glass-stoppered or with TFE-lined closures.

b. Constant-temperature bath: A water bath or electric hot plate capable of temperature control of $\pm 1^\circ\text{C}$ for odor tests at elevated temperatures. The bath must not contribute any odor to the odor flasks.

c. Odor flasks: Glass-stoppered, 500-mL (ST 32) erlenmeyer flasks, to hold sample dilutions during testing.

d. Pipets:

1) *Transfer and volumetric pipets or graduated cylinders:* 200-, 100-, 50-, and 25-mL.

2) *Measuring pipets:* 10-mL, graduated in tenths.

e. Thermometer: Zero to 110°C, chemical or metal-stem dial type.

3. Odor-Free Water

a. Sources: Prepare odor-free water by passing distilled, deionized, or tap water through

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activated carbon. If product water is not odor-free, rebuild or purify the system. In all cases verify quality of product water daily.

b. Odor-free-water generator (Figure 2150:1):*#(20) Make the PVC generator from a 2-ft length of 4-in. PVC pipe approved for use for drinking water purposes (e.g., Schedule 80, or National Water Council-approved in U.K.). Thread pipe end to accept threaded caps. Have a small threaded nipple in the cap center for water inlet or outlet. To retain the activated carbon, place coarse glass wool in top and bottom of generator. Regulate water flow to generator by a needle valve and a pressure regulator to provide the minimum pressure for the desired flow. Use activated carbon of approximately 12 to 40 mesh grain size.†#(21)

c. Generator operation: Pass tap or purified water through odor-free-water generator at rate of 100 mL/min. When generator is started, flush to remove carbon fines and discard product, or pre-rinse carbon.

Check quality of water obtained from the odor-free-water generator daily at 40 and 60°C before use. The life of the carbon will vary with the condition and amount of water filtered. Subtle odors of biological origin often are found if moist carbon filters stand idle between test periods. Detection of odor in the water coming through the carbon indicates that a change of carbon is needed.

4. Procedure

a. Precautions: Carefully select by preliminary tests the persons to make taste or odor tests. Although extreme sensitivity is not required, exclude insensitive persons and concentrate on observers who have a sincere interest in the test. Avoid extraneous odor stimuli such as those caused by smoking and eating before the test or those contributed by scented soaps, perfumes, and shaving lotions. Insure that the tester is free from colds or allergies that affect odor response. Limit frequency of tests to a number below the fatigue level by frequent rests in an odor-free atmosphere. Keep room in which tests are conducted free from distractions, drafts, and odor.² If necessary, set aside a special odor-free room ventilated by air that is filtered through activated carbon and maintained at a constant comfortable temperature and humidity.⁴

For precise work use a panel of five or more testers. Do not allow persons making odor measurements to prepare samples or to know dilution concentrations being evaluated. Familiarize testers with the procedure before they participate in a panel test. Present most dilute sample first to avoid tiring the senses with the concentrated sample. Keep temperature of samples during testing within 1°C of the specified temperature.

Because many raw and waste waters are colored or have decided turbidity that may bias results, use opaque or darkly colored odor flasks, such as red actinic erlenmeyer flasks.

b. Characterization: As part of the threshold test or as a separate test, direct each observer to describe the characteristic sample odor using odor reference standards (see Section 2170). Compile the consensus that may appear among testers and that affords a clue to the origin of the odorous component. The value of the characterization test increases as observers become more experienced with a particular category of odor, e.g., earthy, musty, chlorine.

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c. *Threshold measurement:* (22) The “threshold odor number,” designated by the abbreviation TON, is the greatest dilution of sample with odor-free water yielding a definitely perceptible odor. Bring total volume of sample and odor-free water to 200 mL in each test. Follow dilutions and record corresponding TON presented in Table 2150:I. These numbers have been computed thus:

$$\text{TON} = \frac{A + B}{A}$$

where:

A = mL sample and

B = mL odor-free water.

1) Place proper volume of odor-free water in the flask first, add sample to water (avoiding contact of pipet or sample with lip or neck of flask), mix by swirling, and proceed as follows:

Determine approximate range of the threshold number by adding 200 mL, 50 mL, 12 mL, and 2.8 mL sample to separate 500-mL glass-stoppered erlenmeyer flasks containing odor-free water to make a total volume of 200 mL. Use a separate flask containing only odor-free water as reference for comparison. Heat dilutions and reference to desired test temperature.

2) Shake flask containing odor-free water, remove stopper, and sniff vapors. Test sample containing least amount of odor-bearing water in the same way. If odor can be detected in this dilution, prepare more dilute samples as described in ¶ 5) below. If odor cannot be detected in first dilution, repeat above procedure using sample containing next higher concentration of odor-bearing water, and continue this process until odor is detected clearly.

3) Based on results obtained in the preliminary test, prepare a set of dilutions using Table 2150:II as a guide. Prepare the five dilutions shown on the appropriate line and the three next most concentrated on the next line in Table 2150:II. For example, if odor was first noted in the flask containing 50 mL sample in the preliminary test, prepare flasks containing 50, 35, 25, 17, 12, 8.3, 5.7, and 4.0 mL sample, each diluted to 200 mL with odor-free water. This array is necessary to challenge the range of sensitivities of the entire panel of testers.

Insert two or more blanks in the series near the expected threshold, but avoid any repeated pattern. Do not let tester know which dilutions are odorous and which are blanks. Instruct tester to smell each flask in sequence, beginning with the least concentrated sample, until odor is detected with certainty.

4) Record observations by indicating whether odor is noted in each test flask. For example:

mL Sample Diluted to 200 mL	12	0	17	25	0	35	50

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mL Sample Diluted to 200 mL	12	0	17	25	0	35	50
Response	–	–	–	+	–	+	–

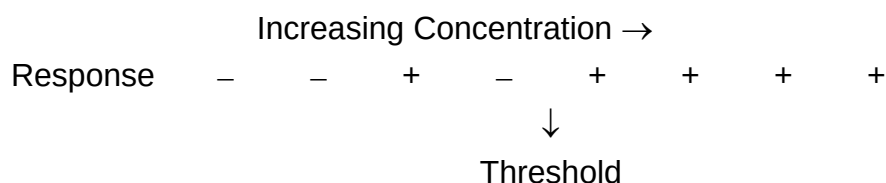
5) If the sample being tested requires more dilution than is provided by Table 2150:II, prepare an intermediate dilution consisting of 20 mL sample diluted to 200 L with odor-free water. Use this dilution for the threshold determination. Multiply TON obtained by 10 to correct for the intermediate dilution. In rare cases more than one tenfold intermediate dilution step may be required.

5. Calculation

The threshold odor number is the dilution ratio at which odor is just detectable. In the example above, 4c4), the first detectable odor occurred when 25 mL sample was diluted to 200 mL. Thus the threshold is 200 divided by 25, or 8. Table 2150:I lists the threshold numbers corresponding to common dilutions.

The smallest TON that can be observed is 1, as in the case where the odor flask contains 200 mL undiluted sample. If no odor is detected at this concentration, report “No odor observed” instead of a threshold number. (In special applications, fractional threshold numbers have been calculated.⁶⁾

Anomalous responses sometimes occur; a low concentration may be called positive and a higher concentration in the series may be called negative. In such a case, designate the threshold as the point after which no further anomalies occur. For instance:



where:

- signifies negative response and
- + signifies positive response.

Occasionally a flask contains residual odor or is contaminated inadvertently. For precise testing repeat entire threshold odor test to determine if the last flask marked “–” was actually a mislabelled blank of odor-free water or if the previous “+” was a contaminated sample.

Use appropriate statistical methods to calculate the most probable average threshold from large numbers of panel results. For most purposes, express the threshold of a group as the

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geometric mean of individual thresholds.

6. Interpretation of Results

A threshold number is not a precise value. In the case of the single observer it represents a judgment at the time of testing. Panel results are more meaningful because individual differences have less influence on the result. One or two observers can develop useful data if comparison with larger panels has been made to check their sensitivity. Do not make comparisons of data from time to time or place to place unless all test conditions have been standardized carefully and there is some basis for comparison of observed intensities.

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2160 TASTE*#(23)

2160 A. Introduction

1. General Discussion

Taste refers only to gustatory sensations called bitter, salty, sour, and sweet that result from chemical stimulation of sensory nerve endings located in the papillae of the tongue and soft palate. Flavor refers to a complex of gustatory, olfactory, and trigeminal sensations resulting from chemical stimulation of sensory nerve endings located in the tongue, nasal cavity, and oral cavity.¹ Water samples taken into the mouth for sensory analysis always produce a flavor, although taste, odor, or mouth-feel may predominate, depending on the chemical substances present. Methods for sensory analysis presented herein require that the sample be taken into the mouth, that is, be tasted, but technically the sensory analysis requires evaluation of the complex sensation called flavor. As used here, taste refers to a method of sensory analysis in which

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samples are taken into the mouth but the resultant evaluations pertain to flavor.

Three methods have been developed for the sensory evaluation of water samples taken into the mouth: the flavor threshold test (FTT), the flavor rating assessment (FRA), and the flavor profile analysis (FPA) (Section 2170). The FTT is the oldest. It has been used extensively and is particularly useful for determining if the overall flavor of a sample of finished water is detectably different from a defined standard.² The FRA is especially valuable for determining if a sample of finished water is acceptable for daily consumption,³ and the FPA is most useful for identifying and characterizing individual flavors in a water sample.⁴

Make flavor tests only on samples known to be safe for ingestion. Do not use samples that may be contaminated with bacteria, viruses, parasites, or hazardous chemicals, that contain dechlorinating agents such as sodium arsenite or that are derived from an unesthetic source. Do not make flavor tests on wastewaters or similar untreated effluents. Observe all sanitary and esthetic precautions with regard to apparatus and containers contacting the sample. Properly clean and sterilize containers before using them. Conduct analyses in a laboratory free from interfering background odors and if possible provide non-odorous carbon-filtered air at constant temperature and humidity. Use the procedure described in Section 2150 with respect to taste- and odor-free water to prepare dilution water and reference samples.

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2160 B. Flavor Threshold Test (FTT)

1. General Discussion

Use the FTT to measure detectable flavor quantitatively. More precisely, use the method to compare the sample flavor objectively with that of specified reference water used as diluent.

The flavor threshold number (FTN) is the greatest dilution of sample with reference water yielding a definitely perceptible difference. The FTN is computed as follows:

$$\text{FTN} = \frac{A + B}{A}$$

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where:

A = sample volume, mL, and

B = reference water (diluent) volume, mL.

Table 2160:I gives the FTNs corresponding to various dilutions.

2. Procedure

a. Panel selection: Carefully select by preliminary trials interested persons to make flavor tests. Exclude insensitive persons and insure that the testers are free from colds or allergies. Familiarize testers with the procedure before they participate in a panel test, but do not let them prepare samples or know dilution concentrations being evaluated. For precise work use a panel of five or more testers.

b. Taste characterization: Have each observer describe the characteristic sample flavor of the most concentrated sample. Compile the consensus that may appear among testers. The value of characterization increases as observers become more experienced with a particular flavor category such as chlorophenolic, grassy, or musty.

c. Preliminary test: To determine approximate range of the FTN, add 200-, 50-, 12-, and 4-mL sample portions to volumes of reference water (see Section 2150) designated in Table 2160:I in separate 300-mL glass beakers to make a total of 200 mL in each beaker, and mix gently with clean stirrer. Use separate beaker containing only reference water for comparison. Keep sample temperature during testing within 1°C of specified temperature. Present samples to each taster in a uniform manner, with the reference water presented first, followed by the most dilute sample. If a flavor can be detected in this dilution, prepare an intermediate sample by diluting 20 mL sample to 200 mL with reference water. Use this dilution for threshold determination and multiply FTN obtained by 10 to correct for intermediate dilution. In rare cases a higher intermediate dilution may be required.

If no flavor is detected in the most dilute sample, repeat using the next concentration. Continue this process until flavor is detected clearly.

d. FTN determination: Based on results obtained in the preliminary test, prepare a set of dilutions using Table 2160:II as a guide. Prepare the seven dilutions shown on the appropriate line. This array is necessary to challenge the range of sensitivities of the entire panel of testers. If the sample being tested requires more dilution than is provided by Table 2160:II, make intermediate dilutions as directed in *c* above.

Use a clean 50-mL beaker filled to the 25-mL level or use an ordinary restaurant-style drinking glass for each dilution and reference sample. Do not use glassware used in sensory testing for other analyses. Between tests, sanitize containers in an automatic dishwasher supplied with water at not less than 60°C.

Maintain samples at $15 \pm 1^\circ\text{C}$. However, if temperature of water in the distribution system is higher than 15°C, select an appropriate temperature. Specify temperature in reporting results.

Present series of samples to each tester in order of increasing concentration. Pair each

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sample with a known reference. Have tester taste sample by taking into the mouth whatever volume is comfortable, moving sample throughout the mouth, holding it for several seconds, and discharging it without swallowing. Have tester compare sample with reference and record whether a flavor or aftertaste is detectable. Insert two or more reference blanks in the series near the expected threshold, but avoid any repeated pattern. Do not let tester know which samples have flavor and which are blanks. Instruct tester to taste each sample in sequence, beginning with the least concentrated sample, until flavor is detected with certainty.

Record observations by indicating whether flavor is noted in each test beaker. For example:

mL Sample Diluted to 200 mL	6	8	12	0	17	25	35	0	50
Response	–	–	–	–	–	+	+	–	+

where:

- signifies negative response and
- + signifies positive response.

3. Calculation

The flavor threshold number is the dilution ratio at which flavor is just detectable. In the example above, the first detectable flavor occurred when 25 mL sample was diluted to 200 mL yielding a threshold number of 8 (Table 2160:I). Reference blanks do not influence calculation of the threshold.

The smallest FTN that can be observed is 1, where the beaker contains 200 mL undiluted sample. If no flavor is detected at this concentration, report “No flavor observed” instead of a threshold number.

Anomalous responses sometimes occur; a low concentration may be called positive and a higher concentration in the series may be called negative. In such cases, designate the threshold as that point after which no further anomalies occur. The following illustrates an approach to an anomalous series (responses to reference blanks are excluded):

	Increasing Concentration →						
Response	–	+	–	+	+	+	+
				↓			
				Threshold			

Calculate mean and standard deviation of all FTNs if the distribution is reasonably symmetrical; otherwise, express the threshold of a group as the median or geometric mean of individual thresholds.

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4. Interpretation of Results

An FTN is not a precise value. In the case of the single observer it represents a judgment at the time of testing. Panel results are more meaningful because individual differences have less influence on the test result. One or two observers can develop useful data if comparison with larger panels has been made to check their sensitivity. Do not make comparisons of data from time to time or place to place unless all test conditions have been standardized carefully and there is some basis for comparison of observed FTNs.

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2160 C. Flavor Rating Assessment (FRA)

1. General Discussion

When the purpose of the test is to estimate acceptability for daily consumption, use the flavor rating assessment described below. This procedure has been used with samples from public sources in laboratory research and consumer surveys to recommend standards governing mineral content in drinking water. Each tester is presented with a list of nine statements about the water ranging on a scale from very favorable to very unfavorable. The tester's task is to select the statement that best expresses his or her opinion. The individual rating is the scale number of the statement selected. The panel rating for a particular sample is an appropriate measure of central tendency of the scale numbers for all testers for that sample.

2. Samples

Sample finished water ready for human consumption or use experimentally treated water if the sanitary requirements given in Section 2160A.1 are met fully. Use taste- and odor-free water as described in Section 2150 and a solution of 2000 mg NaCl/L prepared with taste- and odor-free water as criterion samples.

3. Procedure

a. Panel selection and preparation: Give prospective testers thorough instructions and trial or orientation sessions followed by questions and discussion of procedures. In tasting samples, testers work alone. Select panel members on the basis of performance in these trial sessions. Do not let testers know the composition or source of specific samples.

b. Rating test: A single rating session may be used to evaluate up to 10 samples, including the criterion samples mentioned in ¶ 2 above. Allow at least 30 min rest between repeated rating sessions.

For glassware requirements, see Section 2160B2d.

Present samples at a temperature that the testers will find pleasant for drinking water; maintain this temperature throughout testing. A temperature of 15°C is recommended, but in any case, do not let the test temperature exceed tap water temperatures customary at the time of the test. Specify test temperature in reporting results.

Independently randomize sample order for each tester. Instruct each to complete the following steps: 1) Taste about half the sample by taking water into the mouth, holding it for several seconds, and discharging it without swallowing; 2) Form an initial judgment on the rating scale; 3) Make a second tasting in a similar manner; 4) Make a final rating and record

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result on an appropriate data form; 5) Rinse mouth with reference water; 6) Rest 1 min before repeating Steps 1 through 5 on next sample.

c. *Characterization*: If characterization of flavor also is required, conduct a final rating session wherein each tester is asked to describe the flavor of each sample rated (see Section 2160B.2b).

4. Calculation

Use the following scale for rating. Record ratings as integers ranging from one to nine, with one given the highest quality rating. Calculate mean and standard deviation of all ratings if the distribution is reasonably symmetrical, otherwise express the most typical rating of a group as the median or geometric mean of individual ratings.

Action tendency scale:

- 1) I would be very happy to accept this water as my everyday drinking water.
- 2) I would be happy to accept this water as my everyday drinking water.
- 3) I am sure that I could accept this water as my everyday drinking water.
- 4) I could accept this water as my everyday drinking water.
- 5) Maybe I could accept this water as my everyday drinking water.
- 6) I don't think I could accept this water as my everyday drinking water.
- 7) I could not accept this water as my everyday drinking water.
- 8) I could never drink this water.
- 9) I can't stand this water in my mouth and I could never drink it.

5. Interpretation of Results

Values representing the central tendency and dispersion of quality ratings for a laboratory panel are only estimates of these values for a defined consuming population.

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2170 FLAVOR PROFILE ANALYSIS*#(24)

2170 A. Introduction

1. Discussion

Flavor profile analysis (FPA) is a technique for identifying sample taste(s) and odor(s). For general information on taste see Section 2160; for information on odor see Section 2150.

FPA differs from threshold odor number because the sample is not diluted and each taste or odor attribute is individually characterized and assigned its own intensity rating. The single numerical rating obtained in measuring threshold odor is controlled by the most readily perceived odorant or mixture. Sample dilution may change the odor attribute that is measured.^{1,2} FPA determines the strength or intensity of each perceived taste or odor without dilution or treatment of the sample.

2. References

1. MALLEVIALLE, J. & I.H. SUFFET, eds. 1987. Identification and Treatment of Tastes and Odors in Drinking Water. American Water Works Assoc., Denver, Colo.
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Denver, Colo.

2170 B. Flavor Profile Analysis

1. General Discussion

a. Principle: Flavor profile analysis uses a group of four or five trained panelists to examine the sensory characteristics of samples. Flavor attributes are determined by tasting; odor attributes (aroma) are determined by sniffing the sample. The method allows more than one flavor, odor attribute, or feeling factor (e.g., drying, burning) to be determined per sample and each attribute's strength to be measured.

Panelists must be able to detect and recognize various odors present. Flavor profile analysis requires well-trained panelists and data interpreters. Reproducibility of results depends on the training and experience of the panelists.

Initially, panelists record their perceptions without discussion. Once each individual has made an independent assessment of a sample, the panel discusses its findings and reaches a consensus.¹

b. Interference: Fatigue (adaptation) denotes the decrease in the analyst's sensory acuity with continued exposure to stimulation.¹ Because odor mixtures are more complex than taste mixtures, olfactory adaptation is more serious.² Factors that can induce fatigue include odor intensity, type of odorants (some compounds such as geosmin and chlorine induce more fatigue than others), number of samples tested during a session, and rest interval between samples.¹ The rest interval also is important because it prevents carryover of odors between samples.

Background odors present during analysis affect results. Analyst illness, i.e., cold or allergy, can diminish or otherwise alter perception.

c. Application: Flavor profile analysis has been applied to drinking water sources, finished drinking water, sampling points within the drinking water treatment train, and bottled waters, and for investigating customer complaints.

d. Precautions: Some compounds are health hazards when inhaled or ingested. Do not use this method for industrial wastes or other samples suspected of containing high concentrations of hazardous compounds. Chemically analyze suspect samples to determine whether hazardous chemicals are present before making flavor profile analysis. Do not taste untreated drinking water unless it is certain that no health threat (biological or chemical) exists.

Because flavor profile analysis includes discussion during which panelists reach consensus description, avoid including on the panel a person with a dominating personality.³ The opinions of a senior member or panel leader also may have this undesired effect.³

2. Apparatus⁴⁻⁸

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Reserve apparatus and glassware exclusively for flavor profile analysis. Prepare sample bottles by washing bottle and cap with detergent. Rinse 10 times with hot water. Optionally rinse with HCl (1:1). Rinse with odor-free water (see 2150B.3) at least three times. If there is residual odor, such as chalky, repeat cleaning.

Prepare 500-mL erlenmeyer flasks by either of the two methods described below provided that it imparts no odor to the sample. Make tests with freshly cleaned containers.

1) Without wearing rubber gloves wash flask with soapy water. Additionally, scrub outside of flask with scouring pad to remove body oils. Rinse 10 times with hot water and 3 times with odor-free water. To store flask add 100 to 200 mL odor-free water and stopper. Before use, rinse with 100 mL odor-free water. If there is residual odor, repeat cleaning.

2) Do not handle with rubber gloves. Before use, heat 200 mL odor-free water to boiling; lightly lay stopper over flask opening, permitting water vapor to escape. Discard boiled water and let flask cool to room temperature. If there is residual odor repeat or use cleaning alternative 1). After analyzing a sample, discard sample, rinse 10 times with hot tap water, add 200 mL odor-free water, stopper, and store.

For flasks with persistent odor use commercial cleaning mixture*#(25) or HCl (1:1).

a. *Sample bottles:* Glass, 1-L, with TFE-lined closure.

b. *Containers for odor analysis:*⁴ Select container for odor analysis depending on panelists' preference, temperature of sample, and availability of container.

1) Panel preference⁴⁻⁸—Plastic cups, 7- or 8-ounce disposable, are convenient. Some panels have found that these plastic cups impart a plastic and/or floral aroma to samples. They prefer 500-mL erlenmeyer flasks with ST32 ground-glass stoppers. The panel should determine the acceptable, odor-free, test container.

2) Temperature⁴—If aroma samples are tested at 45°C, do not use plastic. If aroma samples are tested at 25°C, use the same plastic cup and sample for both aroma and flavor analysis.

3) Availability⁴—Containers must be consistently available because the panel responses may change when different containers are used.

c. *Containers for flavor analysis:*⁴ Use either plastic disposable, e.g., polyethylene,†#(26) or glass containers. Do not use wax-lined or paper cups. If using glass containers verify that they contribute no taste to the sample. Wash glass containers as directed above.

d. *Watch glasses.*

e. *Constant-temperature water bath* capable of maintaining a temperature $\pm 1^\circ\text{C}$. The bath must not contribute any odor to the odor flasks or testing room.

f. *Thermometer*, graduated 0 to 110°C, chemical or metal-stem dial type.

g. *Syringes.*

h. *Ice chest.*

i. *Odor-free testing room:*^{3,5} Hold flavor profile analysis sessions in a clean, well-lit quiet,

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aroma-free, and temperature-controlled room. Seat panel members around a common table to facilitate exchange of responses during discussion. Place a blackboard or easel pad in the room so that all panelists are able to see it. Preferably use an easel pad with odor-free markers (such as a wax, china marker).

j. *Refrigerator* capable of maintaining a temperature of 4°C.

3. Reagents

a. *Odor-free water*: See Section 2150B.3.

b. *Crackers*: Use salt- and flavor-free crackers²⁷ to cleanse the palate during taste testing. Before tasting anything, use the crackers and taste-free water to cleanse the palate. Use crackers between samples to reduce carryover of perceptions.

c. *Odor and flavor standards*: Odor and flavor reference standards are being developed for use as a guide for qualitative descriptions (attributes). Aroma reference standards help panelists come to agreement on the description of specific aromas.⁴⁻¹¹ Table 2170:I, Table 2170:II, and Table 2170:III list some of the aroma references in use. New references are being added continually. A taste and odor wheel has been developed from these lists.² For odor identification and training, place several drops of stock solutions (producing moderately strong odors) onto sterile cotton in 25-mL amber-colored vials with TFE-lined caps. Standards can be stored until odor changes.

d. *Taste standards*: Chemicals used for taste standards are sucrose (sweet), citric acid (sour), sodium chloride (salt), and caffeine (bitter). Table 2170:IV lists the chemicals used and their concentrations for basic taste standards at “slight,” “moderate,” and “strong” levels.^{4,5} The taste standards provide reference points for both taste and odor intensity ratings. The panelists compare the intensity of what they are smelling or tasting to the intensity of the standards they have tasted. Make taste standards available during panel training. Because the tastes from all but the sweet standard tend to overpower any subsequent tasting, use only the sweet taste standard during actual sample analysis. If the panel meets as seldom as once a week, make sweet standards available so that panelists can “recalibrate” themselves. Panels that meet more than once a week may not need to “recalibrate.” Make standards fresh each time they are used.⁴

4. Scale

The strength of a taste or odor is judged according to the following scale:

- (odor-free)
- T (threshold)
- 2 (very weak)
- 4 (weak)
- 6
- 8 (moderate)

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12 (strong)

5. Procedure

a. *Sample collection:* Collect sample in cleaned container. When sampling from a tap, remove all screens and aerators, minimize turbulence. Flush tap at least 5 min. Reduce flow rate during sampling. Rinse bottle with sample, then fill it to the top, with no headspace. Chill or refrigerate sample immediately and analyze as soon after collection as possible, preferably within 24 h, but no longer than 48 h.

b. *Sample preparation:*⁴ Pour samples into properly prepared glassware or acceptable disposable containers. Analyze samples at the same temperature. Adjust sample temperatures by placing samples in a water bath 15 min before analysis. Prepare a sample for each panelist. Examine odor samples before flavor samples.

1) Odor analysis—When using 500-mL erlenmeyer flasks for odor analysis, place 200 mL sample in the flask. Make transfer carefully to avoid loss of volatile components. When plastic cups are used, place 60 mL sample in cup and cover it with a watch glass.

2) Taste analysis—Bring sample to 25°C before pouring into containers for tasting. Cover with a watch glass if samples are not tasted immediately. If samples are tested at 25°C for odor, use the same sample for flavor analysis.

c. *Panelists:* Carefully screen and train panelists.⁸

d. *Pre-test considerations:* Notify panelists well in advance of panel session so a substitute can be found if necessary. Panelists who have colds or allergic attacks the day of the panel are unacceptable; they should ask the panel coordinator to find a substitute. Panelists must not smoke or eat for 15 to 30 min before the session. Wearing cologne or perfume or washing hands with scented soap before the session is not permitted.

e. *Sample analysis:* The panel consists of four or five members. If fewer than four panelists are available, store the sample until a full panel can meet.

1) Odor analysis—Heat samples to proper temperature. If erlenmeyer flasks are used, sample temperature is 45°C. If plastic cups are used, sample temperature is 25°C. Give each panelist his/her own sample. If a flask is used, hold it with one hand on the bottom and the other on the stopper. Do not touch flask neck. Gently swirl (do not shake) flask in a circular manner to ensure that volatile compounds are released into the headspace. Bring flask close to nose, remove stopper, and sniff at the flask opening. Record impressions of odor attributes in the order perceived and the assessment of each attribute's intensity. If a cup is used, gently swirl on the table top for a few seconds. Remove watchglass, and keeping hands away from the cup, sniff sample and record perceptions. CAUTION: *Sniff samples only if they are known to be toxicologically safe.* For both the flask and the plastic cup method, smell all samples before going on to the taste test. Do not discuss or interact with other panelists.

2) Taste analysis—When tasting, take sample into the mouth and roll it over the entire

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surface of the tongue. Slurping enhances the odor aspect of flavor. CAUTION: *Taste only samples known to be biologically and toxicologically safe.* Do not discuss or interact with other panelists.

3) Intensity—In both taste and odor analysis, each panelist determines intensity ratings by matching intensity of the flavor or aroma perceived with the defined intensities of the basic taste standards. Initially this may be difficult. Some panels have found it helpful to make basic taste standards available throughout the analysis so that any panelist who wishes to “recalibrate” may do so.

4) Re-examination of samples—First impressions are most important, particularly for intensity. The intensity rating may diminish upon re-examination because of fatigue and volatility of the odorant. However, if an aroma or flavor is difficult for a panelist to describe, the panelist may go back and re-evaluate the sample before recording the results.

5) Individual results—As soon as a sample is tasted or smelled, record individual results. Information recorded includes a description of aromas or flavors perceived, their intensity, and the order in which they were perceived.

6) Rest interval—Sniff odor-free water and rest at least 2 min between samples. Fatigue is a problem¹ that can be dealt with. When samples are expected to cause fatigue, the panel leader can increase the rest interval and limit the number of samples presented. The leader also may try to arrange samples such that samples known to be fatiguing are placed near the end of the sample row. Avoid juxtaposition of such samples. Also, use taste- and odor-free blanks between samples. Five to six samples per session is the maximum.

7) Discussion—When all panelists have had an opportunity to examine the sample, hold a discussion period. Each panelist’s impression of the sample is stated and the panel leader records it to be seen by everyone. By examining the order of appearance, intensity, and description, the panel leader attempts to group responses together, soliciting comments from the panel members as to whether or not they agree. Sometimes panelists detect an aroma that they cannot describe; in the discussion they may see what another panelist called it and decide to agree with that description. With inexperienced panels, several different descriptors may be used for the same aroma. As the panel gains more experience, these differences tend to be reconciled. It is the responsibility of the panel leader to ensure that the panelists are provided with standards that duplicate the sample aromas. Descriptions that fewer than 50% of the panelists use are called “other notes” and are listed separately or are not included in the group results.

8) Recording panel results—Record the following: sample description; time of sampling; identity of panel member; flavor, aroma, and feeling factor descriptions; intensity rating; order of perception; range and average for each descriptor.

6. Calculation and Interpretation¹

In early testing intensity was used to describe the strength of an odor in an ordinal scale from slight to strong.¹² If an odor was recognized, then it had an intensity. The rating scale has been modified to use numbers corresponding to the ordinal categories. Points (other than threshold) on

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the scale can be anchored by the use of taste standards. Thus a certain concentration of a taste standard is defined as a certain point on the rating scale and numerical ratings for nonthreshold intensities are anchored. Threshold ratings are not defined by any standards. Their meaning is different from that of the other ratings and they are difficult to manipulate mathematically.

Calculate averages if at least 50% of the panelists agree on a given description. If a panelist does not give that description, assign an intensity value of zero. For example:

		Intensity									
Description	Panelist						I	II	III	IV	Average
Musty	2	4	0	2	2.0						
Chlorinous	2	4	4	4	3.5						

7. Quality Control¹²

Use odor-free samples and duplicates occasionally. The odor-free sample serves to detect guessing. Duplicates check reproducibility. A standard concentration of an odor reference standard in odor-free water also can be used to check odor recognition and reproducibility of the intensity scale.

Use counterbalancing of samples so that each panelist receives the samples in a different order. This prevents error due to order effects such as fatigue, carryover, and expectation.

8. Precision and Bias

Initial studies of precision and bias have been completed.^{12,13} An odor recognition and reproducibility study by untrained panelists has rated some of the odors of Table 2170:I, Table 2170:II, and Table 2170:III as poor, fair, good, or excellent.¹³

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2310 ACIDITY*#(28)

2310 A. Introduction

Acidity of a water is its quantitative capacity to react with a strong base to a designated pH. The measured value may vary significantly with the end-point pH used in the determination. Acidity is a measure of an aggregate property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known. Strong mineral acids, weak acids such as carbonic and acetic, and hydrolyzing salts such as iron or aluminum sulfates may contribute to the measured acidity according to the method of determination.

Acids contribute to corrosiveness and influence chemical reaction rates, chemical speciation, and biological processes. The measurement also reflects a change in the quality of the source

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water.

2310 B. Titration Method

1. General Discussion

a. Principle: Hydrogen ions present in a sample as a result of dissociation or hydrolysis of solutes react with additions of standard alkali. Acidity thus depends on the end-point pH or indicator used. The construction of a titration curve by recording sample pH after successive small measured additions of titrant permits identification of inflection points and buffering capacity, if any, and allows the acidity to be determined with respect to any pH of interest.

In the titration of a single acidic species, as in the standardization of reagents, the most accurate end point is obtained from the inflection point of a titration curve. The inflection point is the pH at which curvature changes from convex to concave or vice versa.

Because accurate identification of inflection points may be difficult or impossible in buffered or complex mixtures, the titration in such cases is carried to an arbitrary end-point pH based on practical considerations. For routine control titrations or rapid preliminary estimates of acidity, the color change of an indicator may be used for the end point. Samples of industrial wastes, acid mine drainage, or other solutions that contain appreciable amounts of hydrolyzable metal ions such as iron, aluminum, or manganese are treated with hydrogen peroxide to ensure oxidation of any reduced forms of polyvalent cations, and boiled to hasten hydrolysis. Acidity results may be highly variable if this procedure is not followed exactly.

b. End points: Ideally the end point of the acidity titration should correspond to the stoichiometric equivalence point for neutralization of acids present. The pH at the equivalence point will depend on the sample, the choice among multiple inflection points, and the intended use of the data.

Dissolved carbon dioxide (CO₂) usually is the major acidic component of unpolluted surface waters; handle samples from such sources carefully to minimize the loss of dissolved gases. In a sample containing only carbon dioxide-bicarbonates-carbonates, titration to pH 8.3 at 25°C corresponds to stoichiometric neutralization of carbonic acid to bicarbonate. Because the color change of phenolphthalein indicator is close to pH 8.3, this value generally is accepted as a standard end point for titration of total acidity, including CO₂ and most weak acids. Metacresol purple also has an end point at pH 8.3 and gives a sharper color change.

For more complex mixtures or buffered solutions selection of an inflection point may be subjective. Consequently, use fixed end points of pH 3.7 and pH 8.3 for standard acidity determinations via a potentiometric titration in wastewaters and natural waters where the simple carbonate equilibria discussed above cannot be assumed. Bromphenol blue has a sharp color change at its end point of 3.7. The resulting titrations are identified, traditionally, as “methyl orange acidity” (pH 3.7) and “phenolphthalein” or total acidity (pH 8.3) regardless of the actual method of measurement.

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c. Interferences: Dissolved gases contributing to acidity or alkalinity, such as CO_2 , hydrogen sulfide, or ammonia, may be lost or gained during sampling, storage, or titration. Minimize such effects by titrating to the end point promptly after opening sample container, avoiding vigorous shaking or mixing, protecting sample from the atmosphere during titration, and letting sample become no warmer than it was at collection.

In the potentiometric titration, oily matter, suspended solids, precipitates, or other waste matter may coat the glass electrode and cause a sluggish response. Difficulty from this source is likely to be revealed in an erratic titration curve. Do *not* remove interferences from sample because they may contribute to its acidity. Briefly pause between titrant additions to let electrode come to equilibrium or clean the electrodes occasionally.

In samples containing oxidizable or hydrolyzable ions such as ferrous or ferric iron, aluminum, and manganese, the reaction rates at room temperature may be slow enough to cause drifting end points.

Do not use indicator titrations with colored or turbid samples that may obscure the color change at the end point. Residual free available chlorine in the sample may bleach the indicator. Eliminate this source of interference by adding 1 drop of 0.1M sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$).

d. Selection of procedure: Determine sample acidity from the volume of standard alkali required to titrate a portion to a pH of 8.3 (phenolphthalein acidity) or pH 3.7 (methyl orange acidity of wastewaters and grossly polluted waters). Titrate at room temperature using a properly calibrated pH meter, electrically operated titrator, or color indicators.

Use the hot peroxide procedure (§ 4a) to pretreat samples known or suspected to contain hydrolyzable metal ions or reduced forms of polyvalent cation, such as iron pickle liquors, acid mine drainage, and other industrial wastes.

Color indicators may be used for routine and control titrations in the absence of interfering color and turbidity and for preliminary titrations to select sample size and strength of titrant (§ 4b).

e. Sample size: The range of acidities found in wastewaters is so large that a single sample size and normality of base used as titrant cannot be specified. Use a sufficiently large volume of titrant (20 mL or more from a 50-mL buret) to obtain relatively good volumetric precision while keeping sample volume sufficiently small to permit sharp end points. For samples having acidities less than about 1000 mg as calcium carbonate (CaCO_3)/L, select a volume with less than 50 mg CaCO_3 equivalent acidity and titrate with 0.02N sodium hydroxide (NaOH). For acidities greater than about 1000 mg as CaCO_3 /L, use a portion containing acidity equivalent to less than 250 mg CaCO_3 and titrate with 0.1N NaOH. If necessary, make a preliminary titration to determine optimum sample size and/or normality of titrant.

f. Sampling and storage: Collect samples in polyethylene or borosilicate glass bottles and store at a low temperature. Fill bottles completely and cap tightly. Because waste samples may be subject to microbial action and to loss or gain of CO_2 or other gases when exposed to air,

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analyze samples without delay, preferably within 1 d. If biological activity is suspected analyze within 6 h. Avoid sample agitation and prolonged exposure to air.

2. Apparatus

a. Electrometric titrator: Use any commercial pH meter or electrically operated titrator that uses a glass electrode and can be read to 0.05 pH unit. Standardize and calibrate according to the manufacturer's instructions. Pay special attention to temperature compensation and electrode care. If automatic temperature compensation is not provided, titrate at $25 \pm 5^\circ\text{C}$.

b. Titration vessel: The size and form will depend on the electrodes and the sample size. Keep the free space above the sample as small as practicable, but allow room for titrant and full immersion of the indicating portions of electrodes. For conventional-sized electrodes, use a 200-mL, tall-form Berzelius beaker without a spout. Fit beaker with a stopper having three holes, to accommodate the two electrodes and the buret. With a miniature combination glass-reference electrode use a 125-mL or 250-mL erlenmeyer flask with a two-hole stopper.

c. Magnetic stirrer.

d. Pipets, volumetric.

e. Flasks, volumetric, 1000-, 200-, 100-mL.

f. Burets, borosilicate glass, 50-, 25-, 10-mL.

g. Polyolefin bottle, 1-L.

3. Reagents

a. Carbon dioxide-free water: Prepare all stock and standard solutions and dilution water for the standardization procedure with distilled or deionized water that has been freshly boiled for 15 min and cooled to room temperature. The final pH of the water should be ≥ 6.0 and its conductivity should be $<2 \mu\text{mhos/cm}$.

b. Potassium hydrogen phthalate solution, approximately 0.05N: Crush 15 to 20 g primary standard $\text{KHC}_8\text{H}_4\text{O}_4$ to about 100 mesh and dry at 120°C for 2 h. Cool in a desiccator. Weigh $10.0 \pm 0.5 \text{ g}$ (to the nearest mg), transfer to a 1-L volumetric flask, and dilute to 1000 mL.

c. Standard sodium hydroxide titrant, 0.1N: Prepare solution approximately 0.1N as indicated under Preparation of Desk Reagents (see inside front cover). Standardize by titrating 40.00 mL $\text{KHC}_8\text{H}_4\text{O}_4$ solution (§ 3b), using a 25-mL buret. Titrate to the inflection point (§ 1a), which should be close to pH 8.7. Calculate normality of NaOH:

$$\text{Normality} = \frac{A \times B}{204.2 \times C}$$

where:

A = g $\text{KHC}_8\text{H}_4\text{O}_4$ weighed into 1-L flask,

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B = mL $\text{KHC}_8\text{H}_4\text{O}_4$ solution taken for titration, and

C = mL NaOH solution used.

Use the measured normality in further calculations or adjust to 0.1000N; 1 mL = 5.00 mg CaCO_3 .

d. Standard sodium hydroxide titrant, 0.02N: Dilute 200 mL 0.1N NaOH to 1000 mL and store in a polyolefin bottle protected from atmospheric CO_2 by a soda lime tube or tight cap. Standardize against $\text{KHC}_8\text{H}_4\text{O}_4$ as directed in ¶ 3c, using 15.00 mL $\text{KHC}_8\text{H}_4\text{O}_4$ solution and a 50-mL buret. Calculate normality as above (¶ 3c); 1 mL = 1.00 mg CaCO_3 .

e. Hydrogen peroxide, H_2O_2 , 30%.

f. Bromphenol blue indicator solution, pH 3.7 indicator: Dissolve 100 mg bromphenol blue, sodium salt, in 100 mL water.

g. Metacresol purple indicator solution, pH 8.3 indicator: Dissolve 100 mg metacresol purple in 100 mL water.

h. Phenolphthalein indicator solution, alcoholic, pH 8.3 indicator.

i. Sodium thiosulfate, 0.1M: Dissolve 25 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ and dilute to 1000 mL with distilled water.

4. Procedure

If sample is free from hydrolyzable metal ions and reduced forms of polyvalent cations, proceed with analysis according to *b*, *c*, or *d*. If sample is known or suspected to contain such substances, pretreat according to *a*.

a. Hot peroxide treatment: Pipet a suitable sample (see ¶ 1e) into titration flasks. Measure pH. If pH is above 4.0 add 5-mL increments of 0.02N sulfuric acid (H_2SO_4) (Section 2320B.3c) to reduce pH to 4 or less. Remove electrodes. Add 5 drops 30% H_2O_2 and boil for 2 to 5 min. Cool to room temperature and titrate with standard alkali to pH 8.3 according to the procedure of 4d.

b. Color change: Select sample size and normality of titrant according to criteria of ¶ 1e. Adjust sample to room temperature, if necessary, and with a pipet discharge sample into an erlenmeyer flask, while keeping pipet tip near flask bottom. If free residual chlorine is present add 0.05 mL (1 drop) 0.1M $\text{Na}_2\text{S}_2\text{O}_3$ solution, or destroy with ultraviolet radiation. Add 0.2 mL (5 drops) indicator solution and titrate over a white surface to a persistent color change characteristic of the equivalence point. Commercial indicator solutions or solids designated for the appropriate pH range (3.7 or 8.3) may be used. Check color at end point by adding the same concentration of indicator used with sample to a buffer solution at the designated pH.

c. Potentiometric titration curve:

1) Rinse electrodes and titration vessel with distilled water and drain. Select sample size and

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normality of titrant according to the criteria of ¶ 1e. Adjust sample to room temperature, if necessary, and with a pipet discharge sample while keeping pipet tip near the titration vessel bottom.

2) Measure sample pH. Add standard alkali in increments of 0.5 mL or less, such that a change of less than 0.2 pH units occurs per increment. After each addition, mix thoroughly but gently with a magnetic stirrer. Avoid splashing. Record pH when a constant reading is obtained. Continue adding titrant and measure pH until pH 9 is reached. Construct the titration curve by plotting observed pH values versus cumulative milliliters titrant added. A smooth curve showing one or more inflections should be obtained. A ragged or erratic curve may indicate that equilibrium was not reached between successive alkali additions. Determine acidity relative to a particular pH from the curve.

d. Potentiometric titration to pH 3.7 or 8.3: Prepare sample and titration assembly as specified in ¶ 4c1). Titrate to preselected end-point pH (¶ 1d) without recording intermediate pH values. As the end point is approached make smaller additions of alkali and be sure that pH equilibrium is reached before making the next addition.

5. Calculation

$$\text{Acidity, as mg CaCO}_3/\text{L} = \frac{[(A \times B) - (C \times D)] \times 50\,000}{\text{mL sample}}$$

where:

- A = mL NaOH titrant used,
- B = normality of NaOH,
- C = mL H₂SO₄ used (¶ 4a), and
- D = normality of H₂SO₄.

Report pH of the end point used, as follows: “The acidity to pH _____ = _____ mg CaCO₃/L.” If a negative value is obtained, report the value as negative. The absolute value of this negative value should be equivalent to the net alkalinity.

6. Precision and Bias

No general statement can be made about precision because of the great variation in sample characteristics. The precision of the titration is likely to be much greater than the uncertainties involved in sampling and sample handling before analysis.

Forty analysts in 17 laboratories analyzed synthetic water samples containing increments of bicarbonate equivalent to 20 mg CaCO₃/L. Titration according to the procedure of ¶ 4d gave a standard deviation of 1.8 mg CaCO₃/L, with negligible bias. Five laboratories analyzed two

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samples containing sulfuric, acetic, and formic acids and aluminum chloride by the procedures of ¶s 4b and 4d. The mean acidity of one sample (to pH 3.7) was 487 mg CaCO₃/L, with a standard deviation of 11 mg/L. The bromphenol blue titration of the same sample was 90 mg/L greater, with a standard deviation of 110 mg/L. The other sample had a potentiometric titration of 547 mg/L, with a standard deviation of 54 mg/L, while the corresponding indicator result was 85 mg/L greater, with a standard deviation of 56 mg/L. The major difference between the samples was the substitution of ferric ammonium citrate, in the second sample, for part of the aluminum chloride.

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2320 ALKALINITY*(29)

2320 A. Introduction

1. Discussion

Alkalinity of a water is its acid-neutralizing capacity. It is the sum of all the titratable bases. The measured value may vary significantly with the end-point pH used. Alkalinity is a measure of an aggregate property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known.

Alkalinity is significant in many uses and treatments of natural waters and wastewaters. Because the alkalinity of many surface waters is primarily a function of carbonate, bicarbonate, and hydroxide content, it is taken as an indication of the concentration of these constituents. The measured values also may include contributions from borates, phosphates, silicates, or other bases if these are present. Alkalinity in excess of alkaline earth metal concentrations is significant in determining the suitability of a water for irrigation. Alkalinity measurements are used in the interpretation and control of water and wastewater treatment processes. Raw domestic wastewater has an alkalinity less than, or only slightly greater than, that of the water supply. Properly operating anaerobic digesters typically have supernatant alkalinities in the range of 2000 to 4000 mg calcium carbonate (CaCO₃)/L.¹

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2320 B. Titration Method

1. General Discussion

a. Principle: Hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with additions of standard acid. Alkalinity thus depends on the end-point pH used. For methods of determining inflection points from titration curves and the rationale for titrating to fixed pH end points, see Section 2310B.1*a*.

For samples of low alkalinity (less than 20 mg CaCO₃/L) use an extrapolation technique based on the near proportionality of concentration of hydrogen ions to excess of titrant beyond the equivalence point. The amount of standard acid required to reduce pH exactly 0.30 pH unit is measured carefully. Because this change in pH corresponds to an exact doubling of the hydrogen ion concentration, a simple extrapolation can be made to the equivalence point.^{1,2}

b. End points: When alkalinity is due entirely to carbonate or bicarbonate content, the pH at the equivalence point of the titration is determined by the concentration of carbon dioxide (CO₂) at that stage. CO₂ concentration depends, in turn, on the total carbonate species originally present and any losses that may have occurred during titration. The pH values in Table 2320:I are suggested as the equivalence points for the corresponding alkalinity concentrations as milligrams CaCO₃ per liter. “Phenolphthalein alkalinity” is the term traditionally used for the quantity measured by titration to pH 8.3 irrespective of the colored indicator, if any, used in the determination. Phenolphthalein or metacresol purple may be used for alkalinity titration to pH 8.3. Bromcresol green or a mixed bromcresol green-methyl red indicator may be used for pH 4.5.

c. Interferences: Soaps, oily matter, suspended solids, or precipitates may coat the glass electrode and cause a sluggish response. Allow additional time between titrant additions to let electrode come to equilibrium or clean the electrodes occasionally. Do not filter, dilute, concentrate, or alter sample.

d. Selection of procedure: Determine sample alkalinity from volume of standard acid required to titrate a portion to a designated pH taken from ¶ 1*b*. Titrate at room temperature with a properly calibrated pH meter or electrically operated titrator, or use color indicators. If using color indicators, prepare and titrate an indicator blank.

Report alkalinity less than 20 mg CaCO₃/L only if it has been determined by the low-alkalinity method of ¶ 4*d*.

Construct a titration curve for standardization of reagents.

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Color indicators may be used for routine and control titrations in the absence of interfering color and turbidity and for preliminary titrations to select sample size and strength of titrant (see below).

e. Sample size: See Section 2310B.1*e* for selection of size sample to be titrated and normality of titrant, substituting 0.02*N* or 0.1*N* sulfuric (H₂SO₄) or hydrochloric (HCl) acid for the standard alkali of that method. For the low-alkalinity method, titrate a 200-mL sample with 0.02*N* H₂SO₄ from a 10-mL buret.

f. Sampling and storage: See Section 2310B.1 *f*.

2. Apparatus

See Section 2310B.2.

3. Reagents

a. Sodium carbonate solution, approximately 0.05*N*: Dry 3 to 5 g primary standard Na₂CO₃ at 250°C for 4 h and cool in a desiccator. Weigh 2.5 ± 0.2 g (to the nearest mg), transfer to a 1-L volumetric flask, fill flask to the mark with distilled water, and dissolve and mix reagent. Do not keep longer than 1 week.

b. Standard sulfuric acid or hydrochloric acid, 0.1*N*: Prepare acid solution of approximate normality as indicated under Preparation of Desk Reagents. Standardize against 40.00 mL 0.05*N* Na₂CO₃ solution, with about 60 mL water, in a beaker by titrating potentiometrically to pH of about 5. Lift out electrodes, rinse into the same beaker, and boil gently for 3 to 5 min under a watch glass cover. Cool to room temperature, rinse cover glass into beaker, and finish titrating to the pH inflection point. Calculate normality:

$$\text{Normality, } N = \frac{A \times B}{53.00 \times C}$$

where:

A = g Na₂CO₃ weighed into 1-L flask,

B = mL Na₂CO₃ solution taken for titration, and

C = mL acid used.

Use measured normality in calculations or adjust to 0.1000*N*; 1 mL 0.1000*N* solution = 5.00 mg CaCO₃.

c. Standard sulfuric acid or hydrochloric acid, 0.02*N*: Dilute 200.00 mL 0.1000*N* standard acid to 1000 mL with distilled or deionized water. Standardize by potentiometric titration of

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15.00 mL 0.05N Na₂CO₃ according to the procedure of ¶ 3b; 1 mL = 1.00 mg CaCO₃.

d. Bromcresol green indicator solution, pH 4.5 indicator: Dissolve 100 mg bromcresol green, sodium salt, in 100 mL distilled water.

*e. Mixed bromcresol green-methyl red indicator solution:*³ Use either the aqueous or the alcoholic solution:

1) Dissolve 100 mg bromcresol green sodium salt and 20 mg methyl red sodium salt in 100 mL distilled water.

2) Dissolve 100 mg bromcresol green and 20 mg methyl red in 100 mL 95% ethyl alcohol or isopropyl alcohol.

f. Metacresol purple indicator solution, pH 8.3 indicator: Dissolve 100 mg metacresol purple in 100 mL water.

g. Phenolphthalein solution, alcoholic, pH 8.3 indicator.

h. Sodium thiosulfate, 0.1N: See Section 2310B.3i.

4. Procedure

a. Color change: See Section 2310B.4b.

b. Potentiometric titration curve: Follow the procedure for determining acidity (Section 2310B.4c), substituting the appropriate normality of standard acid solution for standard NaOH, and continue titration to pH 4.5 or lower. Do not filter, dilute, concentrate, or alter the sample.

c. Potentiometric titration to preselected pH: Determine the appropriate end-point pH according to ¶ 1b. Prepare sample and titration assembly (Section 2310B.4c). Titrate to the end-point pH without recording intermediate pH values and without undue delay. As the end point is approached make smaller additions of acid and be sure that pH equilibrium is reached before adding more titrant.

d. Potentiometric titration of low alkalinity: For alkalinities less than 20 mg/L titrate 100 to 200 mL according to the procedure of ¶ 4c, above, using a 10-mL microburet and 0.02N standard acid solution. Stop the titration at a pH in the range 4.3 to 4.7 and record volume and exact pH. Carefully add additional titrant to reduce the pH exactly 0.30 pH unit and again record volume.

5. Calculations

a. Potentiometric titration to end-point pH:

$$\text{Alkalinity, mg CaCO}_3/\text{L} = \frac{A \times N \times 50\,000}{\text{mL sample}}$$

where:

A = mL standard acid used and

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N = normality of standard acid

or

$$\text{Alkalinity, mg CaCO}_3/\text{L} = \frac{A \times t \times 1000}{\text{mL sample}}$$

where:

t = titer of standard acid, mg CaCO₃/mL.

Report pH of end point used as follows: “The alkalinity to pH _____ = _____ mg CaCO₃/L” and indicate clearly if this pH corresponds to an inflection point of the titration curve.

b. Potentiometric titration of low alkalinity:

Total alkalinity, mg CaCO₃/L

$$= \frac{(2B - C) \times N \times 50\,000}{\text{mL sample}}$$

where:

B = mL titrant to first recorded pH,

C = total mL titrant to reach pH 0.3 unit lower, and

N = normality of acid.

c. Calculation of alkalinity relationships: The results obtained from the phenolphthalein and total alkalinity determinations offer a means for stoichiometric classification of the three principal forms of alkalinity present in many waters. The classification ascribes the entire alkalinity to bicarbonate, carbonate, and hydroxide, and assumes the absence of other (weak) inorganic or organic acids, such as silicic, phosphoric, and boric acids. It further presupposes the incompatibility of hydroxide and bicarbonate alkalinities. Because the calculations are made on a stoichiometric basis, ion concentrations in the strictest sense are not represented in the results, which may differ significantly from actual concentrations especially at pH > 10. According to this scheme:

1) Carbonate (CO₃²⁻) alkalinity is present when phenolphthalein alkalinity is not zero but is less than total alkalinity.

2) Hydroxide (OH⁻) alkalinity is present if phenolphthalein alkalinity is more than half the total alkalinity.

3) Bicarbonate (HCO₃⁻) alkalinity is present if phenolphthalein alkalinity is less than half the

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total alkalinity. These relationships may be calculated by the following scheme, where P is phenolphthalein alkalinity and T is total alkalinity (§ 1b):

Select the smaller value of P or $(T - P)$. Then, carbonate alkalinity equals twice the smaller value. When the smaller value is P , the balance $(T - 2P)$ is bicarbonate. When the smaller value is $(T - P)$, the balance $(2P - T)$ is hydroxide. All results are expressed as CaCO_3 . The mathematical conversion of the results is shown in Table 2320:II. (A modification of Table 2320:II that is more accurate when $P \approx \frac{1}{2}T$ has been proposed.⁴)

Alkalinity relationships also may be computed nomographically (see Carbon Dioxide, Section 4500- CO_2). Accurately measure pH, calculate OH^- concentration as milligrams CaCO_3 per liter, and calculate concentrations of CO_3^{2-} and HCO_3^- as milligrams CaCO_3 per liter from the OH^- concentration, and the phenolphthalein and total alkalinities by the following equations:

$$\text{CO}_3^{2-} = 2P - 2[\text{OH}^-]$$

$$\text{HCO}_3^- = T - 2P + [\text{OH}^-]$$

Similarly, if difficulty is experienced with the phenolphthalein end point, or if a check on the phenolphthalein titration is desired, calculate phenolphthalein alkalinity as CaCO_3 from the results of the nomographic determinations of carbonate and hydroxide ion concentrations:

$$P = \frac{1}{2} [\text{CO}_3^{2-}] + [\text{OH}^-]$$

6. Precision and Bias

No general statement can be made about precision because of the great variation in sample characteristics. The precision of the titration is likely to be much greater than the uncertainties involved in sampling and sample handling before the analysis.

In the range of 10 to 500 mg/L, when the alkalinity is due entirely to carbonates or bicarbonates, a standard deviation of 1 mg CaCO_3 /L can be achieved. Forty analysts in 17 laboratories analyzed synthetic samples containing increments of bicarbonate equivalent to 120 mg CaCO_3 /L. The titration procedure of § 4b was used, with an end point pH of 4.5. The standard deviation was 5 mg/L and the average bias (lower than the true value) was 9 mg/L.⁵

Sodium carbonate solutions equivalent to 80 and 65 mg CaCO_3 /L were analyzed by 12 laboratories according to the procedure of § 4c.⁶ The standard deviations were 8 and 5 mg/L, respectively, with negligible bias.⁶ Four laboratories analyzed six samples having total alkalinities of about 1000 mg CaCO_3 /L and containing various ratios of carbonate/bicarbonate by the procedures of both § 4a and § 4c. The pooled standard deviation was 40 mg/L, with negligible difference between the procedures.

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2330 CALCIUM CARBONATE SATURATION*#(30)

2330 A. Introduction

1. General Discussion

Calcium carbonate (CaCO_3) saturation indices commonly are used to evaluate the scale-forming and scale-dissolving tendencies of water. Assessing these tendencies is useful in corrosion control programs and in preventing CaCO_3 scaling in piping and equipment such as industrial heat exchangers or domestic water heaters.

Waters oversaturated with respect to CaCO_3 tend to precipitate CaCO_3 . Waters undersaturated with respect to CaCO_3 tend to dissolve CaCO_3 . Saturated waters, i.e., waters in equilibrium with

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CaCO_3 , have neither CaCO_3 -precipitating nor CaCO_3 -dissolving tendencies. Saturation represents the dividing line between “precipitation likely” and “precipitation not likely.”

Several water quality characteristics must be measured to calculate the CaCO_3 saturation indices described here. Minimum requirements are total alkalinity (2320), total calcium (3500-Ca), pH (4500- H^+), and temperature (2550). The ionic strength also must be calculated or estimated from conductivity (2510) or total dissolved solids (2540C) measurements. Measure pH at the system’s water temperature using a temperature-compensated pH meter. If pH is measured at a different temperature, for example in the laboratory, correct the measured pH.¹⁻³ In measuring pH and alkalinity, minimize CO_2 exchange between sample and atmosphere. Ideally, seal the sample from the atmosphere during measurements⁴; at a minimum, avoid vigorous stirring of unsealed samples.

There are two general categories of CaCO_3 saturation indices: indices that determine whether a water has a *tendency* to precipitate CaCO_3 (i.e., is oversaturated) or to dissolve CaCO_3 (i.e., is undersaturated) and indices that estimate the *quantity* of CaCO_3 that can be precipitated from an oversaturated water and the amount that can be dissolved by an undersaturated water. Indices in the second category generally yield more information but are more difficult to determine.

2. Limitations

It is widely assumed that CaCO_3 will precipitate from oversaturated waters and that it cannot be deposited from undersaturated waters. Exceptions may occur. For example, CaCO_3 deposition from oversaturated waters is inhibited by the presence of phosphates (particularly polyphosphates), certain naturally occurring organics, and magnesium.⁵⁻⁷ These materials can act as sequestering agents or as crystal poisons. Conversely, CaCO_3 deposits have been found in pipes conveying undersaturated water. This apparent contradiction is caused by high pH (relative to the bulk water pH) in the immediate vicinity of certain areas (cathodes) of corroding metal surfaces. A locally oversaturated condition may occur even if the bulk water is undersaturated. A small, but significant, amount of CaCO_3 can be deposited.

The calculations referred to here, even the most sophisticated computerized calculations, do not adequately describe these exceptions. For this reason, do not consider saturation indices as absolutes. Rather, view them as guides to the behavior of CaCO_3 in aqueous systems and supplement them, where possible, with experimentally derived information.

Similarly, the effects predicted by the indices do not always conform to expectations. The relationship between the indices and corrosion rates is a case in point. Conceptually, piping is protected when CaCO_3 is precipitated on its surfaces. CaCO_3 is believed to inhibit corrosion by clogging reactive areas and by providing a matrix to retain corrosion products, thus further sealing the surfaces. Waters with positive indices traditionally have been assumed to be protective while waters with negative indices have been assumed to be not protective, or

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corrosive. The expected relationship is observed sometimes,^{8,9} but not always.^{10,11} Unexpected results may be due in part to limited capability to predict CaCO_3 behavior. Also, water characteristics not directly involved in the calculation of the indices (e.g., dissolved oxygen, buffering intensity, chloride, sulfate, and water velocity) may influence corrosion rates appreciably.^{9,12-16} Thus, do not estimate corrosion rates on the basis of CaCO_3 indices alone.

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2330 B. Indices Indicating Tendency of a Water to Precipitate CaCO_3 or Dissolve CaCO_3

1. General Discussion

Indices that indicate CaCO_3 precipitation or dissolution tendencies define whether a water is oversaturated, saturated, or undersaturated with respect to CaCO_3 . The most widely used indices are the Saturation Index (SI); the Relative Saturation (RS), also known as the Driving Force Index (DFI); and the Ryznar Index (RI). The SI is by far the most commonly used and will be described here. The RS and SI are related (see Equation 6, Section 2330D). The RI¹ has been used for many years, sometimes with good results. Because it is semi-empirical it may be less reliable than the SI.

2. Saturation Index by Calculation

SI is determined from Equation 1.

$$SI = pH - pH_s \quad (1)$$

where:

pH = measured pH and

pH_s = pH of the water if it were in equilibrium with CaCO_3 at the existing calcium ion $[\text{Ca}^{2+}]$ and bicarbonate ion $[\text{HCO}_3^-]$ concentrations.

A positive SI connotes a water oversaturated with respect to CaCO_3 . A negative SI signifies an undersaturated water. An SI of zero represents a water in equilibrium with CaCO_3 .

a. *Analytical solution for pH_s* : Determine pH_s as follows²:

$$pH_s = pK_2 - pK_s + p[\text{Ca}^{2+}] + p[\text{HCO}_3^-] + 5 p f_m \quad (2)$$

where:

K_2 = second dissociation constant for carbonic acid, at the water temperature,

K_s = solubility product constant for CaCO_3 at the water temperature,

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- $[Ca^{2+}]$ = calcium ion concentration, g-moles/L,
 $[HCO_3^-]$ = bicarbonate ion concentration, g-moles/L, and
 f_m = activity coefficient for monovalent species at the specified temperature.

In Equation 2, p preceding a variable designates $-\log_{10}$ of that variable.

Calculate values of pK_2 , pK_s , and pf_m required to solve Equation 2 from the equations in Table 2330:I. To save computation time, values for pK_2 and pK_s have been precalculated for selected temperatures (see Table 2330:II).

Table 2330:II gives several values for pK_s . Different isomorphs of $CaCO_3$ can form in aqueous systems, including calcite, aragonite, and vaterite. Each has somewhat different solubility properties. These differences can be accommodated when computing pH_s simply by using the pK_s for the compound most likely to form. The form of $CaCO_3$ most commonly found in fresh water is calcite. Use the pK_s for calcite unless it is clear that a different form of $CaCO_3$ controls $CaCO_3$ solubility.

Estimate calcium ion concentration from total calcium measurements with Equation 3.

$$[Ca^{2+}] = Ca_t - Ca_{ip} \quad (3)$$

where:

Ca_t = total calcium, g-moles/L, and

Ca_{ip} = calcium associated with ion pairs such as $CaHCO_3^+$, $CaSO_4^0$, and $CaOH^+$.

Calcium associated with ion pairs is not available to form $CaCO_3$.

Estimate $[HCO_3^-]$, the bicarbonate ion concentration, from Equation 4.

$$[HCO_3^-] = \frac{Alk_t - Alk_o + 10^{(pf_m - pH)} - 10^{(pH + pf_m - pK_w)}}{1 + 0.5 \times 10^{(pH - pK_2)}} \quad (4)$$

where:

Alk_t = total alkalinity, as determined by acid titration to the carbonic acid end point, g-equivalents/L,

K_w = dissociation constant for water, at the water temperature, and

Alk_o = alkalinity contributed by NH_3^0 , $H_3SiO_4^-$, HPO_4^{2-} , $B(OH)_4^-$, CH_3COO^- (acetate), HS^- , and ion pairs such as $CaHCO_3^+$ and $MgOH^+$. These contributions usually are small compared to the contributions of components normally considered (HCO_3^- , CO_3^{2-} , OH^- ,

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and H^+).

Calculations can be simplified. For example, in Equation 4, terms containing exponents (e.g., $10^{(pH + pf_m - p_{kw})}$) usually can be neglected for waters that are approximately neutral (pH 6.0 to 8.5) with alkalinity greater than about 50 mg/L as $CaCO_3$. The terms Ca_{ip} in Equation 3 and Alk_o in Equation 4 are difficult to calculate without computers. Therefore they usually are neglected for hand calculations. The simplified version of Equation 2 under such conditions is:

$$pH_s = pK_2 - pK_s + p[Ca_t] + p[Alk_t] + 5 pf_m$$

1) Sample calculation—The calculation is best illustrated by working through an example. Assume that calcite controls $CaCO_3$ solubility and determine the SI for a water of the following composition:

Constituent	Concentration			
	mg	÷	mg mole	= g-moles/L
Calcium	152		40 000	3.80×10^{-3}
Magnesium	39		24 312	1.60×10^{-3}
Sodium	50		22 989	2.18×10^{-3}
Potassium	5		39 102	1.28×10^{-4}
Chloride	53		35 453	1.49×10^{-3}
Alkalinity (as $CaCO_3$)	130		50 000	$2.60 \times 10^{-3*}$
Sulfate	430		96 060	4.48×10^{-3}
Silica (as SiO_2)	15		60 084	2.50×10^{-4}

*g-equivalents.

Water temperature = 20°C (293.2°K); pH = 9.00.

Before evaluating pf_m in Equation 2, determine the ionic strength (I) and another constant (A). Estimate ionic strength from the first equation of Table 2330:I, assuming all the alkalinity is due to bicarbonate ion. Use the alkalinity concentration (2.60×10^{-3}) and the bicarbonate charge (-1) to calculate the contribution of alkalinity to ionic strength. Assume silica is mostly H_4SiO_4 . Because H_4SiO_4 has zero charge, silica does not contribute to ionic strength.

$$I = 0.5 [4(3.80 \times 10^{-3}) + 4(1.60 \times 10^{-3}) + 2.18 \times 10^{-3} +$$

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$$\begin{aligned}
 &1.28 \times 10^{-4} + 1.49 \times 10^{-3} + 2.60 \times 10^{-3} \\
 &+ 4(4.48 \times 10^{-3}) \\
 &= 2.29 \times 10^{-2} \text{ g-moles/L}
 \end{aligned}$$

In the absence of a complete water analysis, estimate ionic strength from conductivity or total dissolved solids measurements (see alternative equations, Table 2330:I).

Estimate A from the equation in Table 2330:I, after first determining the dielectric constant E from the formula in the same table. Alternatively, use precalculated values of A in Table 2330:II. In Table 2330:II, $A = 0.506$ at 20°C .

Next estimate pf_m from the equation in Table 2330:I

$$\begin{aligned}
 pf_m &= 0.506 \\
 &\times \left[\frac{\sqrt{2.29 \times 10^{-2}}}{1 + \sqrt{2.29 \times 10^{-2}}} - 0.3 (2.29 \times 10^{-2}) \right] \\
 &= 0.063
 \end{aligned}$$

Determine $[\text{HCO}_3^-]$ from Equation 4. Neglect Alk_o , but because the pH exceeds 8.5, calculate the other terms. From Table 2330:II, $pK_2 = 10.38$ and $pK_w = 14.16$.

$$\begin{aligned}
 [\text{HCO}_3^-] &= \frac{2.60 \times 10^{-3} + 10^{(0.063 - 9.0)} - 10^{(9.0 + 0.063 - 14.16)}}{1 + 0.5 \times 10^{(9.0 - 10.38)}} \\
 &= 2.54 \times 10^{-3} \text{ g-moles/L}
 \end{aligned}$$

Therefore $p[\text{HCO}_3^-] = 2.60$.

Determine $[\text{Ca}^{2+}]$ from Equation 3. Neglect Ca_{ip} .

$$[\text{Ca}^{2+}] = Ca_t = 3.80 \times 10^{-3} \text{ g-moles/L}$$

Therefore $p[\text{Ca}^{2+}] = 2.42$.

From Table 2330:II, pK_s for calcite is 8.45.

Determine pH_s from Equation 2:

$$pH_s = 10.38 - 8.45 + 2.42 + 2.60 + 5 (0.063) = 7.27$$

And finally, determine SI from Equation 1:

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$$SI = 9.00 - 7.27 = 1.73$$

The positive SI indicates the water is oversaturated with respect to calcite.

2) Effect of neglecting Ca_{ip} and Alk_o —If Ca_{ip} is neglected, pH_s is underestimated and SI is overestimated by an amount equal to $p(1 - Y_{Ca_{ip}})$, where $Y_{Ca_{ip}}$ is the fraction of total calcium in ion pairs. For example, if $Y_{Ca_{ip}} = 0.30$ then the estimate for SI is 0.15 units too high. Similarly, if Alk_o is neglected, SI is overestimated by an amount equal to $p(1 - Y_{Alk_o})$, where Y_{Alk_o} is the fraction of total alkalinity contributed by species other than HCO_3^- , CO_3^{2-} , OH^- , and H^+ . The effects of neglecting Ca_{ip} and Alk_o are additive.

Ca_{ip} and Alk_o may be neglected if the factors $Y_{Ca_{ip}}$ and Y_{Alk_o} are small and do not interfere with interpretation of the SI . The factors are small for waters of low and neutral pH, but they increase as pH values approach and exceed 9. At high pH values, however, the SI is typically much larger than its overestimate, so neglecting Ca_{ip} and Alk_o causes no problem. To return to the example above, when calculations were done with a computerized water chemistry code (SEQUIL) (see Table 2330:III) that considers Ca_{ip} and Alk_o , the SI was 1.48, i.e., 0.25 units lower than the result obtained by hand calculations. In this instance, neglecting Ca_{ip} and Alk_o did not interfere with interpreting the result. Both calculations showed the water to be strongly oversaturated.

The potential for misinterpretation is most acute in nearly-saturated waters of high sulfate concentration. Recirculating cooling water is an example. Calcium is sequestered by the robust $CaSO_4^0$ ion pair and the SI can be overestimated by as much as 0.3 to 0.5 units, even at neutral pH. Under these conditions, the SI may be thought to be zero (neither scale-forming or corrosive) when in fact it is negative.

Resolve this problem by determining pH_s using computerized water chemistry codes that consider ion pairs and the other forms of alkalinity. Section 2330D provides information about water chemistry codes. The most accurate calculations are obtained when a complete mineral analysis is provided.

An alternative but somewhat less rigorous procedure involves direct measurement of (Ca^{2+}) , the calcium ion activity, with a calcium specific-ion electrode.⁹ Use Equation 5 to determine $p[Ca^{2+}]$; then use $p[Ca^{2+}]$ in Equation 2.

$$p[Ca^{2+}] = p(Ca^{2+}) - 4p f_m \quad (5)$$

This approach eliminates the need to determine Ca_{ip} . However, no equivalent procedure is available to bypass the determination of Alk_o .

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b. Graphical solutions for pH_s : Caldwell-Lawrence diagrams can be used to determine pH_s .¹⁰⁻¹² The diagrams are particularly useful for estimating chemical dosages needed to achieve desired water conditions. Consult the references for descriptions of how to use the diagrams; see Section 2330D for additional information about the diagrams.

3. Saturation Index by Experimental Determination

a. Saturometry: Saturometers were developed to measure the relative saturation of seawater with respect to CaCO_3 . A water of known calcium and pH is equilibrated with CaCO_3 in a sealed flask containing a pH electrode. The water temperature is controlled with a constant-temperature bath. During equilibration the pH decreases if CaCO_3 precipitates and increases if CaCO_3 dissolves. When the pH stops changing, equilibrium is said to have been achieved. The initial pH and calcium values and the final pH value are used to calculate the relative saturation (RS).¹³ Equation 6, Section 2330D, may then be used to determine SI.

A major advantage of this method is that the approach to equilibrium can be tracked by measuring pH, thus minimizing uncertainty about the achievement of equilibrium. The method is most sensitive in the range of minimum buffering intensity (pH 7.5 to 8.5). The calculations do not consider ion pairs or noncarbonate alkalinity, except borate. The technique has been used for *in situ* oceanographic measurements¹⁴ as well as in the laboratory.

The saturometry calculations discussed above use K_s of the CaCO_3 phase assumed to control solubility. Uncertainties occur if the identity of the controlling solid is unknown. Resolve these uncertainties by measuring K_s of the controlling solid. It is equal to the CaCO_3 activity product, $[\text{Ca}^{2+}] \times [\text{CO}_3^{2-}]$, at equilibrium. Calculate the latter from the equilibrium pH and initial calcium, alkalinity, and pH measurements.¹⁵

*b. Alkalinity difference technique:*¹⁶ SI also can be determined by equilibrating water of known pH, calcium, and alkalinity with CaCO_3 in a sealed, constant-temperature system. The CaCO_3 activity product before equilibration is determined from initial calcium, pH, and alkalinity (or total carbonate) values. The CaCO_3 solubility product constant (K_s) equals the CaCO_3 activity product after equilibration, which is determined by using the alkalinity change that occurred during equilibration. RS is found by dividing the initial activity product by K_s . Calculate SI by using Equation 6 (see Section 2330D.1). The advantage of this method is that it makes no assumptions about the identity of the CaCO_3 phase. However it is more difficult to determine when equilibrium is achieved with this method than with the saturometry method.

Whatever the method used, use the temperatures that are the same as the temperature of the water source. Alternatively, correct test results to the temperature of the water source.¹⁶

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2330 C. Indices Predicting the Quantity of CaCO_3 That Can Be Precipitated or Dissolved

The Calcium Carbonate Precipitation Potential (CCPP) predicts both tendency to precipitate or to dissolve CaCO_3 and quantity that may be precipitated or dissolved. The CCPP also is known by other names, e.g., Calcium Carbonate Precipitation Capacity (CCPC).

The CCPP is defined as the quantity of CaCO_3 that theoretically can be precipitated from oversaturated waters or dissolved by undersaturated waters during equilibration.¹ The amount that actually precipitates or dissolves may be less, because equilibrium may not be achieved. The CCPP is negative for undersaturated waters, zero for saturated waters, and positive for oversaturated waters.

1. Calculating CCPP

The CCPP does not lend itself to hand calculations. Preferably calculate CCPP with computerized water chemistry models and Caldwell-Lawrence diagrams (see Section 2330D).

The most reliable calculations consider ion pairs and the contribution to alkalinity of other species besides HCO_3^- , CO_3^{2-} , OH^- , and H^+ . Models that do not consider these factors overestimate the amount of CaCO_3 that can be precipitated and underestimate the amount of CaCO_3 that can be dissolved.

2. Experimental Determination of CCPP

Estimate CCPP by one of several experimental techniques.

- a. *Saturation*: See Section 2330B. The CCPP is determined as part of the RS calculation.
- b. *Alkalinity difference technique*: See Section 2330B. The CCPP equals the difference between alkalinity (or calcium) values of the initial and equilibrated water, when they are expressed as CaCO_3 .
- c. *Marble test*: The marble test¹⁻⁵ is similar to the alkalinity difference technique. The CCPP equals the change in alkalinity (or calcium) values during equilibration, when they are expressed as CaCO_3 .
- d. *Enslow test*: The Enslow test⁵ is a continuous version of the alkalinity difference or marble tests. Water is fed continuously to a leveling bulb or separatory funnel partly filled with CaCO_3 . The effluent from this device is filtered through crushed marble so that the filtrate is assumed to be in equilibrium with CaCO_3 . The CCPP equals the change in alkalinity (or calcium) values that occurs during passage through the apparatus.
- e. *Calcium carbonate deposition test*:⁶ The calcium carbonate deposition test (CCDT) is an electrochemical method that measures the electric current produced when dissolved oxygen is

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reduced on a rotating electrode. When an oversaturated water is placed in the apparatus, CaCO_3 deposits on the electrode. The deposits interfere with oxygen transfer and the current diminishes. The rate of CaCO_3 deposition is directly proportional to the rate at which the current declines. The CCDT and the CCPP are related, but they are not the same. The CCDT is a rate, and the CCPP is a quantity.

For realistic assessments of the CCPP (or CCDT) keep test temperature the same as the temperature of the water source. Alternatively, correct test results to the temperature of the water source.

3. References

1. MERRILL, D.T. & R.L. SANKS. 1978, 1979. Corrosion control by deposition of CaCO_3 films: A practical approach for plant operators. *J. Amer. Water Works Assoc.* 70:592; 70:634 & 71:12.
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2330 D. Diagrams and Computer Codes for CaCO_3 Indices

1. Description

Table 2330:III lists diagrams and computer codes that can be used to determine the SI and CCPP. It also provides a brief description of their characteristics.

Many computer codes do not calculate *SI* directly, but instead calculate the relative saturation (*RS*). When *RS* data are presented, calculate the *SI* from:¹

$$SI = \log_{10} RS \quad (6)$$

where:

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RS = ratio of CaCO_3 activity product to CaCO_3 solubility product constant.

The diagrams and a few of the codes define pH_s as the pH the water would exhibit if it were in equilibrium with CaCO_3 at the existing calcium and total alkalinity concentrations.² This definition of pH_s differs from the definition following Equation 1 because alkalinity is used instead of bicarbonate. Within the pH range 6 to 9, alkalinity-based pH_s and bicarbonate-based pH_s are virtually equal, because total alkalinity is due almost entirely to bicarbonate ion. Above pH 9 they differ and Equation 6 no longer applies if SI is calculated with alkalinity-based pH_s . However, if SI is determined from bicarbonate-based pH, Equation 6 continues to apply.

Furthermore, calculating SI with alkalinity-based pH_s reverses the sign of the SI above pH values of approximately pK_2 , i.e., a positive, not the usual negative, SI connotes an undersaturated water.³ With bicarbonate-based pH_s or RS , sign reversal does not occur, thereby eliminating the confusing sign change. For these reasons, bicarbonate-based pH_s or RS is preferred. Table 2330:III lists the definition of pH_s used for each code.

Some models calculate only the amount of CaCO_3 that can be precipitated but not the amount of CaCO_3 that can be dissolved. Others calculate both.

The diagrams and codes can be used to determine many more parameters than the CaCO_3 saturation indices. A fee may be charged for computer software or graphs. The information in Table 2330:III describes parameters each code uses to calculate SI . Contact the sources listed below the table for current information.

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2340 HARDNESS*#(31)

2340 A. Introduction

1. Definition

Originally, water hardness was understood to be a measure of the capacity of water to precipitate soap. Soap is precipitated chiefly by the calcium and magnesium ions present. Other polyvalent cations also may precipitate soap, but they often are in complex forms, frequently with organic constituents, and their role in water hardness may be minimal and difficult to define. In conformity with current practice, total hardness is defined as the sum of the calcium and magnesium concentrations, both expressed as calcium carbonate, in milligrams per liter.

When hardness numerically is greater than the sum of carbonate and bicarbonate alkalinity, that amount of hardness equivalent to the total alkalinity is called “carbonate hardness”; the

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amount of hardness in excess of this is called “noncarbonate hardness.” When the hardness numerically is equal to or less than the sum of carbonate and bicarbonate alkalinity, all hardness is carbonate hardness and noncarbonate hardness is absent. The hardness may range from zero to hundreds of milligrams per liter, depending on the source and treatment to which the water has been subjected.

2. Selection of Method

Two methods are presented. Method B, hardness by calculation, is applicable to all waters and yields the higher accuracy. If a mineral analysis is performed, hardness by calculation can be reported. Method C, the EDTA titration method, measures the calcium and magnesium ions and may be applied with appropriate modification to any kind of water. The procedure described affords a means of rapid analysis.

3. Reporting Results

When reporting hardness, state the method used, for example, “hardness (calc.)” or “hardness (EDTA).”

2340 B. Hardness by Calculation

1. Discussion

The preferred method for determining hardness is to compute it from the results of separate determinations of calcium and magnesium.

2. Calculation

Hardness, mg equivalent $\text{CaCO}_3/\text{L} = 2.497 [\text{Ca, mg/L}] + 4.118 [\text{Mg, mg/L}]$

2340 C. EDTA Titrimetric Method

1. General Discussion

a. Principle: Ethylenediaminetetraacetic acid and its sodium salts (abbreviated EDTA) form a chelated soluble complex when added to a solution of certain metal cations. If a small amount of a dye such as Eriochrome Black T or Calmagite is added to an aqueous solution containing calcium and magnesium ions at a pH of 10.0 ± 0.1 , the solution becomes wine red. If EDTA is added as a titrant, the calcium and magnesium will be complexed, and when all of the magnesium and calcium has been complexed the solution turns from wine red to blue, marking the end point of the titration. Magnesium ion must be present to yield a satisfactory end point. To insure this, a small amount of complexometrically neutral magnesium salt of EDTA is added to the buffer; this automatically introduces sufficient magnesium and obviates the need for a blank correction.

The sharpness of the end point increases with increasing pH. However, the pH cannot be

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increased indefinitely because of the danger of precipitating calcium carbonate, CaCO_3 , or magnesium hydroxide, $\text{Mg}(\text{OH})_2$, and because the dye changes color at high pH values. The specified pH of 10.0 ± 0.1 is a satisfactory compromise. A limit of 5 min is set for the duration of the titration to minimize the tendency toward CaCO_3 precipitation.

b. Interference: Some metal ions interfere by causing fading or indistinct end points or by stoichiometric consumption of EDTA. Reduce this interference by adding certain inhibitors before titration. MgCDTA [see 2b3)], selectively complexes heavy metals, releases magnesium into the sample, and may be used as a substitute for toxic or malodorous inhibitors. It is useful only when the magnesium substituted for heavy metals does not contribute significantly to the total hardness. With heavy metal or polyphosphate concentrations below those indicated in Table 2340:I, use Inhibitor I or II. When higher concentrations of heavy metals are present, determine calcium and magnesium by a non-EDTA method (see Section 3500-Ca and Section 3500-Mg) and obtain hardness by calculation. The figures in Table 2340:I are intended as a rough guide only and are based on using a 25-mL sample diluted to 50 mL.

Suspended or colloidal organic matter also may interfere with the end point. Eliminate this interference by evaporating the sample to dryness on a steam bath and heating in a muffle furnace at 550°C until the organic matter is completely oxidized. Dissolve the residue in 20 mL 1N hydrochloric acid (HCl), neutralize to pH 7 with 1N sodium hydroxide (NaOH), and make up to 50 mL with distilled water; cool to room temperature and continue according to the general procedure.

c. Titration precautions: Conduct titrations at or near normal room temperature. The color change becomes impractically slow as the sample approaches freezing temperature. Indicator decomposition becomes a problem in hot water.

The specified pH may produce an environment conducive to CaCO_3 precipitation. Although the titrant slowly redissolves such precipitates, a drifting end point often yields low results. Completion of the titration within 5 min minimizes the tendency for CaCO_3 to precipitate. The following three methods also reduce precipitation loss:

1) Dilute sample with distilled water to reduce CaCO_3 concentration. This simple expedient has been incorporated in the procedure. If precipitation occurs at this dilution of 1 + 1 use modification 2) or 3). Using too small a sample contributes a systematic error due to the buret-reading error.

2) If the approximate hardness is known or is determined by a preliminary titration, add 90% or more of titrant to sample *before* adjusting pH with buffer.

3) Acidify sample and stir for 2 min to expel CO_2 *before* pH adjustment. Determine alkalinity to indicate amount of acid to be added.

2. Reagents

a. Buffer solution:

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1) Dissolve 16.9 g ammonium chloride (NH_4Cl) in 143 mL conc ammonium hydroxide (NH_4OH). Add 1.25 g magnesium salt of EDTA (available commercially) and dilute to 250 mL with distilled water.

2) If the magnesium salt of EDTA is unavailable, dissolve 1.179 g disodium salt of ethylenediaminetetraacetic acid dihydrate (analytical reagent grade) and 780 mg magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) or 644 mg magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) in 50 mL distilled water. Add this solution to 16.9 g NH_4Cl and 143 mL conc NH_4OH with mixing and dilute to 250 mL with distilled water. To attain the highest accuracy, adjust to exact equivalence through appropriate addition of a small amount of EDTA or MgSO_4 or MgCl_2 .

Store Solution 1) or 2) in a plastic or borosilicate glass container for no longer than 1 month. Stopper tightly to prevent loss of ammonia (NH_3) or pickup of carbon dioxide (CO_2). Dispense buffer solution by means of a bulb-operated pipet. Discard buffer when 1 or 2 mL added to the sample fails to produce a pH of 10.0 ± 0.1 at the titration end point.

3) Satisfactory alternate “odorless buffers” also are available commercially. They contain the magnesium salt of EDTA and have the advantage of being relatively odorless and more stable than the NH_4Cl - NH_4OH buffer. They usually do not provide as good an end point as NH_4Cl - NH_4OH because of slower reactions and they may be unsuitable when this method is automated. Prepare one of these buffers by mixing 55 mL conc HCl with 400 mL distilled water and then, slowly and with stirring, adding 300 mL 2-aminoethanol (free of aluminum and heavier metals). Add 5.0 g magnesium salt of EDTA and dilute to 1 L with distilled water.

b. Complexing agents: For most waters no complexing agent is needed. Occasionally water containing interfering ions requires adding an appropriate complexing agent to give a clear, sharp change in color at the end point. The following are satisfactory:

1) *Inhibitor I:* Adjust acid samples to pH 6 or higher with buffer or 0.1N NaOH. Add 250 mg sodium cyanide (NaCN) in powder form. Add sufficient buffer to adjust to pH 10.0 ± 0.1 . (CAUTION: NaCN is extremely poisonous. Take extra precautions in its use. Flush solutions containing this inhibitor down the drain with large quantities of water after insuring that no acid is present to liberate volatile poisonous hydrogen cyanide.)

2) *Inhibitor II:* Dissolve 5.0 g sodium sulfide nonahydrate ($\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$) or 3.7 g $\text{Na}_2\text{S} \cdot 5\text{H}_2\text{O}$ in 100 mL distilled water. Exclude air with a tightly fitting rubber stopper. This inhibitor deteriorates through air oxidation. It produces a sulfide precipitate that obscures the end point when appreciable concentrations of heavy metals are present. Use 1 mL in ¶ 3b below.

3) *MgCDTA:* Magnesium salt of 1, 2-cyclohexanediaminetetraacetic acid. Add 250 mg per 100 mL sample and dissolve completely before adding buffer solution. Use this complexing agent to avoid using toxic or odorous inhibitors when interfering substances are present in concentrations that affect the end point but will not contribute significantly to the hardness value.

Commercial preparations incorporating a buffer and a complexing agent are available. Such

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mixtures must maintain pH 10.0 ± 0.1 during titration and give a clear, sharp end point when the sample is titrated.

c. Indicators: Many types of indicator solutions have been advocated and may be used if the analyst demonstrates that they yield accurate values. The prime difficulty with indicator solutions is deterioration with aging, giving indistinct end points. For example, alkaline solutions of Eriochrome Black T are sensitive to oxidants and aqueous or alcoholic solutions are unstable. In general, use the least amount of indicator providing a sharp end point. It is the analyst's responsibility to determine individually the optimal indicator concentration.

1) *Eriochrome Black T:* Sodium salt of 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid; No. 203 in the Color Index. Dissolve 0.5 g dye in 100 g 2,2',2''-nitrilotriethanol (also called triethanolamine) or 2-methoxymethanol (also called ethylene glycol monomethyl ether). Add 2 drops per 50 mL solution to be titrated. Adjust volume if necessary.

2) *Calmagite:* 1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid. This is stable in aqueous solution and produces the same color change as Eriochrome Black T, with a sharper end point. Dissolve 0.10 g Calmagite in 100 mL distilled water. Use 1 mL per 50 mL solution to be titrated. Adjust volume if necessary.

3) Indicators 1 and 2 can be used in dry powder form if care is taken to avoid excess indicator. Prepared dry mixtures of these indicators and an inert salt are available commercially.

If the end point color change of these indicators is not clear and sharp, it usually means that an appropriate complexing agent is required. If NaCN inhibitor does not sharpen the end point, the indicator probably is at fault.

d. Standard EDTA titrant, 0.01M: Weigh 3.723 g analytical reagent-grade disodium ethylenediaminetetraacetate dihydrate, also called (ethylenedinitrilo)tetraacetic acid disodium salt (EDTA), dissolve in distilled water, and dilute to 1000 mL. Standardize against standard calcium solution (§ 2e) as described in § 3b below.

Because the titrant extracts hardness-producing cations from soft-glass containers, store in polyethylene (preferable) or borosilicate glass bottles. Compensate for gradual deterioration by periodic restandardization and by using a suitable correction factor.

e. Standard calcium solution: Weigh 1.000 g anhydrous CaCO_3 powder (primary standard or special reagent low in heavy metals, alkalis, and magnesium) into a 500-mL erlenmeyer flask. Place a funnel in the flask neck and add, a little at a time, 1 + 1 HCl until all CaCO_3 has dissolved. Add 200 mL distilled water and boil for a few minutes to expel CO_2 . Cool, add a few drops of methyl red indicator, and adjust to the intermediate orange color by adding 3N NH_4OH or 1 + 1 HCl, as required. Transfer quantitatively and dilute to 1000 mL with distilled water; 1 mL = 1.00 mg CaCO_3 .

f. Sodium hydroxide, NaOH, 0.1N.

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3. Procedure

a. Pretreatment of polluted water and wastewater samples: Use nitric acid-sulfuric acid or nitric acid-perchloric acid digestion (Section 3030).

b. Titration of sample: Select a sample volume that requires less than 15 mL EDTA titrant and complete titration within 5 min, measured from time of buffer addition.

Dilute 25.0 mL sample to about 50 mL with distilled water in a porcelain casserole or other suitable vessel. Add 1 to 2 mL buffer solution. Usually 1 mL will be sufficient to give a pH of 10.0 to 10.1. The absence of a sharp end-point color change in the titration usually means that an inhibitor must be added at this point (§ 2b et seq.) or that the indicator has deteriorated.

Add 1 to 2 drops indicator solution or an appropriate amount of dry-powder indicator formulation [§ 2c3)]. Add standard EDTA titrant slowly, with continuous stirring, until the last reddish tinge disappears. Add the last few drops at 3- to 5-s intervals. At the end point the solution normally is blue. Daylight or a daylight fluorescent lamp is recommended highly because ordinary incandescent lights tend to produce a reddish tinge in the blue at the end point.

If sufficient sample is available and interference is absent, improve accuracy by increasing sample size, as described in § 3c below.

c. Low-hardness sample: For ion-exchanger effluent or other softened water and for natural waters of low hardness (less than 5 mg/L), take a larger sample, 100 to 1000 mL, for titration and add proportionately larger amounts of buffer, inhibitor, and indicator. Add standard EDTA titrant slowly from a microburet and run a blank, using redistilled, distilled, or deionized water of the same volume as the sample, to which identical amounts of buffer, inhibitor, and indicator have been added. Subtract volume of EDTA used for blank from volume of EDTA used for sample.

4. Calculation

$$\text{Hardness (EDTA) as mg CaCO}_3\text{/L} = \frac{A \times B \times 1000}{\text{mL sample}}$$

where:

A = mL titration for sample and

B = mg CaCO₃ equivalent to 1.00 mL EDTA titrant.

5. Precision and Bias

A synthetic sample containing 610 mg/L total hardness as CaCO₃ contributed by 108 mg Ca/L and 82 mg Mg/L, and the following supplementary substances: 3.1 mg K/L, 19.9 mg Na/L, 241 mg Cl⁻/L, 0.25 mg NO₂⁻-N/L, 1.1 mg NO₃⁻-N/L, 259 mg SO₄²⁻/L, and 42.5 mg total alkalinity/L (contributed by NaHCO₃) in distilled water was analyzed in 56 laboratories by the

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EDTA titrimetric method with a relative standard deviation of 2.9% and a relative error of 0.8%.

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2350 OXIDANT DEMAND/REQUIREMENT*(32)

2350 A. Introduction

1. Significance and Chemistry

Oxidants are added to water supplies and wastewater primarily for disinfection. Other beneficial uses include slime removal, oxidation of undesirable inorganic species (e.g., ferrous ion, reduced manganese, sulfide, and ammonia) and oxidation of organic constituents (e.g., taste- and odor-producing compounds). Oxidant demand refers to the difference between the added oxidant dose and the residual oxidant concentration measured after a prescribed contact time at a given pH and temperature. Oxidant requirement refers to the oxidant dose required to achieve a given oxidant residual at a prescribed contact time, pH, and temperature.

The fate of oxidants in water and wastewater is complex. For example, chlorine reacts with sample constituents by three general pathways: oxidation, addition, and substitution. First, chlorine can oxidize reduced species, such as Fe^{2+} , Mn^{2+} , and sulfide. In these reactions, chlorine is reduced to inorganic chloride (Cl^-). Second, chlorine can add to olefins and other double-bond-containing organic compounds to produce chlorinated organic compounds. Third, chlorine can substitute onto chemical substrates. The addition and substitution reactions produce

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organochlorine species (e.g., chlorination of phenol to chlorophenols) or active chlorine species (e.g., chlorination of ammonia to produce monochloramine). Chlorine reacts with naturally occurring organic compounds by a combination of these mechanisms to generate such products as trihalomethanes. See Section 4500-Cl (chlorine), 4500-ClO₂ (chlorine dioxide), and 4500-O₃ (ozone) for additional information.

Oxidant demand and oxidant requirement are significantly affected by the chemical and physical characteristics of the sample and the manner in which the oxidant consumption is measured. In particular, oxidant reactivity is influenced by temperature, pH, contact time, and oxidant dose. Oxidant demand and oxidant requirement are defined operationally by the analytical method used to determine the residual oxidant concentration. *Report sample temperature, pH, contact time, oxidant dose, and analytical method with oxidant demand or oxidant requirement.* Sample temperature strongly affects reaction kinetics and thus the demand exerted in a given contact time. Sample pH affects the form of the oxidant and the nature and extent of the demand. For example, ozone is unstable at high pH values and ozone demand is especially sensitive to sample pH. Oxidant demand increases with time and therefore the demand must be defined for a given contact time. Oxidant demand also is dependent on oxidant dose. Increasing oxidant dose usually will increase demand, but it is incorrect to assume that doubling the oxidant dose will double the oxidant demand. For these reasons, it is difficult to extrapolate oxidant demand data from one set of conditions to another. *Always study oxidant consumption under the range of conditions expected in the field.*

Oxidant consumption is used to evaluate oxidant demand and oxidant requirement. Report consumption values according to the objective of the study. For example, report chlorine *demand* as follows: “The sample dosed at 5.0 mg/L consumed 3.9 mg/L after 24 h at 20°C and pH 7.1, as measured by amperometric titration.” By contrast, report ozone *requirement* as follows: “The sample required a dose of 2.1 mg/L to achieve an ozone residual of 0.5 mg/L after 20 min at 15°C and pH 6.5, as measured by the indigo method.”

2. Selection of Method

Select a method to measure oxidant residuals used in the demand calculation that is specific and has adequate sensitivity. Some oxidant residual measurement techniques are subject to interferences from oxidation-produced oxidants. Interferences affect oxidant demand measurements because the concentrations of the interferents may change as the oxidant residual changes. Thus, calculate free chlorine demand in municipal wastewater as the difference between free chlorine dose and free chlorine residual measured after a desired contact time at a given temperature, pH, and chlorine dose for a specified analytical method. Chlorination of non-nitrified municipal wastewater probably produces chloramines. If the analytical method for free chlorine is subject to interferences from chloramines, then the free chlorine residual measurement will be too large (see Section 4500-Cl.A.3), and the resulting free chlorine demand value will be incorrectly low. It is sometimes difficult to predict the manner in which oxidant-produced oxidants will affect the demand measurement. The best approach is to use the analytical method most specific to the oxidant of interest but always indicate the method with the

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result.

The addition of reagents may cause loss of oxidant residual or other changes in oxidant demand. The loss of total chlorine upon addition of acid and KI is discussed in Section 4500-Cl.A.3a.

2350 B. Chlorine Demand/Requirement

1. General Discussion

a. Principle: The sample is divided into subsamples and each is dosed with the standardized oxidant (chlorine) solution to yield a series of increasing doses. After the appropriate contact time, oxidant residual, pH, and temperature are measured and the demand/requirement determined by difference between initial and final concentrations.

b. Selection of method: Chlorine consumption tests may be made to examine the demand or requirement for total chlorine, free chlorine, combined chlorine, monochloramine, or dichloramine. Specify the chlorine species consumed in the chlorine demand/requirement test. The analytical method should exhibit minimal interferences for the species examined. For demand/requirement studies with free chlorine, use only amperometric titration (Section 4500-Cl.D) or DPD methods (Section 4500-Cl.F and Section 4500-Cl.G).

c. Interference: Refer to Section 4500-Cl.D.1b (amperometric titration), Section 4500-Cl.F.1d (DPD ferrous titrimetric method), or Section 4500-Cl.G.1b (DPD colorimetric method). Pay special attention to interferences caused by oxidation products such as MnO_2 , NH_2Cl , and NHCl_2 . If the ammonia or organic nitrogen content of the water is significant, combined chlorine may form. See Section 4500-Cl for details. Under these conditions, expect interferences in the measurement of free chlorine by combined chlorine.

d. Minimum detectable concentration: Because it is calculated by difference, the minimum detectable chlorine demand/requirement is $\sqrt{2}$ times the minimum chlorine residual detectable by the analytical method. For minimum detectable chlorine residual, see Section 4500-Cl.F.1e (DPD ferrous titrimetric method) or Section 4500-Cl.G.1c (DPD colorimetric method). Minimum detectable demand also is influenced by amount of oxidant consumed relative to oxidant dose (see ¶ 6 below).

e. Sampling: Most reliable results are obtained on fresh samples that contain low amounts of suspended solids. If samples will be analyzed within 24 h of collection, refrigerate unacidified at 4°C immediately after collection. To preserve for up to 28 d, freeze unacidified samples at -20°C. Warm chilled samples to desired test condition before analysis.

2. Apparatus

See Section 4500-Cl.D (amperometric titration) or Section 4500-Cl.G (DPD colorimetric method).

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3. Reagents

a. *Chlorine-demand-free water*: See Section 4500-Cl.C.3m. Alternatively, prepare dilutions, blanks, and dosing solutions from high-quality distilled water (preferably carbon-filtered redistilled water).

b. *Acetic acid*, conc (glacial).

c. *Potassium iodide*, KI, crystals.

d. *Standard sodium thiosulfate titrant*, 0.025N: See Section 4500-Cl.B.2d.

e. *Starch indicator solution*: See Section 4500-Cl.B.2e.

f. *Reagents for determining residual chlorine*: See Section 4500-Cl.D.3 (amperometric titration), Section 4500-Cl.F.2 (DPD ferrous titrimetric method), or Section 4500-Cl.G.3 (DPD colorimetric method).

g. *Standard chlorine solution*: Prepare by bubbling chlorine gas through distilled water or by diluting commercially available 5–7% (50 000–70 000 mg/L) sodium hypochlorite. Store in the dark or in a brown, glass-stoppered bottle. Standardize each day of use. A suitable strength of chlorine solution usually will be between 100 and 1000 mg/L, preferably about 100 times estimated chlorine demand. Use a solution of sufficient concentration so that adding the chlorine solution will not increase the volume of the treated portions by more than 5%.

Standardization—Place 2 mL acetic acid and 10 to 15 mL chlorine-demand-free water in a flask. Add about 1 g KI. Measure into the flask a suitable volume of chlorine solution. In choosing a convenient volume, note that 1 mL 0.025N $\text{Na}_2\text{S}_2\text{O}_3$ titrant is equivalent to about 0.9 mg chlorine as Cl_2 . Select volumes that will require no more than 20 mL titrant.

Titrate with standardized 0.025N $\text{Na}_2\text{S}_2\text{O}_3$ titrant until the yellow iodine color almost disappears. Add 1 to 2 mL starch indicator solution and continue titrating until the blue color disappears.

Determine the blank by adding identical quantities of acid, KI, and starch indicator to a volume of chlorine-demand-free water corresponding to the sample used for titration. Perform whichever blank titration applies, according to Section 4500-Cl.B.3d. Calculate the chlorine stock concentration as described in Section 4500-Cl.B.4.

4. Procedure

Measure sample temperature and pH. Keep sample and sample portions at desired temperature and protect from light throughout the procedure. If pH adjustment is desired, prepare a blank in distilled water containing the same amount of buffer as in the sample. Carry the blank throughout the procedure.

Measure 5*(33) equal sample portions of 200 mL†(34) each into glass-stoppered bottles or flasks of ample capacity to permit mixing. Add increasing amounts of standard chlorine solution (¶ 3g) to successive portions in the series. Try to bracket the estimated demand/requirement and satisfy criteria of ¶ 5a. Increase dosage between portions in increments of 0.1 mg/L for

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determining low demands/requirements and up to 1.0 mg/L or more for higher demands. Mix while adding. Dose sample portions according to a staggered schedule that will permit determining the residual after predetermined contact times.

Conduct test over desired contact period. Record contact time. At end of contact period, measure sample temperature, sample pH, and residual chlorine. Record residual measurement method used.

5. Calculation

a. *Chlorine demand*: Select sample portion with a residual at the end of the contact period that satisfies the following criteria:

- 1) $R_s < D_s - 1.4 R_{min}$,
- 2) $R_s > R_{min}$, and
- 3) Dose is most similar to the dosage range expected in the field

where:

R_s = residual after contact time, mg/L,

D_s = dose, mg/L, and

R_{min} = minimum residual measurable by the method, mg/L.

The first two criteria insure that the chlorine residual and demand are greater than their respective minimum detection limits. If no sample portion satisfies all criteria, repeat the test and adjust doses accordingly. Calculate chlorine demand as follows:

$$\text{Chlorine demand, mg/L} = (D_s - R_s) - (D_B - R_B)$$

where R_s and D_s are defined as above, and:

R_B = residual of blank after contact time, mg/L, and

D_B = blank dose, mg/L.

When reporting chlorine demand, include dose, contact time, sample temperature, sample pH, and analytical method.

b. *Chlorine requirement*: Report the chlorine dose that produced the target residual after the desired contact time. When reporting chlorine requirement, include the target residual, contact time, sample temperature, sample pH, and analytical method. Report chlorine demand of blank if it is greater than 10% of the difference between the requirement and the target residual.

6. Precision and Bias

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For data on precision and bias of concentration measurements, see analytical method used. Because demand is calculated by difference, the uncertainty associated with the demand value will be greater than the uncertainty of the individual residual measurements. If the standard deviations of the dose measurement and residual measurements are the same, then the standard deviation and minimum detection limit of the oxidant demand will be $\sqrt{2}$ (approximately 1.4) times the standard deviation and minimum detection limit of the measurement technique, respectively.

The chlorine dose and amount consumed affect the precision and bias of demand calculation in two ways. First, the amount consumed must be sufficiently large, relative to the dose, to minimize errors associated with a value calculated from the difference of two numbers of approximately equal value. Second, the amount consumed must be small enough, relative to the dose, to prevent the residual concentration from being too small.

7. Bibliography

See Section 4500-Cl.D.7 or Section 4500-Cl.F.6, according to analytical method used.

2350 C. Chlorine Dioxide Demand/Requirement

1. General Discussion

a. *Principle:* See Section 2350B.1. Chlorine dioxide consumption studies are made by dosing samples from a ClO_2 stock solution.

b. *Selection of method:* Use the amperometric method II (Section 4500- ClO_2 .E) because of its high degree of accuracy and minimal interferences.

c. *Interference:* See Section 4500- ClO_2 .E.1b.

d. *Minimum detectable concentration:* The minimum detectable chlorine dioxide demand/requirement is $\sqrt{2}$ times the minimum chlorine dioxide residual detectable by the analytical method (see Section 2350B.1d and Section 2350B.6).

2. Apparatus

See Section 4500- ClO_2 .E.2.

3. Reagents

See Section 4500- ClO_2 .B.2 to prepare and standardize ClO_2 .

See Section 4500- ClO_2 .E.3 for reagents required to determine ClO_2 residual.

4. Procedure

Follow procedure of Section 2350B.4, using ClO_2 solution, rather than chlorine solution, for

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dosing sample portions.

Follow procedure of Section 4500-ClO₂.E.4 to measure ClO₂ residual.

5. Calculation

a. *Chlorine dioxide demand*: See Section 2350B.5a.

b. *Chlorine dioxide requirement*: See Section 2350B.5b.

6. Precision and Bias

See Section 2350B.6.

7. Bibliography

See Section 4500-ClO₂.E.6 and Section 4500-ClO₂.E.7.

2350 D. Ozone Demand/Requirement—Batch Method

1. General Discussion

a. *Principle*: See Section 2350B.1. Samples can be ozonated in batch and semi-batch modes. In batch ozone consumption studies, an ozone stock solution is used to add ozone to the samples. In semi-batch ozone consumption studies, a stream of ozone gas is added continuously to the sample.

Ozone decomposes at high pH. Thus, even pH-buffered distilled water has a non-zero ozone demand/requirement. Analyze blanks with all ozone consumption tests. Do not subtract the ozone demand of the blank from the ozone demand of the sample, but report it separately.

b. *Selection of method*: Ozone produces oxidants that interfere with iodometric methods. The indigo method (Section 4500-O₃.B) is recommended for measuring ozone residuals in ozone consumption studies. The indigo method measures only the demand for ozone; it does not measure the demand for ozone-produced oxidants such as the hydroxyl radical.

c. *Interference*: See Section 4500-O₃.B.1b.

d. *Minimum detectable concentration*: The minimum detectable ozone demand/requirement is $\sqrt{2}$ times the minimum ozone residual detectable by the analytical method (see Section 2350B.1d and Section 2350B.6).

For minimum detectable ozone residuals, see Section 4500-O₃.B.1c.

2. Apparatus

a. *Ozone generator*: Use a laboratory-scale ozonator, capable of providing up to about 5% ozone in the gas phase at a gas flow of up to about 1 L/min.

b. *Apparatus for measuring residual ozone*: See Section 4500-O₃.B.2.

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3. Reagents

a. *Ozone-demand-free water*: Ozonate reagent water (see Section 1080) for at least 1 h and purge with nitrogen gas for at least 1 h. CAUTION: *Conduct all laboratory ozonations under a vented hood.*

b. *Standard ozone solution*: Put about 800 mL of ozone-demand-free water in a 1-L flask. Bubble ozone (approximately 1 to 5% O₃ in the gas phase) through the water for about 30 min while stirring. At room temperature the ozone solution will contain about 10 to 20 mg O₃/L. If the flask is cooled in an ice bath throughout the procedure, the ozone concentration will be about 30 to 40 mg O₃/L. Standardize the ozone solution by the indigo method. Use a small sample volume (typically 1 mL) as directed in Section 4500-O₃.B.4a3).

4. Procedure

Follow procedure of Section 2350B.4, using standard ozone solution, rather than chlorine solution, for dosing sample portions. Carry a reagent blank through the procedure.

Follow procedure of Section 4500-O₃.B.4 to measure O₃ residual.

5. Calculation

a. *Ozone demand*: See Section 2350B.5a for selection of the proper sample portion. Calculate ozone demand in sample as follows:

$$\text{Ozone demand, mg/L} = D_S - R_S$$

where:

R_S = oxidant residual of sample after contact time, mg/L, and

D_S = sample oxidant dose, mg/L.

Calculate ozone demand in the blank separately.

$$\text{Ozone demand of blank, mg/L} = D_B - R_B$$

where:

R_B = oxidant residual of blank after contact time, mg/L, and

D_B = blank oxidant dose, mg/L.

Report the ozone demand and the ozone demand of the blank, ozone dose, contact time, sample temperature, sample pH, and analytical method.

b. *Ozone requirement*: See Section 2350B.5b.

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6. Precision and Bias

See Section 2350B.6.

7. Bibliography

See Section 4500-O₃.B.7 and Section 4500-O₃.B.8.

2350 E. Ozone Demand/Requirement—Semi-Batch Method

1. General Discussion

See Section 2350D.1.

The semi-batch method involves determination of ozone demand with the continuous addition of gaseous ozone to a batch reactor. The results obtained in this method depend on the mass-transfer characteristics of the reactor. In addition, some compounds that consume ozone may volatilize during the test.

2. Apparatus

All apparatus listed in Section 2350D.2 is required, plus:

- Gas washing bottles*, borosilicate glass, minimum volume 250 mL.
- Tubing*: Use only stainless steel or TFE tubing.
- Glassware*: Buret, 50 mL; beaker, 400 mL; graduated cylinder, 250 mL.
- Wash bottle*, 500 mL.
- Magnetic stirrer* (optional).

3. Reagents

- Ozone-demand-free water*: See Section 2350D.3a.
- Sulfuric acid*, H₂SO₄, 2*N*: Cautiously add 56 mL conc H₂SO₄ to 800 mL ozone-demand-free water in a 1-L volumetric flask. Mix thoroughly, cool, add up to mark with ozone-demand-free water.
- Potassium iodide*, KI: Dissolve 20 g KI in about 800 mL of ozone-demand-free water in a 1-L volumetric flask. Make up to mark with ozone-demand-free water.
- Standard sodium thiosulfate titrant*, Na₂S₂O₃, 0.1*N*: See Section 4500-Cl.B.2c.
- Standard sodium thiosulfate titrant*, Na₂S₂O₃, 0.005*N*: Dilute the proper volume (approximately 50 mL) of standardized 0.1*N* Na₂S₂O₃ to 1 L.
- Starch indicator solution*: See Section 4500-Cl.B.2e.

4. Procedure

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Determine the output of the ozone generator by passing the ozone gas through two serial KI traps (Traps A and B) for about 10 min. For best results, keep gas flow below approximately 1 L/min. Each trap is a gas washing bottle containing a known volume (at least 200 mL) of 2% KI. Quantitatively transfer contents of each trap into a beaker, add 10 mL of 2N H₂SO₄, and titrate with standardized 0.005N Na₂S₂O₃ until the yellow iodine color almost disappears. Add 1 to 2 mL starch indicator solution and continue titrating to the disappearance of blue color.

Put a known volume (at least 200 mL) of sample in a separate gas washing bottle (label gas washing bottles to avoid contaminating the reaction vessel with iodide). Direct ozone gas through this reaction vessel. For ozone demand studies, direct gas stream leaving reaction vessel through a KI trap (Trap C) prepared as above. Ozonate sample for a given contact time. For ozone demand studies, turn ozonator off at end of contact time and pour contents of Trap C into a beaker. Add 10 mL 2N H₂SO₄ and titrate with 0.005N Na₂S₂O₃ as described above. For ozone requirement studies, remove a portion from the reaction vessel at the end of contact time and measure residual ozone concentration by the indigo method.

5. Calculation

a. Ozone dose:

$$\text{Ozone dose, mg/min} = \frac{(A + B) \times N \times 24}{T}$$

where:

A = mL titrant for Trap A,
B = mL titrant for Trap B,
N = normality of Na₂S₂O₃, and
T = ozonation time, min.

b. Ozone demand:

$$\text{Ozone demand, mg/min} = \text{ozone dose, mg/min} - \frac{C \times N \times 24}{T}$$

where:

C = mL titrant for Trap C.

Report sample ozone demand and blank ozone demand, ozone dose, ozonation time, sample temperature, sample pH, sample volume, and analytical method. Because the ozone transfer rate is highly dependent on experimental conditions, also report vessel volume, vessel type, gas flow rate, and sample volume.

c. Ozone requirement: The ozone requirement in the semi-batch test is the ozone dose,

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mg/min, required to obtain the target ozone residual after the desired ozonation time. See Section 2350E.5a to calculate dose. When reporting ozone requirement, also include target oxidant residual as well as other experimental characteristics listed in ¶ 5b above.

6. Precision and Bias

See Section 2350B.6.

7. Bibliography

See Section 4500-O₃.B.7 and Section 4500-O₃.B.8.

2510 CONDUCTIVITY*(35)

2510 A. Introduction

Conductivity, k , is a measure of the ability of an aqueous solution to carry an electric current. This ability depends on the presence of ions; on their total concentration, mobility, and valence; and on the temperature of measurement. Solutions of most inorganic compounds are relatively good conductors. Conversely, molecules of organic compounds that do not dissociate in aqueous solution conduct a current very poorly, if at all.

1. Definitions and Units of Expression

Conductance, G , is defined as the reciprocal of resistance, R :

$$G = \frac{1}{R}$$

where the unit of R is ohm and G is ohm⁻¹ (sometimes written mho). Conductance of a solution is measured between two spatially fixed and chemically inert electrodes. To avoid polarization at the electrode surfaces the conductance measurement is made with an alternating current signal.¹ The conductance of a solution, G , is directly proportional to the electrode surface area, A , cm², and inversely proportional to the distance between the electrodes, L , cm. The constant of proportionality, k , such that:

$$G = k \left(\frac{A}{L} \right)$$

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is called “conductivity” (preferred to “specific conductance”). It is a characteristic property of the solution between the electrodes. The units of k are 1/ohm-cm or mho per centimeter.

Conductivity is customarily reported in micromhos per centimeter ($\mu\text{mho/cm}$).

In the International System of Units (SI) the reciprocal of the ohm is the siemens (S) and conductivity is reported as millisiemens per meter (mS/m); 1 mS/m = 10 $\mu\text{mhos/cm}$ and 1 $\mu\text{S/cm}$ = 1 $\mu\text{mho/cm}$. To report results in SI units of mS/m divide $\mu\text{mhos/cm}$ by 10.

To compare conductivities, values of k are reported relative to electrodes with $A = 1 \text{ cm}^2$ and $L = 1 \text{ cm}$. Absolute conductances, G_s , of standard potassium chloride solutions between electrodes of precise geometry have been measured; the corresponding standard conductivities, k_s , are shown in Table 2510:I.

The equivalent conductivity, Λ , of a solution is the conductivity per unit of concentration. As the concentration is decreased toward zero, Λ approaches a constant, designated as Λ° . With k in units of micromhos per centimeter it is necessary to convert concentration to units of equivalents per cubic centimeter; therefore:

$$\Lambda = 0.001k/\text{concentration}$$

where the units of Λ , k , and concentration are mho-cm²/equivalent, $\mu\text{mho/cm}$, and equivalent/L, respectively. Equivalent conductivity, Λ , values for several concentrations of KCl are listed in Table 2510:I. In practice, solutions of KCl more dilute than 0.001M will not maintain stable conductivities because of absorption of atmospheric CO₂. Protect these dilute solutions from the atmosphere.

2. Measurement

a. Instrumental measurements: In the laboratory, conductance, G_s , (or resistance) of a standard KCl solution is measured and from the corresponding conductivity, k_s , (Table 2510:I) a cell constant, C , cm⁻¹, is calculated:

$$C = \frac{k_s}{G_s}$$

Most conductivity meters do not display the actual solution conductance, G , or resistance, R ; rather, they generally have a dial that permits the user to adjust the internal cell constant to match the conductivity, k_s , of a standard. Once the cell constant has been determined, or set, the conductivity of an unknown solution,

$$k_u = CG_u$$

will be displayed by the meter.

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Distilled water produced in a laboratory generally has a conductivity in the range 0.5 to 3 $\mu\text{mhos/cm}$. The conductivity increases shortly after exposure to both air and the water container.

The conductivity of potable waters in the United States ranges generally from 50 to 1500 $\mu\text{mhos/cm}$. The conductivity of domestic wastewaters may be near that of the local water supply, although some industrial wastes have conductivities above 10 000 $\mu\text{mhos/cm}$. Conductivity instruments are used in pipelines, channels, flowing streams, and lakes and can be incorporated in multiple-parameter monitoring stations using recorders.

Most problems in obtaining good data with conductivity monitoring equipment are related to electrode fouling and to inadequate sample circulation. Conductivities greater than 10 000 to 50 000 $\mu\text{mho/cm}$ or less than about 10 $\mu\text{mho/cm}$ may be difficult to measure with usual measurement electronics and cell capacitance. Consult the instrument manufacturer's manual or published references.^{1,5,6}

Laboratory conductivity measurements are used to:

- Establish degree of mineralization to assess the effect of the total concentration of ions on chemical equilibria, physiological effect on plants or animals, corrosion rates, etc.
- Assess degree of mineralization of distilled and deionized water.
- Evaluate variations in dissolved mineral concentration of raw water or wastewater. Minor seasonal variations found in reservoir waters contrast sharply with the daily fluctuations in some polluted river waters. Wastewater containing significant trade wastes also may show a considerable daily variation.
- Estimate sample size to be used for common chemical determinations and to check results of a chemical analysis.
- Determine amount of ionic reagent needed in certain precipitation and neutralization reactions, the end point being denoted by a change in slope of the curve resulting from plotting conductivity against buret readings.
- Estimate total dissolved solids (mg/L) in a sample by multiplying conductivity (in micromhos per centimeter) by an empirical factor. This factor may vary from 0.55 to 0.9, depending on the soluble components of the water and on the temperature of measurement. Relatively high factors may be required for saline or boiler waters, whereas lower factors may apply where considerable hydroxide or free acid is present. Even though sample evaporation results in the change of bicarbonate to carbonate the empirical factor is derived for a comparatively constant water supply by dividing dissolved solids by conductivity.
- Approximate the milliequivalents per liter of either cations or anions in some waters by multiplying conductivity in units of micromhos per centimeter by 0.01.

b. Calculation of conductivity: For naturally occurring waters that contain mostly Ca^{2+} , Mg^{2+} , Na^+ , K^+ , HCO_3^- , SO_4^{2-} , and Cl^- and with TDS less than about 2500 mg/L, the following procedure can be used to calculate conductivity from measured ionic concentrations.⁷ The abbreviated water analysis in Table 2510:II illustrates the calculation procedure.

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At infinite dilution the contribution to conductivity by different kinds of ions is additive. In general, the relative contribution of each cation and anion is calculated by multiplying equivalent conductances, λ°_+ and λ°_- , mho-cm²/equivalent, by concentration in equivalents per liter and correcting units. Table 2510:III contains a short list of equivalent conductances for ions commonly found in natural waters.⁸ Trace concentrations of ions generally make negligible contribution to the overall conductivity. A temperature coefficient of 0.02/deg is applicable to all ions, except H⁺ (0.0139/deg) and OH⁻ (0.018/deg).

At finite concentrations, as opposed to infinite dilution, conductivity per equivalent decreases with increasing concentration (see Table 2510:I). For solutions composed of one anion type and one cation type, e.g., KCl as in Table 2510:I, the decrease in conductivity per equivalent with concentration can be calculated, $\pm 0.1\%$, using an ionic-strength-based theory of Onsager.⁹ When mixed salts are present, as is nearly always the case with natural and wastewaters, the theory is quite complicated.¹⁰ The following semiempirical procedure can be used to calculate conductivity for naturally occurring waters:

First, calculate infinite dilution conductivity (Table 2510:II, Column 4):

$$k^\circ = \sum |z_i|(\lambda^\circ_{+i})(mM_i) + \sum |z_i|(\lambda^\circ_{-i})(mM_i)$$

where:

$|z_i|$ = absolute value of the charge of the i -th ion,

mM_i = millimolar concentration of the i -th ion, and

λ°_{+i} , λ°_{-i} = equivalent conductance of the i -th ion.

If mM is used to express concentration, the product, $(\lambda^\circ_{+i})(mM_i)$ or $(\lambda^\circ_{-i})(mM_i)$, corrects the units from liters to cm³. In this case k° is 578.2 $\mu\text{mho/cm}$ (Table 2510:II, Column 4).

Next, calculate ionic strength, IS in molar units:

$$IS = \sum z_i^2(mM_i)/2000$$

The ionic strength is $15.33/2000 = 0.00767\text{ M}$ (Table 2510:II, Column 5).

Calculate the monovalent ion activity coefficient, y , using the Davies equation for $IS \leq 0.5\text{ M}$ and for temperatures from 20 to 30°C.^{9,11}

$$y = 10^{-0.5[IS^{1/2}/(1 + IS^{1/2}) - 0.3IS]}$$

In the present example $IS = 0.00767\text{ M}$ and $y = 0.91$.

Finally, obtain the calculated value of conductivity, k_{calc} , from:

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$$k_{\text{calc}} = k^{\circ}y^2$$

In the example being considered, $k_{\text{calc}} = 578.2 \times 0.91^2 = 478.8 \mu\text{mho/cm}$ versus the reported value as measured by the USGS of $477 \mu\text{mho/cm}$.

For 39 analyses of naturally occurring waters,^{7,12} conductivities calculated in this manner agreed with the measured values to within 2%.

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2510 B. Laboratory Method

1. General Discussion

See Section 2510A.

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2. Apparatus

a. *Self-contained conductivity instruments:* Use an instrument capable of measuring conductivity with an error not exceeding 1% or 1 $\mu\text{mho}/\text{cm}$, whichever is greater.

b. *Thermometer,* capable of being read to the nearest 0.1°C and covering the range 23 to 27°C. Many conductivity meters are equipped to read an automatic temperature sensor.

c. *Conductivity cell:*

1) Platinum-electrode type—Conductivity cells containing platinized electrodes are available in either pipet or immersion form. Cell choice depends on expected range of conductivity. Experimentally check instrument by comparing instrumental results with true conductivities of the KCl solutions listed in Table 2510:I. Clean new cells, not already coated and ready for use, with chromic-sulfuric acid cleaning mixture [see Section 2580B.3a2)] and platinize the electrodes before use. Subsequently, clean and replatinize them whenever the readings become erratic, when a sharp end point cannot be obtained, or when inspection shows that any platinum black has flaked off. To platinize, prepare a solution of 1 g chloroplatinic acid, $\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$, and 12 mg lead acetate in 100 mL distilled water. A more concentrated solution reduces the time required to platinize electrodes and may be used when time is a factor, e.g., when the cell constant is 1.0/cm or more. Immerse electrodes in this solution and connect both to the negative terminal of a 1.5-V dry cell battery. Connect positive side of battery to a piece of platinum wire and dip wire into the solution. Use a current such that only a small quantity of gas is evolved. Continue electrolysis until both cell electrodes are coated with platinum black. Save platinizing solution for subsequent use. Rinse electrodes thoroughly and when not in use keep immersed in distilled water.

2) Nonplatinum-electrode type—Use conductivity cells containing electrodes constructed from durable common metals (stainless steel among others) for continuous monitoring and field studies. Calibrate such cells by comparing sample conductivity with results obtained with a laboratory instrument. Use properly designed and mated cell and instrument to minimize errors in cell constant. Very long meter leads can affect performance of a conductivity meter. Under such circumstances, consult the manufacturer's manual for appropriate correction factors if necessary.

3. Reagents

a. *Conductivity water:* Any of several methods can be used to prepare reagent-grade water. The methods discussed in Section 1080 are recommended. The conductivity should be small compared to the value being measured.

b. *Standard potassium chloride solution, KCl, 0.0100M:* Dissolve 745.6 mg anhydrous KCl in conductivity water and dilute to 1000 mL in a class A volumetric flask at 25°C and store in a CO_2 -free atmosphere. This is the standard reference solution, which at 25°C has a conductivity of 1412 $\mu\text{mhos}/\text{cm}$. It is satisfactory for most samples when the cell has a constant between 1

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and 2 cm^{-1} . For other cell constants, use stronger or weaker KCl solutions listed in Table 2510:I. Care must be taken when using KCl solutions less than $0.001M$, which can be unstable because of the influence of carbon dioxide on pure water. For low conductivity standards, Standard Reference Material 3190, with a certified conductivity of $25.0 \mu\text{S/cm} \pm 0.3 \mu\text{S/cm}$, may be obtained from NIST. Store in a glass-stoppered borosilicate glass bottle.

4. Procedure

a. Determination of cell constant: Rinse conductivity cell with at least three portions of $0.01M$ KCl solution. Adjust temperature of a fourth portion to $25.0 \pm 0.1^\circ\text{C}$. If a conductivity meter displays resistance, R , ohms, measure resistance of this portion and note temperature. Compute cell constant, C :

$$C, \text{ cm}^{-1} = (0.001412)(R_{\text{KCl}})[1 + 0.0191(t - 25)]$$

where:

R_{KCl} = measured resistance, ohms, and

t = observed temperature, $^\circ\text{C}$.

Conductivity meters often indicate conductivity directly. Commercial probes commonly contain a temperature sensor. With such instruments, rinse probe three times with $0.0100M$ KCl, as above. Adjust temperature compensation dial to 0.0191 C^{-1} . With probe in standard KCl solution, adjust meter to read $1412 \mu\text{mho/cm}$. This procedure automatically adjusts cell constant internal to the meter.

b. Conductivity measurement: Thoroughly rinse cell with one or more portions of sample. Adjust temperature of a final portion to about 25°C . Measure sample resistance or conductivity and note temperature to $\pm 0.1^\circ\text{C}$.

5. Calculation

The temperature coefficient of most waters is only approximately the same as that of standard KCl solution; the more the temperature of measurement deviates from 25.0°C , the greater the uncertainty in applying the temperature correction. Report temperature-compensated conductivities as “ $\mu\text{mho/cm } 25.0^\circ\text{C}$.”

a. When sample resistance is measured, conductivity at 25°C is:

$$k = \frac{(1\,000\,000)(C)}{R_m[1 + 0.0191(t - 25)]}$$

where:

k = conductivity, $\mu\text{mhos/cm}$,

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C = cell constant, cm^{-1} ,
 R_m = measured resistance of sample, ohms, and
 t = temperature of measurement.

b. When sample conductivity is measured without internal temperature compensation conductivity at 25°C is:

$$k, \mu\text{mho/cm} = \frac{(k_m)}{1 + 0.0191(t - 25)}$$

where:

k_m = measured conductivity in units of $\mu\text{mho/cm}$ at $t^\circ\text{C}$, and other units are defined as above.

For instruments with automatic temperature compensation and readout directly in $\mu\text{mho/cm}$ or similar units, the readout automatically is corrected to 25.0°C. Report displayed conductivity in designated units.

6. Precision and Bias

The precision of commercial conductivity meters is commonly between 0.1 and 1.0%. Reproducibility of 1 to 2% is expected after an instrument has been calibrated with such data as is shown in Table 2510:I.

2520 SALINITY*#(36)

2520 A. Introduction

1. General Discussion

Salinity is an important unitless property of industrial and natural waters. It was originally conceived as a measure of the mass of dissolved salts in a given mass of solution. The experimental determination of the salt content by drying and weighing presents some difficulties due to the loss of some components. The only reliable way to determine the true or absolute salinity of a natural water is to make a complete chemical analysis. However, this method is time-consuming and cannot yield the precision necessary for accurate work. Thus, to determine salinity, one normally uses indirect methods involving the measurement of a physical property such as conductivity, density, sound speed, or refractive index. From an empirical relationship of salinity and the physical property determined for a standard solution it is possible

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to calculate salinity. The resultant salinity is no more accurate than the empirical relationship. The precision of the measurement of a physical property will determine the precision in salinity. Following are the precisions of various physical measurements and the resultant salinity presently attainable with commercial instruments:

Property	Precision of Measurement	Precision of Salinity
Conductivity	± 0.0002	± 0.0002
Density	$\pm 3 \times 10^{-6} \text{ g/cm}^3$	± 0.004
Sound speed	$\pm 0.02 \text{ m/s}$	± 0.01

Although conductivity has the greatest precision, it responds only to ionic solutes. Density, although less precise, responds to all dissolved solutes.

2. Selection of Method

In the past, the salinity of seawater was determined by hydrometric and argentometric methods, both of which were included in previous editions of *Standard Methods*. In recent years the conductivity (2520B) and density (2520C) methods have been used because of their high sensitivity and precision. These two methods are recommended for precise field and laboratory work.

3. Quality Assurance

Calibrate salinometer or densimeter against standards of KCl or standard seawater. Expected precision is better than ± 0.01 salinity units with careful analysis and use of bracketing standards.

2520 B. Electrical Conductivity Method

1. Determination

See Conductivity, Section 2510. Because of its high sensitivity and ease of measurement, the conductivity method is most commonly used to determine salinity.¹ For seawater measurements use the Practical Salinity Scale 1978.²⁻⁵ This scale was developed relative to a KCl solution. A seawater with a conductivity, C , at 15°C equal to that of a KCl solution containing a mass of 32.4356 g in a mass of 1 kg of solution is defined as having a practical salinity of 35. This value was determined as an average of three independent laboratory studies. The salinity dependence of the conductivity ratio, R_t , as a function of temperature ($t^\circ\text{C}$, International Practical Temperature Scale 1968) of a given sample to a standard $S = 35$ seawater is used to determine the salinity

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$$S = a_0 + a_1 R_t^{1/2} + a_2 R_t + a_3 R_t^{3/2} + a_4 R_t^2 + a_5 R_t^{5/2} + S$$

where S is given by

$$\Delta S = \left[\frac{t - 15}{1 + 0.0162 (t - 15)} \right] (b_0 + b_1 R_t^{1/2} + b_2 R_t + b_3 R_t^{3/2} + b_4 R_t^2 + b_5 R_t^{5/2})$$

and:

$a_0 = 0.0080$	$b_0 = 0.0005$
$a_1 = -0.1692$	$b_1 = -0.0056$
$a_2 = 25.3851$	$b_2 = -0.0066$
$a_3 = 14.0941$	$b_3 = -0.0375$
$a_4 = -7.0261$	$b_4 = 0.0636$
$a_5 = 2.7081$	$b_5 = -0.0144$

valid from $S = 2$ to 42, where:

$$R_t = \frac{C \text{ (sample at } t\text{)}}{C \text{ (KCl solution at } t\text{)}}$$

To measure the conductivity, use a conductivity bridge calibrated with standard seawater*#(37) with a known conductivity relative to KCl, following manufacturer's instructions and the procedures noted in Section 2510. If the measurements are to be made in estuarine waters, make secondary calibrations of weight-diluted seawater of known conductivity to ensure that the bridge is measuring true conductivities.

The Practical Salinity Scale recently has been extended to low salinities⁶ to give an equation that is valid from 0 to 40 salinity. The equation is:

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$$S = S_{PSS} - \frac{a_0}{1 + 1.5X + X^2} - \frac{b_0 f(t)}{1 + Y^{1/2} + Y^{3/2}}$$

where:

S_{PSS} = value determined from the Practical Salinity Scale given earlier,

$a_0 = 0.008$,

$b_0 = 0.0005$,

$X = 400R_p$,

$Y = 100R_p$, and

$f(t) = (t-15)/[1 + 0.0162(t-15)]$

The practical salinity breaks with the old salinity-chlorinity relationship, $S = 1.80655 Cl$. Although the scale can be used for estuarine waters⁷⁻¹⁰ and brines¹¹⁻¹³, there are limitations.^{12,14-22}

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2520 C. Density Method

1. Determination

With a precise vibrating flow densimeter, it is possible to make rapid measurements of the density of natural waters. The measurements are made bypassing the sample through a vibrating tube encased in a constant-temperature jacket. The solution density (ρ) is proportional to the square of the period of the vibration (τ).

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$$\rho = A + B\tau^2$$

where A and B are terms determined by calibration, B being determined by calibration with a densimeter with standard seawater. The difference between the density of the sample and that of pure water is given by:

$$\rho - \rho_0 = B(\tau^2 - \tau_0^2)$$

where τ and τ_0 are, respectively, the periods of the sample and water. The system is calibrated with two solutions of known density. Follow manufacturer's recommendations for calibration. These two solutions can be nitrogen gas and water or standard seawater and water. The salinity of the sample can be determined from the 1 atm international equation of state for seawater. This equation relates $(\rho - \rho_0)$ to the practical salinity (S) as a function of temperature.¹

$$\rho \text{ (kg/m}^3\text{)} = \rho_0 + AS + BS^{3/2} + CS^2$$

where:

$$\begin{aligned} A &= 8.244\,93 \times 10^{-1} - 4.0899 \times 10^{-3}t \\ &\quad + 7.6438 \times 10^{-5}t^2 - 8.2467 \times 10^{-7}t^3 + 5.3875 \times 10^{-9}t^4, \\ B &= -5.724\,66 \times 10^{-3} + 1.0227 \times 10^{-4}t - 1.6546 \times 10^{-6}t^2, \\ C &= 4.8314 \times 10^{-4}, \end{aligned}$$

and the density of water is given by:

$$\begin{aligned} \rho_0 &= 999.842\,594 + 6.793\,952 \times 10^{-2}t - 9.095\,290 \times 10^{-3}t^2 \\ &\quad + 1.001\,685 \times 10^{-4}t^3 - 1.120\,083 \times 10^{-6}t^4 + 6.536\,332 \\ &\quad \times 10^{-9}t^5 \end{aligned}$$

Perform simple iteration by adjusting S until it gives the measured $\rho - \rho_0$ at a given temperature. If the measurements are made at 25°C, the salinity can be determined from the following equation:

$$S = 1.3343 (\rho - \rho_0) + 2.155\,306 \times 10^{-4} (\rho - \rho_0)^2 - 1.171\,16 \times 10^{-5} (\rho - \rho_0)^3$$

which has a $\tau = 0.0012$ in S . Approximate salinities also can be determined from densities or specific gravities obtained with a hydrometer at a given temperature (Section 210B, 16th edition).

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2. Reference

1. MILLERO, F.J. & A. POISSON. 1981. International one-atmosphere equation of state of seawater. *Deep Sea Res.* 28:625.

2520 D. Algorithm of Practical Salinity

Because all practical salinity measurements are carried out in reference to the conductivity of standard seawater (corrected to $S = 35$), it is the quantity R_t that will be available for salinity calculations. R_t normally is obtained directly by laboratory salinometers, but *in situ* measurements usually produce the quantity R , the ratio of the *in situ* conductivity to the standard conductivity at $S = 35$, $t = 15^\circ\text{C}$, $p = 0$ (where p is the pressure above one standard atmosphere and the temperature is on the 1968 International Temperature Scale). R is factored into three parts, i.e.,

$$R = R_p r_t R_t$$

where:

R_p = ratio of *in situ* conductivity to conductivity of the same sample at the same temperature, but at $p = 0$ and r_t = ratio of conductivity of reference seawater, having a practical salinity of 35, at temperature t , to its conductivity at $t = 15^\circ\text{C}$. From R_p and r_t calculate R_t using the *in situ* results, i.e.,

$$R_t = \frac{R}{R_p r_t}$$

R_p and r_t can be expressed as functions of the numerical values of the *in situ* parameters, R , t , and p , when t is expressed in $^\circ\text{C}$ and p in bars (10^5 Pa), as follows:

$$R_p = 1 + \frac{p(e_1 + e_2 p + e_3 p^2)}{1 + d_1 t + d_2 t^2 + (d_3 + d_4 t)R}$$

where:

$$e_1 = 2.070 \times 10^{-4}, \quad d_1 = 3.426 \times 10^{-2},$$

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$$\begin{array}{ll} e_2 = -6.370 \times 10^{-8}, & d_2 = 4.464 \times 10^{-4}, \\ e_3 = 3.989 \times 10^{-12}, & d_3 = 4.215 \times 10^{-1}, \\ \text{and} & d_4 = -3.107 \times 10^{-3}, \end{array}$$

and

$$r_t = c_0 + c_1 t + c_2 t^2 + c_3 t^3 + c_4 t^4$$

where:

$$\begin{array}{l} c_0 = 0.676\,609\,7, \\ c_1 = 2.005\,64 \times 10^{-2}, \\ c_2 = 1.104\,259 \times 10^{-4}, \\ c_3 = -6.9698 \times 10^{-7}, \text{ and} \\ c_4 = 1.0031 \times 10^{-9}. \end{array}$$

2530 FLOATABLES#(38)*

2530 A. Introduction

One important criterion for evaluating the possible effect of waste disposal into surface waters is the amount of floatable material in the waste. Two general types of floating matter are found: particulate matter that includes “grease balls,” and liquid components capable of spreading as a thin, highly visible film over large areas. Floatable material in wastewaters is important because it accumulates on the surface, is often highly visible, is subject to wind-induced transport, may contain pathogenic bacteria and/or viruses associated with individual particles, and can significantly concentrate metals and chlorinated hydrocarbons such as pesticides and PCBs. Colloidally dispersed oil and grease behave like other dispersed organic matter and are included in the material measured by the COD, BOD, and TOC tests. The floatable oil test indicates the readily separable fraction. The results are useful in designing oil and grease separators, in ascertaining the efficiency of operating separators, and in monitoring raw and treated wastewater streams. Many cities and districts have specified floatable oil and grease limits for wastewater discharged to sewers.

2530 B. Particulate Floatables (GENERAL)

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1. Discussion

a. Principle: This method depends on the gravity separation of particles having densities less than that of the surrounding water. Particles that collect on the surface and can be filtered out and dried at 103 to 105°C are defined by this test as floatable particles.

b. Application: This method is applicable to raw wastewater, treated primary and secondary effluent, and industrial wastewater. Because of the limited sensitivity, it is not applicable to tertiary effluents or receiving waters, whether freshwater or seawater.

c. Precautions: Even slight differences in sampling and handling during and after collection can give large differences in the measured amount of floatable material. Additionally, uniformity of the TFE#(39)* coating of the separation funnel is critical to obtaining reliable results. For a reproducible analysis treat all samples uniformly, preferably by mixing them in a standard manner, before flotation and use consistently prepared separation funnels as much as possible. Because the procedure relies on the difference in specific gravity between the liquid and the floating particles, temperature variations may affect the results. Conduct the test at a constant temperature the same as that of the receiving water body, and report temperature with results.

d. Minimum detectable concentration: The minimum reproducible detectable concentration is approximately 1 mg/L. Although the minimum levels that can be measured are below 1 mg/L, the results are not meaningful within the current established accuracy of the test.

2. Apparatus

a. Floatables sampler with mixer: Use a metal container of at least 5 L capacity equipped with a propeller mixer on a separate stand (Figure 2530:1), and with a 20-mm-ID bottom outlet cocked at an angle of 45° to the container wall in the direction of fluid movement. The 45° angle assures that even large particles will flow from the container into the flotation funnels where the sample is withdrawn. Fit exterior of bottom outlet with a short piece of tubing and a pinch clamp to allow unrestricted flow through the outlet. Coat inside of container with TFE as uniformly as possible, using a TFE spray to prevent oil and grease from sticking to the surface.

b. Flotation funnel: Use an Imhoff cone provided with a TFE stopcock at the bottom and extended at the top to a total volume of 3.5 L (Figure 2530:2). Coat inside of flotation funnel with TFE as uniformly as possible to prevent floatable grease particles sticking to the sides. Mount flotation funnels as shown in Figure 2530:3 with a light behind the bottom of the funnels to aid in reading levels.

c. Filter holder: Coat inside of top of a standard 500-mL membrane filter holder with TFE, again taking all possible precautions to obtain a uniform TFE coating.

d. Filters, glass fiber, fine porosity.#(40)†

e. Vacuum flask, 500 mL.

f. TFE coating: Follow instructions that accompany commercially available coating kits. Alternatively, have necessary glassware coated commercially. Uniform coatings are key to the

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reliability of the test results, but in practice are difficult to obtain.

3. Procedure

a. *Preparation of glass fiber filters:* See Section 2540D.3a.

b. *Sample collection and treatment:* Collect sample in the floatables sampler at a point of complete mixing, transport to the laboratory, and place 3.0 L in the flotation funnel within 2 h after sample collection to minimize changes in the floatable material. While the flotation funnel is being filled, mix sampler contents with a small propeller mixer. Adjust mixing speed to provide uniform distribution of floating particles throughout the liquid but avoid extensive air entrapment through formation of a large vortex.

c. *Correction for density and for concentration effects:* When a receiving water has a density and ion concentration different from that of the waste, adjust sample density and ion concentration to that of the receiving water. For example, if the receiving water is ocean water, place 1.5 L sample in flotation funnel and add 1.5 L filtered seawater from the receiving area together with mixture of 39.8 g NaCl, 8.0 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, and 2.3 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. The final mixture contains the amount of floatables in a 1.5-L sample in a medium of approximately the same density and ion concentration as seawater.

d. *Flotation:* Mix flotation funnel contents at 40 rpm for 15 min using a paddle mixer (Figure 2530:3). Let settle for 5 min, mix at 100 rpm for 1 min, and let settle for 30 min. Discharge 2.8 L through bottom stopcock at a rate of 500 mL/min. Do not disturb the sample surface in the flotation funnel during discharge. With distilled water from a wash bottle, wash down any floatable material sticking to sides of stirring paddle and funnel. Let remaining 200 mL settle for 15 min and discharge settled solids and liquid down to the 40-mL mark on the Imhoff cone. Let settle again for 10 min and discharge until only 10 mL liquid and the floating particles remain in funnel. Add 500 mL distilled water and stir by hand to separate entrapped settleable particles from the floatable particles. Let settle for 15 min, then discharge to the 40-mL mark. Let settle for 10 min, then discharge dropwise to the 10-mL mark. Filter remaining 10 mL and floating particles through a preweighed glass fiber filter. Wash sides of flotation funnel with distilled water to transfer all floatable material to filter.

e. *Weighing:* Dry and weigh glass fiber filter at 103 to 105° C for exactly 2 h (see Section 2540D.3c).

4. Calculation

$$\text{mg particulate floatables/L} = \frac{(A - B)}{C}$$

where:

A = weight of filter + floatables, mg,

B = weight of filter, mg, and

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C = sample volume, L. (Do not include volume used for density or concentration correction, if used.)

5. Precision and Bias

Precision varies with the concentration of suspended matter in the sample. There is no completely satisfactory procedure for determining the bias of the method for wastewater samples but approximate recovery can be determined by running a second test for floatables on all water discharged throughout the procedure, with the exception of the last 10 mL. Precision and bias are summarized in Table 2530:I. Experience with the method at one municipal treatment plant indicates that the practical lower limit of detection is approximately 1 mg/L.

6. Bibliography

- HEUKELEKIAN, H. & J. BALMAT. 1956. Chemical composition of the particulate fractions of domestic sewage. *Sewage Ind. Wastes* 31:413.
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- BRACEWELL, L.W. 1976. Contribution of Wastewater Discharges to Surface Films and Other Floatables on the Ocean Surface. Thesis, Univ. California, Berkeley.
- BRACEWELL, L.W., R.E. SELLECK & R. CARTER. 1980. Contribution of wastewater discharges to ocean surface particulates. *J. Water Pollut. Control Fed.* 52:2230.

2530 C. Trichlorotrifluoroethane-Soluble Floatable Oil and Grease (GENERAL)

1. Discussion

The floatable oil and grease test does not measure a precise class of substances; rather, the results are determined by the conditions of the test. The fraction measured includes oil and grease, both floating and adhering to the sides of the test vessel. The adhering and the floating portions are of similar practical significance because it is assumed that most of the adhering

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portion would otherwise float under receiving water conditions. The results have been found to represent well the amount of oil removed in separators having overflow rates equivalent to test conditions.

2. Apparatus

a. *Floatable oil tube* (Figure 2530:4): Before use, carefully clean tube by brushing with a mild scouring power. Water must form a smooth film on inside of cleaned glass. Do not use lubricant on stopcock.

b. *Conical flask*, 300 mL.

3. Reagents

a. *1,1,2-trichloro-1,2,2-trifluoroethane* #(41)*: See Section 5520B.3b.

b. *Hydrochloric acid*, HCl, 6N.

c. *Filter paper* #(42)†

4. Procedure

a. *Sampling*: Collect samples at a place where there is a strong turbulence in the water and where floating material is not trapped at the surface. Fill floatable oil tube to mark by dipping into water. *Do not use samples taken to the laboratory in a bottle, because oil and grease cannot be redispersed to their original condition.*

b. *Flotation*: Support tube in a vertical position. Start flotation period at sampling site immediately after filling tube. The standard flotation time is 30 min. If a different time is used, state this variation in reporting results. At end of flotation period, discharge the first 900 mL of water carefully through bottom stopcock, stopping before any surface oil or other floating material escapes. Rotate tube slightly back and forth about its vertical axis to dislodge sludge from sides, and let settle for 5 min. Completely discharge sludge that has settled to the bottom or that comes down from the sides with the liquid. Scum on top of the liquid may mix with the water as it moves down the tube. If mixing occurs, stop drawing off water before any floatables have been lost. Let settle for 5 min before withdrawing remainder of water. After removing water, return tube to laboratory to complete test.

c. *Extraction*: Acidify to pH 2 or lower with a few drops of 6N HCl, add 50 to 100 mL trichlorotrifluoroethane, and shake vigorously. Let settle and draw off solvent into a clean dry beaker. Filter solvent through a dry filter paper into a tared 300-mL conical flask, taking care not to get any water on filter paper. Add a second 50-mL portion of trichlorotrifluoroethane and repeat extraction, settling, and filtration into the same 300-mL flask. A third extraction may be needed if the amount of floatables in sample exceeds 4 mg/L. Wash filter paper carefully with fresh solvent discharged from a wash bottle with a fine tip. Evaporate solvent from flask as described in Section 5520B.4. For each solvent batch, determine weight of residue left after evaporation from the same volume as used in the analysis.

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5. Calculations

Report results as “soluble floatable oil and grease, 30 min (or other specified) settling time, mg/L.”

Trichlorotrifluoroethane-soluble floatable oil and grease, 30 min settling time, mg/L

$$= \frac{(A - B) \times 1000}{\text{mL sample}}$$

where:

A = total gain in weight of tared flask, mg, and

B = calculated residue from solvent blank of the same volume as that used in the test, mg.

6. Precision and Bias

There is no standard against which bias of this test can be determined. Variability of replicates is influenced by sample heterogeneity. If large grease particles are present, the element of chance in sampling may be a major factor. One municipal wastewater discharge and two meat-packing plant discharges, both containing noticeable particles of grease, were analyzed in triplicate. Averages for the three wastewaters were 48, 57, and 25 mg/L; standard deviations averaged 11%. An oil refinery made duplicate determinations of its separator effluent on 15 consecutive days, obtaining results ranging from 5.1 to 11.2 mg/L. The average difference between pairs of samples was 0.37 mg/L.

7. Bibliography

POMEROY, R.D. 1953. Floatability of oil and grease in wastewaters. *Sewage Ind. Wastes* 25:1304.

2540 SOLIDS#(43)*

2540 A. Introduction

Solids refer to matter suspended or dissolved in water or wastewater. Solids may affect water or effluent quality adversely in a number of ways. Waters with high dissolved solids generally are of inferior palatability and may induce an unfavorable physiological reaction in the transient consumer. For these reasons, a limit of 500 mg dissolved solids/L is desirable for drinking waters. Highly mineralized waters also are unsuitable for many industrial applications. Waters high in suspended solids may be esthetically unsatisfactory for such purposes as bathing. Solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory agency wastewater effluent limitations.

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1. Definitions

“Total solids” is the term applied to the material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Total solids includes “total suspended solids,” the portion of total solids retained by a filter, and “total dissolved solids,” the portion that passes through the filter.

The type of filter holder, the pore size, porosity, area, and thickness of the filter and the physical nature, particle size, and amount of material deposited on the filter are the principal factors affecting separation of suspended from dissolved solids. “Dissolved solids” is the portion of solids that passes through a filter of 2.0 μm (or smaller) nominal pore size under specified conditions. “Suspended solids” is the portion retained on the filter.

“Fixed solids” is the term applied to the residue of total, suspended, or dissolved solids after heating to dryness for a specified time at a specified temperature. The weight loss on ignition is called “volatile solids.” Determinations of fixed and volatile solids do not distinguish precisely between inorganic and organic matter because the loss on ignition is not confined to organic matter. It includes losses due to decomposition or volatilization of some mineral salts. Better characterization of organic matter can be made by such tests as total organic carbon (Section 5310), BOD (Section 5210), and COD (Section 5220).

“Settleable solids” is the term applied to the material settling out of suspension within a defined period. It may include floating material, depending on the technique (Section 2540F.3b).

2. Sources of Error and Variability

Sampling, subsampling, and pipeting two-phase or three-phase samples may introduce serious errors. Make and keep such samples homogeneous during transfer. Use special handling to insure sample integrity when subsampling. Mix small samples with a magnetic stirrer. If suspended solids are present, pipet with wide-bore pipets. If part of a sample adheres to the sample container, consider this in evaluating and reporting results. Some samples dry with the formation of a crust that prevents water evaporation; special handling is required to deal with this. Avoid using a magnetic stirrer with samples containing magnetic particles.

The temperature at which the residue is dried has an important bearing on results, because weight losses due to volatilization of organic matter, mechanically occluded water, water of crystallization, and gases from heat-induced chemical decomposition, as well as weight gains due to oxidation, depend on temperature and time of heating. Each sample requires close attention to desiccation after drying. Minimize opening desiccator because moist air enters. Some samples may be stronger desiccants than those used in the desiccator and may take on water.

Residues dried at 103 to 105°C may retain not only water of crystallization but also some mechanically occluded water. Loss of CO_2 will result in conversion of bicarbonate to carbonate. Loss of organic matter by volatilization usually will be very slight. Because removal of occluded water is marginal at this temperature, attainment of constant weight may be very slow.

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Residues dried at $180 \pm 2^{\circ}\text{C}$ will lose almost all mechanically occluded water. Some water of crystallization may remain, especially if sulfates are present. Organic matter may be lost by volatilization, but not completely destroyed. Loss of CO_2 results from conversion of bicarbonates to carbonates and carbonates may be decomposed partially to oxides or basic salts. Some chloride and nitrate salts may be lost. In general, evaporating and drying water samples at 180°C yields values for dissolved solids closer to those obtained through summation of individually determined mineral species than the dissolved solids values secured through drying at the lower temperature.

To rinse filters and filtered solids and to clean labware use Type III water. Special samples may require a higher quality water; see Section 1080.

Results for residues high in oil or grease may be questionable because of the difficulty of drying to constant weight in a reasonable time.

To aid in quality assurance, analyze samples in duplicate. Dry samples to constant weight if possible. This entails multiple drying-cooling-weighing cycles for each determination.

Analyses performed for some special purposes may demand deviation from the stated procedures to include an unusual constituent with the measured solids. Whenever such variations of technique are introduced, record and present them with the results.

3. Sample Handling and Preservation

Use resistant-glass or plastic bottles, provided that the material in suspension does not adhere to container walls. Begin analysis as soon as possible because of the impracticality of preserving the sample. Refrigerate sample at 4°C up to the time of analysis to minimize microbiological decomposition of solids. Preferably do not hold samples more than 24 h. In no case hold sample more than 7 d. Bring samples to room temperature before analysis.

4. Selection of Method

Methods B through F are suitable for the determination of solids in potable, surface, and saline waters, as well as domestic and industrial wastewaters in the range up to 20 000 mg/L.

Method G is suitable for the determination of solids in sediments, as well as solid and semisolid materials produced during water and wastewater treatment.

5. Bibliography

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2540 B. Total Solids Dried at $103\text{--}105^{\circ}\text{C}$

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1. General Discussion

a. *Principle:* A well-mixed sample is evaporated in a weighed dish and dried to constant weight in an oven at 103 to 105°C. The increase in weight over that of the empty dish represents the total solids. The results may not represent the weight of actual dissolved and suspended solids in wastewater samples (see above).

b. *Interferences:* Highly mineralized water with a significant concentration of calcium, magnesium, chloride, and/or sulfate may be hygroscopic and require prolonged drying, proper desiccation, and rapid weighing. Exclude large, floating particles or submerged agglomerates of nonhomogeneous materials from the sample if it is determined that their inclusion is not desired in the final result. Disperse visible floating oil and grease with a blender before withdrawing a sample portion for analysis. Because excessive residue in the dish may form a water-trapping crust, limit sample to no more than 200 mg residue (see Section 2540A.2).

2. Apparatus

a. *Evaporating dishes:* Dishes of 100-mL capacity made of one of the following materials:

- 1) Porcelain, 90-mm diam.
- 2) Platinum—Generally satisfactory for all purposes.
- 3) High-silica glass. # (44)*

b. *Muffle furnace* for operation at 550°C.

c. *Steam bath.*

d. *Desiccator*, provided with a desiccant containing a color indicator of moisture concentration or an instrumental indicator.

e. *Drying oven*, for operation at 103 to 105°C.

f. *Analytical balance*, capable of weighing to 0.1 mg.

g. *Magnetic stirrer* with TFE stirring bar.

h. *Wide-bore pipets.* # (45)†

i. *Graduated cylinder.*

j. *Low-form beaker.* # (46)‡

3. Procedure

a. *Preparation of evaporating dish:* If volatile solids are to be measured ignite clean evaporating dish at 550°C for 1 h in a muffle furnace. If only total solids are to be measured, heat clean dish to 103 to 105°C for 1 h. Store and cool dish in desiccator until needed. Weigh immediately before use.

b. *Sample analysis:* Choose a sample volume that will yield a residue between 2.5 and 200 mg. Pipet a measured volume of well-mixed sample, during mixing, to a preweighed dish. For homogeneous samples, pipet from the approximate midpoint of the container but not in the vortex. Choose a point both middepth and midway between wall and vortex. Evaporate to

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dryness on a steam bath or in a drying oven. Stir sample with a magnetic stirrer during transfer. If necessary, add successive sample portions to the same dish after evaporation. When evaporating in a drying oven, lower temperature to approximately 2°C below boiling to prevent splattering. Dry evaporated sample for at least 1 h in an oven at 103 to 105°C, cool dish in desiccator to balance temperature, and weigh. Repeat cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained, or until weight change is less than 4% of previous weight or 0.5 mg, whichever is less. When weighing dried sample, be alert to change in weight due to air exposure and/or sample degradation. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight.

4. Calculation

$$\text{mg total solids/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

where:

A = weight of dried residue + dish, mg, and

B = weight of dish, mg.

5. Precision

Single-laboratory duplicate analyses of 41 samples of water and wastewater were made with a standard deviation of differences of 6.0 mg/L.

6. Bibliography

SYMONS, G.E. & B. MOREY. 1941. The effect of drying time on the determination of solids in sewage and sewage sludges. *Sewage Works J.* 13:936.

2540 C. Total Dissolved Solids Dried at 180°C

1. General Discussion

a. Principle: A well-mixed sample is filtered through a standard glass fiber filter, and the filtrate is evaporated to dryness in a weighed dish and dried to constant weight at 180°C. The increase in dish weight represents the total dissolved solids. This procedure may be used for drying at other temperatures.

The results may not agree with the theoretical value for solids calculated from chemical analysis of sample (see above). Approximate methods for correlating chemical analysis with dissolved solids are available.¹ The filtrate from the total suspended solids determination (Section 2540D) may be used for determination of total dissolved solids.

b. Interferences: See Section 2540A.2 and Section 2540B.1. Highly mineralized waters

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with a considerable calcium, magnesium, chloride, and/or sulfate content may be hygroscopic and require prolonged drying, proper desiccation, and rapid weighing. Samples high in bicarbonate require careful and possibly prolonged drying at 180°C to insure complete conversion of bicarbonate to carbonate. Because excessive residue in the dish may form a water-trapping crust, limit sample to no more than 200 mg residue.

2. Apparatus

Apparatus listed in Section 2540B.2a - h is required, and in addition:

- a. *Glass-fiber filter disks* (47)* without organic binder.
- b. *Filtration apparatus*: One of the following, suitable for the filter disk selected:
 - 1) *Membrane filter funnel*.
 - 2) *Gooch crucible*, 25-mL to 40-mL capacity, with Gooch crucible adapter.
 - 3) *Filtration apparatus* with reservoir and coarse (40- to 60- μ m) fritted disk as filter support. (48)†
- c. *Suction flask*, of sufficient capacity for sample size selected.
- d. *Drying oven*, for operation at $180 \pm 2^\circ\text{C}$.

3. Procedure

a. *Preparation of glass-fiber filter disk*: If pre-prepared glass fiber filter disks are used, eliminate this step. Insert disk with wrinkled side up into filtration apparatus. Apply vacuum and wash disk with three successive 20-mL volumes of reagent-grade water. Continue suction to remove all traces of water. Discard washings.

b. *Preparation of evaporating dish*: If volatile solids are to be measured, ignite cleaned evaporating dish at 550°C for 1 h in a muffle furnace. If only total dissolved solids are to be measured, heat clean dish to $180 \pm 2^\circ\text{C}$ for 1 h in an oven. Store in desiccator until needed. Weigh immediately before use.

c. *Selection of filter and sample sizes*: Choose sample volume to yield between 2.5 and 200 mg dried residue. If more than 10 min are required to complete filtration, increase filter size or decrease sample volume.

d. *Sample analysis*: Stir sample with a magnetic stirrer and pipet a measured volume onto a glass-fiber filter with applied vacuum. Wash with three successive 10-mL volumes of reagent-grade water, allowing complete drainage between washings, and continue suction for about 3 min after filtration is complete. Transfer total filtrate (with washings) to a weighed evaporating dish and evaporate to dryness on a steam bath or in a drying oven. If necessary, add successive portions to the same dish after evaporation. Dry evaporated sample for at least 1 h in an oven at $180 \pm 2^\circ\text{C}$, cool in a desiccator to balance temperature, and weigh. Repeat drying cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% of previous weight or 0.5 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their

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average weight. If volatile solids are to be determined, follow procedure in Section 2540E.

4. Calculation

$$\text{mg total dissolved solids/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

where:

A = weight of dried residue + dish, mg, and

B = weight of dish, mg.

5. Precision

Single-laboratory analyses of 77 samples of a known of 293 mg/L were made with a standard deviation of differences of 21.20 mg/L.

6. Reference

1. SOKOLOFF, V.P. 1933. Water of crystallization in total solids of water analysis. *Ind. Eng. Chem., Anal. Ed.* 5:336.

7. Bibliography

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2540 D. Total Suspended Solids Dried at 103–105°C

1. General Discussion

a. Principle: A well-mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103 to 105°C. The increase in weight of the filter represents the total suspended solids. If the suspended material clogs the filter and prolongs filtration, it may be necessary to increase the diameter of the filter or decrease the sample volume. To obtain an estimate of total suspended solids, calculate the difference between total dissolved solids and total solids.

b. Interferences: See Section 2540A.2 and Section 2540B.1. Exclude large floating particles or submerged agglomerates of nonhomogeneous materials from the sample if it is determined that their inclusion is not representative. Because excessive residue on the filter may form a

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water-entrapping crust, limit the sample size to that yielding no more than 200 mg residue. For samples high in dissolved solids thoroughly wash the filter to ensure removal of dissolved material. Prolonged filtration times resulting from filter clogging may produce high results owing to increased colloidal materials captured on the clogged filter.

2. Apparatus

Apparatus listed in Section 2540B.2 and Section 2540C.2 is required, except for evaporating dishes, steam bath, and 180°C drying oven. In addition:

Aluminum weighing dishes.

3. Procedure

a. Preparation of glass-fiber filter disk: If pre-prepared glass fiber filter disks are used, eliminate this step. Insert disk with wrinkled side up in filtration apparatus. Apply vacuum and wash disk with three successive 20-mL portions of reagent-grade water. Continue suction to remove all traces of water, turn vacuum off, and discard washings. Remove filter from filtration apparatus and transfer to an inert aluminum weighing dish. If a Gooch crucible is used, remove crucible and filter combination. Dry in an oven at 103 to 105°C for 1 h. If volatile solids are to be measured, ignite at 550°C for 15 min in a muffle furnace. Cool in desiccator to balance temperature and weigh. Repeat cycle of drying or igniting, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less. Store in desiccator until needed.

b. Selection of filter and sample sizes: Choose sample volume to yield between 2.5 and 200 mg dried residue. If volume filtered fails to meet minimum yield, increase sample volume up to 1 L. If complete filtration takes more than 10 min, increase filter diameter or decrease sample volume.

c. Sample analysis: Assemble filtering apparatus and filter and begin suction. Wet filter with a small volume of reagent-grade water to seat it. Stir sample with a magnetic stirrer at a speed to shear larger particles, if practical, to obtain a more uniform (preferably homogeneous) particle size. Centrifugal force may separate particles by size and density, resulting in poor precision when point of sample withdrawal is varied. While stirring, pipet a measured volume onto the seated glass-fiber filter. For homogeneous samples, pipet from the approximate midpoint of container but not in vortex. Choose a point both middepth and midway between wall and vortex. Wash filter with three successive 10-mL volumes of reagent-grade water, allowing complete drainage between washings, and continue suction for about 3 min after filtration is complete. Samples with high dissolved solids may require additional washings. Carefully remove filter from filtration apparatus and transfer to an aluminum weighing dish as a support. Alternatively, remove the crucible and filter combination from the crucible adapter if a Gooch crucible is used. Dry for at least 1 h at 103 to 105°C in an oven, cool in a desiccator to balance temperature, and weigh. Repeat the cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until the weight change is less than 4% of the previous weight or 0.5 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree

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within 5% of their average weight. If volatile solids are to be determined, treat the residue according to 2540E.

4. Calculation

$$\text{mg total suspended solids/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

where:

A = weight of filter + dried residue, mg, and

B = weight of filter, mg.

5. Precision

The standard deviation was 5.2 mg/L (coefficient of variation 33%) at 15 mg/L, 24 mg/L (10%) at 242 mg/L, and 13 mg/L (0.76%) at 1707 mg/L in studies by two analysts of four sets of 10 determinations each.

Single-laboratory duplicate analyses of 50 samples of water and wastewater were made with a standard deviation of differences of 2.8 mg/L.

6. Bibliography

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determination. *J. Water Pollut. Control Fed.* 50:2370.

2540 E. Fixed and Volatile Solids Ignited at 550°C

1. General Discussion

a. Principle: The residue from Method B, C, or D is ignited to constant weight at 550°C. The remaining solids represent the fixed total, dissolved, or suspended solids while the weight lost on ignition is the volatile solids. The determination is useful in control of wastewater treatment plant operation because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge, and industrial wastes.

b. Interferences: Negative errors in the volatile solids may be produced by loss of volatile matter during drying. Determination of low concentrations of volatile solids in the presence of high fixed solids concentrations may be subject to considerable error. In such cases, measure for suspect volatile components by another test, for example, total organic carbon (Section 5310). Highly alkaline residues may react with silica in sample or silica-containing crucibles.

2. Apparatus

See Section 2540B.2, Section 2540C.2, and Section 2540D.2.

3. Procedure

Ignite residue produced by Method 2540B, C, or D to constant weight in a muffle furnace at a temperature of 550°C. Ignite a blank glass fiber filter along with samples. Have furnace up to temperature before inserting sample. Usually, 15 to 20 min ignition are required for 200 mg residue. However, more than one sample and/or heavier residues may overtax the furnace and necessitate longer ignition times. Let dish or filter disk cool partially in air until most of the heat has been dissipated. Transfer to a desiccator for final cooling in a dry atmosphere. Do not overload desiccator. Weigh dish or disk as soon as it has cooled to balance temperature. Repeat cycle of igniting, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% or 0.5 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight. Weight loss of the blank filter is an indication of unsuitability of a particular brand or type of filter for this analysis.

4. Calculation

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$$\text{mg volatile solids/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$
$$\text{mg fixed solids/L} = \frac{(B - C) \times 1000}{\text{sample volume, mL}}$$

where:

A = weight of residue + dish before ignition, mg,

B = weight of residue + dish or filter after ignition, mg, and

C = weight of dish or filter, mg.

5. Precision

The standard deviation was 11 mg/L at 170 mg/L volatile total solids in studies by three laboratories on four samples and 10 replicates. Bias data on actual samples cannot be obtained.

2540 F. Settleable Solids

1. General Discussion

Settleable solids in surface and saline waters as well as domestic and industrial wastes may be determined and reported on either a volume (mL/L) or a weight (mg/L) basis.

2. Apparatus

The volumetric test requires only an Imhoff cone. The gravimetric test requires all the apparatus listed in Section 2540D.2 and a glass vessel with a minimum diameter of 9 cm.

3. Procedure

a. Volumetric: Fill an Imhoff cone to the 1-L mark with a well-mixed sample. Settle for 45 min, gently agitate sample near the sides of the cone with a rod or by spinning, settle 15 min longer, and record volume of settleable solids in the cone as milliliters per liter. If the settled matter contains pockets of liquid between large settled particles, estimate volume of these and subtract from volume of settled solids. The practical lower limit of measurement depends on sample composition and generally is in the range of 0.1 to 1.0 mL/L. Where a separation of settleable and floating materials occurs, do not estimate the floating material as settleable matter. Replicates usually are not required.

Where biological or chemical floc is present, the gravimetric method (3b) is preferred.

b. Gravimetric:

1) Determine total suspended solids as in Section 2540D.

2) Pour a well-mixed sample into a glass vessel of not less than 9 cm diam using not less

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than 1 L and sufficient sample to give a depth of 20 cm. Alternatively use a glass vessel of greater diameter and a larger volume of sample. Let stand quiescent for 1 h and, without disturbing the settled or floating material, siphon 250 mL from center of container at a point halfway between the surface of the settled material and the liquid surface. Determine total suspended solids (milligrams per liter) of this supernatant liquor (Section 2540D). These are the nonsettleable solids.

4. Calculation

$$\text{mg settleable solids/L} = \text{mg total suspended solids/L} - \text{mg nonsettleable solids/L}$$

5. Precision and Bias

Precision and bias data are not now available.

6. Bibliography

FISCHER, A.J. & G.E. SYMONS. 1944. The determination of settleable sewage solids by weight. *Water Sewage Works* 91:37.

2540 G. Total, Fixed, and Volatile Solids in Solid and Semisolid Samples

1. General Discussion

a. Applicability: This method is applicable to the determination of total solids and its fixed and volatile fractions in such solid and semisolid samples as river and lake sediments, sludges separated from water and wastewater treatment processes, and sludge cakes from vacuum filtration, centrifugation, or other sludge dewatering processes.

b. Interferences: The determination of both total and volatile solids in these materials is subject to negative error due to loss of ammonium carbonate and volatile organic matter during drying. Although this is true also for wastewater, the effect tends to be more pronounced with sediments, and especially with sludges and sludge cakes. The mass of organic matter recovered from sludge and sediment requires a longer ignition time than that specified for wastewaters, effluents, or polluted waters. Carefully observe specified ignition time and temperature to control losses of volatile inorganic salts if these are a problem. Make all weighings quickly because wet samples tend to lose weight by evaporation. After drying or ignition, residues often are very hygroscopic and rapidly absorb moisture from the air. Highly alkaline residues may react with silica in the samples or silica-containing crucibles.

2. Apparatus

All the apparatus listed in Section 2540B.2 is required except that a magnetic stirrer and pipets are not used and a balance capable of weighing to 10 mg may be used.

3. Procedure

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a. Total solids:

1) Preparation of evaporating dish—If volatile solids are to be measured, ignite a clean evaporating dish at 550°C for 1 h in a muffle furnace. If only total solids are to be measured, heat dish at 103 to 105°C for 1 h in an oven. Cool in desiccator, weigh, and store in desiccator until ready for use.

2) Sample analysis

a) Fluid samples—If the sample contains enough moisture to flow more or less readily, stir to homogenize, place 25 to 50 g in a prepared evaporating dish, and weigh. Evaporate to dryness on a water bath, dry at 103 to 105°C for 1 h, cool to balance temperature in an individual desiccator containing fresh desiccant, and weigh. Repeat heating, cooling, desiccating, and weighing procedure until the weight change is less than 4% or 50 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight.

b) Solid samples—If the sample consists of discrete pieces of solid material (dewatered sludge, for example), take cores from each piece with a No. 7 cork borer or pulverize the entire sample coarsely on a clean surface by hand, using rubber gloves. Place 25 to 50 g in a prepared evaporating dish and weigh. Place in an oven at 103 to 105°C overnight. Cool to balance temperature in a desiccator and weigh. Repeat drying (1 h), cooling, weighing, and desiccating steps until weight change is less than 4% or 50 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight.

b. Fixed and volatile solids: Transfer the dried residue from 2)a) above to a cool muffle furnace, heat furnace to 550°C, and ignite for 1 h. (If the residue contains large amounts of organic matter, first ignite it over a gas burner and under an exhaust hood in the presence of adequate air to lessen losses due to reducing conditions and to avoid odors in the laboratory.) Cool in desiccator to balance temperature and weigh. Repeat igniting (30 min), cooling, desiccating and weighing steps until the weight change is less than 4% or 50 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight.

4. Calculation

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$$\% \text{ total solids} = \frac{(A - B) \times 100}{C - B}$$

$$\% \text{ volatile solids} = \frac{(A - D) \times 100}{A - B}$$

$$\% \text{ fixed solids} = \frac{(D - B) \times 100}{A - B}$$

where:

A = weight of dried residue + dish, mg,

B = weight of dish,

C = weight of wet sample + dish, mg, and

D = weight of residue + dish after ignition, mg.

5. Precision and Bias

Precision and bias data are not now available.

6. Bibliography

GOODMAN, B.L. 1964. Processing thickened sludge with chemical conditioners. Pages 78 et seq. *in* Sludge Concentration, Filtration and Incineration. Univ. Michigan Continued Education Ser. No. 113, Ann Arbor.

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2550 TEMPERATURE#(49)*

2550 A. Introduction

Temperature readings are used in the calculation of various forms of alkalinity, in studies of saturation and stability with respect to calcium carbonate, in the calculation of salinity, and in general laboratory operations. In limnological studies, water temperatures as a function of depth often are required. Elevated temperatures resulting from discharges of heated water may have significant ecological impact. Identification of source of water supply, such as deep wells, often is possible by temperature measurements alone. Industrial plants often require data on water temperature for process use or heat-transmission calculations.

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2550 B. Laboratory and Field Methods

1. Laboratory and Other Non-Depth Temperature Measurements

Normally, temperature measurements may be made with any good mercury-filled Celsius thermometer. As a minimum, the thermometer should have a scale marked for every 0.1°C, with markings etched on the capillary glass. The thermometer should have a minimal thermal capacity to permit rapid equilibration. Periodically check the thermometer against a precision thermometer certified by the National Institute of Standards and Technology (NIST, formerly National Bureau of Standards) that is used with its certificate and correction chart. For field operations use a thermometer having a metal case to prevent breakage.

Thermometers are calibrated for total immersion or partial immersion. One calibrated for total immersion must be completely immersed to the depth of the etched circle around the stem just below the scale level.

2. Depth Temperature Measurements

Depth temperature required for limnological studies may be measured with a reversing thermometer, thermophone, or thermistor. The thermistor is most convenient and accurate; however, higher cost may preclude its use. Calibrate any temperature measurement devices with a NIST-certified thermometer before field use. Make readings with the thermometer or device immersed in water long enough to permit complete equilibration. Report results to the nearest 0.1 or 1.0°C, depending on need.

The thermometer commonly used for depth measurements is of the reversing type. It often is mounted on the sample collection apparatus so that a water sample may be obtained simultaneously. Correct readings of reversing thermometers for changes due to differences between temperature at reversal and temperature at time of reading. Calculate as follows:

$$\Delta T = \left[\frac{(T^1 - t)(T^1 + V_0)}{K} \right] \times \left[1 + \frac{(T^1 - t)(T^1 + V_0)}{K} \right] + L$$

where:

ΔT = correction to be added algebraically to uncorrected reading,

T^1 = uncorrected reading at reversal,

t = temperature at which thermometer is read,

V_0 = volume of small bulb end of capillary up to 0°C graduation,

K = constant depending on relative thermal expansion of mercury and glass (usual

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value of $K = 6100$), and

L = calibration correction of thermometer depending on T^1 .

If series observations are made it is convenient to prepare graphs for a thermometer to obtain ΔT from any values of T^1 and t .

3. Bibliography

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2560 PARTICLE COUNTING AND SIZE DISTRIBUTION (PROPOSED)#(51)*

2560 A. Introduction

1. General Discussion

Particles are ubiquitous in natural waters and in water and wastewater treatment streams. Particle counting and size distribution analysis can help to determine the makeup of natural waters, treatment plant influent, process water, and finished water. Similarly, it can aid in designing treatment processes, making decisions about changes in operations, and/or determining process efficiency. Methods for measuring particle size distribution included herein depend on electronic measurement devices because manual methods are likely to be too slow for routine analysis. However, when particle size analysis is to include size distribution of large (>500- μm) aggregates, use direct microscopic counting and sizing. Principles of various types of instruments capable of producing both size and number concentration information on particulate dispersions are included. Unless explicitly stated otherwise, the term “size distribution” means an absolute size distribution, i.e., one that includes the number concentration or count.

In most particle-counting instruments, particles pass through a sensing zone where they are measured individually; the only exception included is the static type of light-scattering instrument. Instruments create an electronic pulse (voltage, current, or resistance) that is proportional to a characteristic size of the particle. The instrument responses (pulse height, width, or area) are classified by magnitude and counted in each class to yield the particle size distribution.

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2. Selection of Method

Three instrument types are included: electrical sensing zone instruments, light-blockage instruments, and light-scattering instruments.

Select instrument consistent with expected use of the particle size analysis. Instruments vary in the particle characteristic being sensed, lower and upper size limits of detection, degree of resolution of the size distribution, particle number concentration range that can be measured accurately, amount of shear to which a sample is subjected before measurement, amount of sample preparation, operator skill required, and the ease with which data can be obtained and manipulated into the desired forms. See Section 2560B.1, Section 2560C.1, and Section 2560D.1, and manufacturers' literature for information on characteristics of each type of instrumentation.

Some instruments can be set up for either continuous-flow or batch sampling. Others can be used only for batch analysis. For instruments usable in both modes, check that no systematic differences in particle size distributions occur between continuous-flow measurements and batch samples taken at or near the intake point for continuous-flow samples.

3. Sample Collection and Handling

a. Batch samples: Use extreme care in obtaining, handling, and preparing batch samples to avoid changing total particle count and size distribution.

Choose representative times and locations for sampling. Ensure that particles are not subjected to greater physical forces during collection than in their natural setting. Collect samples from a body of water with submerged vessels to minimize turbulence and bubble entrainment. If sampling from particular depths, use standard samplers designed for that purpose. For flowing systems, make sure that the velocity into the opening of the sampling device is the same as that of the flowing stream (isokinetic sampling) and that the opening diameter is at least 50 times as large as the particles to be measured. For sampling from a tap, let water flow slowly and continuously down the side of the collection vessel.

Minimize particle contamination from the air, dilution water (or, for electrical sensing zone instruments, electrolyte solution) (see ¶ 4 below), and any vessel or glassware that comes in contact with the sample. Minimize exposure to air by keeping sample in a closed container and by minimizing time between sampling and analysis.

Preferably use glass bottles and other vessels with bottle cap liners of TFE.

Clean all glassware scrupulously by automatic dishwashing, vigorous hand brushing, and/or ultrasonication. Rinse glassware immediately before use with particle-free water. Between samples, rinse any part of the instrument that comes in contact with samples with either clean water or the upcoming sample. Alternatively, run multiple replicates and discard the first results.

To avoid breakup of aggregates of particles or flocs, sample and make dilutions very slowly using wide-bore pipets, needles, or other sampling devices; cut off pipet tips to avoid high velocities at the entrance. If sample dilution is required, add sample to dilution water, not vice

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versa, by submerging the pipet tip in the dilution water and releasing sample slowly. Use minimum intensity and duration of mixing adequate to dilute the suspension into the dilution water. Avoid mechanical stirrers inside the sample or ultrasonication. Simultaneously gently rotate and partially invert entire sample in a closed bottle. Use cylindrical dilution bottles to avoid sharp corners. Leave less than approximately 25% air space during mixing. To avoid sedimentation, make measurements immediately after mixing. Do not mix during measurement unless absolutely necessary to prevent sedimentation.

Most surface and ground waters contain relatively stable particles that aggregate slowly. Particle size distribution in biologically active waters or waters that have been treated with coagulants is more likely to change over short time periods. To minimize flocculation, minimize time between sampling and measurement. In highly flocculent systems, maximum holding time should be only a few minutes; for more stable samples, a few hours may be acceptable. Dilution slows flocculation kinetics and, in some cases, makes flocculation less likely. Make dilutions only immediately before measurement.

Samples measured at different temperature or pressure than when collected and those with biological activity may develop entrained air bubbles that interfere with measurement accuracy. If any gas bubbles are visible, let sample stand for a short time to degas naturally or use a mild vacuum to speed degassing. Use ultrasound to aid degassing, but only if floc breakup is not a problem.

Minimize time between sampling and analysis; if at all possible, make measurements immediately after sampling. If storage is unavoidable, refrigerate at 4°C but restore samples to room temperature, preferably in water bath, before measurement.

b. Continuous-flow: Using a particle counter as a continuous-flow monitor may be desirable. Many more samples can be processed by automated particle counting than by batch sample analysis. All the instruments mentioned in Methods B through D can be used in this mode, although instruments and samples not requiring dilution are easier to set up. For some instruments, this type of operation requires custom hardware; for others there is commercially available hardware.

Problems of batch samples are equally relevant to continuous analysis. Other critical considerations include selection of sampling point, velocity at entrance and throughout sample line, maintenance of stable sample flow rate, position of instrument's sensor in the sample line, and absence of flow-modifying devices upstream of the sensor.

Choose a sampling point that is representative and away from wall surfaces. Place entrance to sample line facing the flow, with the velocity at the opening nearly the same as the surrounding flow.

Sample preservation within the instrument is difficult because both deposition (or temporary holdup) of particles and floc breakup must be avoided. Deposition occurs by gravity settling of particles onto horizontal surfaces. Floc breakup occurs because of excessive shear forces. Minimize length of transmission lines, especially in horizontal components, and preferably have no horizontal lines. Avoid drastic changes in flow direction or velocity. Do not use

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flow-modifying devices such as pumps, fittings with irregular surfaces, sharp angle changes, flow controllers, etc., between the sampling point and the sensor. Locate pumps after the sensor. Preferably, provide curved sample lines (e.g., flexible tubing or bent glass tubing) rather than right-angle fittings.

For most instruments, flow rate is specified. Because calibration (see ¶ 5) can change with flow rate within the range, flow control within very narrow limits is essential. Calibrate and use the sensor at the same flow rate. For continuous particle size distribution measurements, flow control, not simply flow monitoring, is necessary. Do not make measurements at a flow rate different from that used for calibration. Provide a flow control system downstream of the sensor, maintain a constant rate, and do not introduce turbulence or pulsations before or through the sensor.

4. Dilution Water

Particle-free water is virtually impossible to obtain, but it is possible to produce water containing very few particles within the size range to be measured. Produce “particle-free” or “clean” water from distilled, deionized water or water taken from the same source as the samples. If all samples are from the same source or have similar chemical characteristics, preferably filter sample water to produce “particle-free” water with no change in chemical environment. Distilled, deionized water produced by ion exchange and cartridge filtration may produce acceptable “particle free” dilution water, but preferably use continuous closed-loop membrane filtration.

Dilution water preparation systems are available from particle-counter manufacturers. Alternatively, assemble a system similar to that shown in Figure 2560:1 (or any system that produces a water of sufficient quality). In the system shown, a pump draws the water from a bottle and puts it through the in-line filter. Use membrane filters with nominal pore sizes no more than 10% of the smallest particle size expected; alternatively use cartridge filters. Pass water through the filter several times. The system lets water be passed directly from the product-water bottle to the source-water bottle by opening of the clamps and three-way stopcock. Use glass tubing in the bottles, but use flexible tubing to allow draining product-water bottle into source-water bottle. Attach a glass wool or membrane filter to air inlets. Dilute samples by drawing water directly from the three-way stopcock into the bottle to be used in sample analysis.

A simpler system with one-pass filtration directly into the sample bottle may be adequate for many samples, depending on the particle size range to be measured. Such a system would omit the product-water bottle, the connections between the two bottles and associated stopcock and clamps of Figure 2560:1. Dilution water is produced on demand and the effluent is put directly, without additional handling, into the sample container.

Guard against biological growth within filtration systems by frequent disassembly and adequate washing or replacement of components. For many samples, do not use chemical disinfectants because they might change the particle count and size distribution by their oxidizing potential.

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5. Calibration

As a particle is detected in the sensing zone of any instrument, an electrical response is generated and sorted into a channel of the instrument based on its magnitude.

Calibrate by determining the channel number into which particles of known size are sorted by the instrument. Use spherical particles manufactured for this purpose. Calibration particles are available in suspension or as dry particles. Over time, suspensions are likely to undergo some aggregation; use ultrasound to break up flocs before calibration. Calibration particles are nearly monodisperse but do exhibit a small inherent (true) variance.

The precision (see Section 1030A.1) and resolution of the instrument influence its ability to sort particles into different channels. Resolution is a measure of the ability of an instrument to distinguish between particles of similar but different sizes. An instrument with high precision and good resolution will measure monodisperse particles in a very narrow size range; some instruments have sufficient precision and resolution so that calibration particles with extremely narrow size distributions (very small variance) are sorted into a few adjacent channels. In such cases, the true variance of the particle size and the measured variance will be nearly equal. An instrument with low precision but high resolution will yield a wide distribution on the same particles (i.e., measured variance > true variance). An instrument with high precision but low resolution (e.g., few channels) may yield a narrow measured distribution (e.g., all the particles in the same channel) even though the true distribution is broader (i.e., measured variance < true variance).

Use at least three sizes of calibration particles in reasonably similar number concentrations to calibrate a sensor. To analyze different-size particles in a mixture, ensure that the different sizes do not interfere with one another. Calibrate under conditions identical with those of sample measurements, e.g., settings on the instrument, flow rate, type of sample cell, dilution water or electrolyte solution, and mixing during measurement. Do not exceed the maximum concentration for the sensor during calibration.

The calibration curve depends on the characteristic of the particle measured by the instrument (diameter, area, or volume) and whether the pulses are sorted into channels on an arithmetic or logarithmic basis. For example, if an electrical sensing zone instrument (which responds to particle volume) is used in a logarithmic mode, the calibration curve will be the logarithm of particle volume vs. channel number.

Generally, increments between channels are equal on an arithmetic or logarithmic basis. In most light-blockage and light-scattering instruments, the lower and upper limits for each channel can be set by the user. Because most samples have broad particle size distributions, spanning at least one order of magnitude, and often two or three, preferably use larger increments for larger sizes. This is consistent with equal logarithmic spacing, although other less systematic intervals that preserve the characteristic of larger increments for larger sizes are permissible. Such intervals also are consistent with the resolution capabilities of most available instruments, which respond to a characteristic particle area or volume (proportional to square or cube of diameter, respectively), and therefore require greater resolution for larger particles than for smaller ones on

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a diameter basis.

At a given set of settings for a given sensor, each channel represents a certain average size and size increment. After measuring the channel number associated with several sizes of calibration particles, use a calibration equation to assign average sizes or, in some cases, the lower limit of size, to all other channels. Knowledge of the average size and both the arithmetic and logarithmic increment of each channel is necessary for reporting. In contrast to calibration particles, environmental particles are rarely spherical. Generally report measurements in terms of “equivalent spherical diameter,” i.e., size of any particle taken as that of a sphere that would give the same response in the instrument. Because most sample particles are nonspherical and different instruments respond to different characteristics of particles, different measured particle size distributions result from different instruments.

Some light-scattering instruments calculate particle size from first principles and do not require calibration per se. For those instruments, periodically adjust the system optics. Make frequent checks that the instrument response is consistent by following the calibration procedures for other instruments.

6. Data Reporting

a. Particle concentrations: Number, surface, or volume concentration over a specified size range is particularly valuable as a summary factor for particle counting and size distribution. Its variation with some changes in an independent variable in a natural or engineered system may be of interest.

When reporting the number concentration (number per milliliter) of particles, report also the size range measured (both lower and upper size limits). The lower limit is particularly important, because most samples have large number concentrations near the lower limit of detection of currently available instruments. Never state or imply a lower size limit of zero.

In some studies a surface area concentration ($\mu\text{m}^2/\text{mL}$) or volume concentration ($\mu\text{m}^3/\text{mL}$) may be more relevant; convert number concentration to these forms by multiplying by the area or volume, respectively, of a sphere with the mean diameter for each size class. (This is an approximation based on the assumption that particles are spherical.) In such cases, also report lower and upper limits of size class.

b. Tabulated size distributions: If the distribution itself (i.e., the variation of particle number, surface area, or volume concentration with particle size) is to be shown in tabular format, give for each instrument channel (or grouping of channels) the number concentration and the associated size range (lower and upper limits).

c. Graphical size distributions: For graphical reporting, preferably use the count information (on a number, surface area, or volume basis) as the ordinate and particle size as the abscissa.

For the count information, use absolute, rather than relative, scales because they indicate both concentration and size distribution. Also, preferably use differential, rather than cumulative, distributions because they show directly what size range contained the most particles. For ease of plotting, associate the number (or surface area or volume) concentration with a mean size for

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each channel, rather than with the size range (the lower and upper limit). To account for that change without losing information, normalize the data by dividing the concentration of particles in a given size class by the size interval for that class (on either an arithmetic or a logarithmic basis). Normalization prevents creation of artificial (or apparent) peaks or valleys in a distribution and ensures that the same distribution measured by different instruments with different size increments will be the same graphically. If data are not normalized, plot size distribution data (absolute and differential) as a discrete histogram (i.e., as a bar chart with the upper limit of one channel being identical to the lower limit of the next).

Preferably show particle sizes with a logarithmic scale. Most samples have broad distributions and most analyzers use larger increments of diameter with increasing size; these characteristics are consistent with a logarithmic scale, which also intrinsically avoids showing a zero size. Produce the logarithmic scale by showing the log of diameter on an arithmetic scale or the arithmetic values of diameter on a logarithmic scale.

d. Example calculations: A calculation layout (with example data) useful for preparing either tabular or graphical presentation is shown in Table 2560:I. This format may be abbreviated or modified to suit the data presentation being developed.

Columns A through G represent calibration information. Which of these values are set (or determined from calibration by the user) and which are calculated from this primary information depends on the type of instrument. In constructing such a table, preferably place primary information first. In most light-blockage and light-scattering instruments, the lower and upper size limits for each channel are known; calculate mean diameter as the arithmetic or logarithmic (geometric) mean of these limits, depending on whether instrument channels represent arithmetic or logarithmic increments. In electrical sensing zone instruments, the mean size (or log size) is determined directly from the calibration equation and increment width (arithmetic, Δd_{pi} , or logarithmic, $\Delta \log d_{pi}$) is pre-set; calculate lower and upper limits as mean size $\pm$ one half the arithmetic or logarithmic increment.

Columns H through O represent counting information for a sample. The corrected counts (Column H) are the direct counts from the particle size analyzer minus the background count for each channel. Adjust background count for dilution and differences in sample measured and dilution water measured for background count. Adjust this figure for sample dilution and volume analyzed to obtain the number concentration of particles (Column I). For example, for corrected count n , a 1:10 dilution, and 0.5 mL analyzed, number concentration is calculated as $(n \times 10)/0.5 = 20n/\text{mL}$. Volume concentration (Column J) may be approximated as the number of particles multiplied by the volume of a spherical particle with the mean diameter for the channel ($\pi d_p^3/6$). Values in the remaining columns are calculated as shown in the table. The normalized, differential, absolute distribution functions obtained in Columns K (particle size distribution function) through O give the values needed for graphic presentations as follows:

K: particle numbers (arithmetic scale) vs. diameter (arithmetic scale)

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L: particle volume (arithmetic scale) vs. diameter (arithmetic scale)

M: particle number (arithmetic scale) vs. diameter (logarithmic scale)

N: particle volume (arithmetic scale) vs. diameter (logarithmic scale)

O: log of particle number (arithmetic scale) vs. diameter (logarithmic scale).

(Alternatively, plot values from Column K directly on a logarithmic scale.)

If distribution K, L, M, or N is plotted, the area under the resulting graph between any two values of diameter represents the total number or volume concentration between those size limits. The logarithmic graph (O) shows the entire distribution better than other distributions, but can hide differences between similar distributions. It often is linear over specified size ranges.

7. Quality Control

See Section 1020 and Section 1030. Observe precautions for sampling and handling discussed in ¶ 3 above. Particle counting and size analysis requires experienced analysts capable of judgment in recognizing unusual behavior of an instrument. Ability to recognize partial blockage of an aperture or sensor or electronic noise is essential.

Electronic noise can be detected directly in some instruments by making a particle count when no flow is being put through a sensor. Ensure that the noise is not environmental, i.e., due to other nearby instruments, poor grounding, or inconsistent electrical supply. Noise also can be created by excessive cable length between the sensor and instrument or worn cables and connectors. Perform noise checks periodically.

Analyze sample blanks, handled identically to real samples, daily. Discard data from all channels that yield counts in the blanks greater than 5% of the counts for real samples. This procedure accounts for both particle contamination and electronic noise. Develop a maximum acceptable total count for blanks for each sensor (or for each set of standard settings for one sensor); if blanks give more than this maximum, discontinue particle counting until the contamination or noise is eliminated. Set maximum so that the 5% rule is met for all channels of interest (i.e., down to some minimum size limit acceptable in the laboratory for the sensor in use and the samples being measured). Recognize that the lower size limit of measurement for every sensor and every instrument is more likely to be dictated by electronic noise and particle contamination than by electronic settings.

Test cleaning and rinsing for sample bottles by partially filling the container with clean water, swirling the water to contact all sides, and performing a particle count. When the container has been shown to contribute negligible counts, check cleanliness of the cap similarly. Develop and document the standard washing and rinsing procedure, verify its validity as indicated, and follow the procedure without exception.

Also develop standard procedure for mixing samples and dilution water. Test mixing by varying the mixing intensity and/or duration to determine the minimum that gives adequate reproducibility (a sign of uniform concentration) and to determine if greater mixing increases the total particle counts (a sign of floc breakup). Follow an established mixing procedure without

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exception.

To determine precision, make replicate measurements on at least 5% of the samples. Report standard deviation (or range) of the total particle number concentration for each sensor or the total of all sensors, if more than one is used. See Section 1030.

Particle counting and size distribution analyzers are calibrated for size, not concentration. Standards for calibrating (or checking) the ability of an instrument to measure particle concentration accurately are under development. Currently, only precision, not bias, can be measured well for particle concentration. Obtain an indication of bias by preparing standards of particles of known density at a known suspended solids concentration; multiply total measured particle volume concentration by the known density to estimate the suspended solids concentration from the particle measurements. Compare this estimate with the known suspended solids concentration as an indicator of the combined instrument and laboratory bias.

Periodically calibrate with particles traceable to the National Institute of Standards and Technology (NIST).

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2560 B. Electrical Sensing Zone Method

1. General Discussion

a. Principle: In electrical sensing zone instruments, particles are suspended in an electrolyte solution and pass through a small orifice. A constant current or potential is applied between electrodes on either side of the orifice; the change in resistance caused by the particle taking up volume in the orifice causes a change in potential or current (whichever is not being held constant electronically). The pulse is proportional to the particle volume.

The voltage or current pulses are amplified and sorted into size classes or channels based on their maximum height. Some instruments have fixed channels; others permit selection of number of size classes, size width of each channel, and/or lowest size to be measured.

b. Interferences: Interferences are caused by contamination with particles, the presence of gas bubbles, and electronic noise. See Section 2560A.3*a* and *b* for sampling precautions, and Section 2560A.4 for particle-free water preparation. Minimize electronic noise as directed in Section 2560A.7. Increasing electrolyte strength is another method for minimizing electronic noise.

c. Detectable sizes and concentrations: The lower size limit of measurement depends on both electronic noise and aperture size. Manufacturers claim that the lower size limit (diameter) is approximately 2% of the aperture size, but not lower than approximately 0.4 μm , but values as low as approximately 0.7 μm have been reported. For the upper size limit, manufacturers state the maximum size is 40 to 60% of the aperture size, but a realistic upper limit for measuring flocs is 20% of the aperture size.

The minimum concentration limit depends on the ability to distinguish particles from background counts. For each channel, background count should be less than 5% of total count. In size ranges in which the count is very low (zero or nearly so), counts may be statistically unreliable. Determine if a larger sample volume provides more satisfactory (less scattered) data. Grouping data from several adjacent channels, thereby increasing the size increment, also can give a more accurate size distribution.

The maximum concentration limit depends on the rate at which the instrument can process different pulses and the need to avoid more than one particle in the sensing zone at the same time (coincidence). Follow manufacturers' instructions for upper limits on particle counts for each aperture size. Some instruments include a capability for correcting for overly concentrated samples, but do not use this feature because each particle is not measured individually and errors in absolute size distribution may result.

2. Apparatus

a. Particle counter and size-distribution analyzer.

b. Glassware: For glassware preparation, see Section 2560A.3*a* and Section 2560A.7.

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3. Reagents

a. Particle-free electrolyte solution: Prepare an electrolyte solution of NaCl, CaCl₂, NaNO₃, Ca(NO₃)₂, or some other simple inorganic salt at a concentration of 1 to 10% by weight. Pass solution through a continuous-flow membrane microfiltration system (see Section 2560A.4). Alternatively, use a commercially prepared electrolyte solution (filtration also may be required).

b. Calibration particles: See Section 2560A.5.

4. Procedure

a. Preparation: Let instrument warm up according to manufacturer's instructions. Select proper size aperture for particle sizes of interest; use more than one aperture if size distribution is wider than the range that can be measured with one aperture. Rinse aperture with acid solution, distilled water, and finally clean electrolyte solution. Install aperture. Choose settings on the instrument for each type of choice, including linear or logarithmic mode (spacing of sizes for each channel; logarithmic is preferable for environmental samples), desired size width of each channel, total number of channels and which channels are to be included, and method of starting and stopping counting. Counting can be started and stopped manually, by switches built into the manometer representing set sample volumes, by a set maximum count in any one channel, or stopped by time after a manual start. For reporting absolute measurements, choose a method that measures volume sampled.

b. Calibration: See Section 2560A.5. Calibrate each aperture with at least three particle sizes, determining the channel number at which the maximum count for each size is located. Handle calibration samples identically to those used for measurement. Because calibration can change, check it at each use of the instrument. Plot results as the volume of calibration particles vs. channel number (linear mode) or as the logarithm (base 10 or e) of the volume of calibration particles vs. channel number (logarithmic mode). The results should plot as a straight line. Use an equation for that line to assign an average size to each channel. Convert results from particle volume to particle diameter for reporting.

c. Blank sample: Measure at least one blank sample of the electrolyte solution. Carry a sample bottle with only electrolyte solution through procedures identical to those used for samples. Subtract counts from the blank solution (i.e., background counts), channel by channel, from the counts of the samples. See Section 2560A.7.

d. Measurement of samples: Prepare diluted sample, ensuring that the volume of sample is no greater than the volume of the electrolyte solution (dilution water); if a more concentrated suspension is necessary, prepare dilution water with a higher electrolyte concentration. Mix gently (see Section 2560A.3a). Insert sample container into the instrument. Start vacuum to control the mercury column and start particle counting. During counting, continuously watch the monitor provided on the instrument to check for blockage of the aperture by oversized particles. Check that the time for the specified volume (if the count is controlled by the stop and start switches on the manometer) is consistent with that found for particle-free electrolyte solution.

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When count is complete, check that the concentration did not exceed the manufacturer's recommendations. Repeat with a greater dilution if necessary. When a series of samples with various concentrations is to be measured and sample order is not critical, measure samples in order of expected increasing particle concentration, rinsing with next sample.

5. Calculation

See Section 2560A.6.

2560 C. Light-Blockage Methods

1. General Discussion

a. Principle: In light-blockage instruments, a focused beam of light shines from one side of the measurement zone toward a photovoltaic cell on the other side. The illuminated volume of liquid constitutes the sensing zone. Particles pass through the sensing zone at a known velocity. The blockage of light by the particle creates a change in the voltage at the photovoltaic cell. In different instruments, different characteristics of the resulting signal are used to determine size: light obscuration instruments use pulse height (related to the cross-sectional area of the particle) while time-of-transition instruments use the pulse width (proportional to a characteristic length of the particle). In all cases, measurements usually are reported in terms of equivalent spherical diameter.

For light-obscuration instruments, consult manufacturer's literature to determine the relationship between height of the voltage pulse and particle size (and increment of particle size).

Time-of-transition devices typically utilize a laser beam as the light source. The beam may scan a stationary sample at a fixed velocity as it sweeps out an optical sensing zone. Alternatively, a suspension can be passed through a fixed optical sensing zone. In the latter case, the measured particle size is sensitive to flow rate through the sensor.

Devices using light blockage principles vary in the number of channels (size classes) into which particles are sorted. In some cases, these are pre-set by the manufacturer; in others, they can be set by the analyst. Different (interchangeable) sensors are available to measure different size ranges and different particle concentrations.

b. Interferences: Interferences are caused by contamination with particles, the presence of gas bubbles, and electronic noise. See Section 2560A.3*a* and *b* for sampling precautions and Section 2560A.4 for particle-free dilution water preparation. Dilution water is necessary only for samples with particle number concentrations exceeding the capacity of the sensor to distinguish different particles. If dilution water is to be used, analyze it without sample and subtract particle counts in each channel from sample counts.

For devices in which sample flows through the sensor, some electronic noise can be detected by counting with clean water in the sensor and no flow (that is, with no true particle counts). If noise is present, it is likely to occur as a very large number of counts in the smallest size range (lowest channel). Eliminate this noise by re-setting the size range of the smallest channel

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(increasing the lowest size range to be measured) high enough to avoid significant counts in the lowest channel. For further measures to minimize electronic noise, see Section 2560A.7.

c. Detectable sizes and concentrations: The lower size limit of measurement depends on both electronic noise and sensor capability. Devices using the principle of light obscuration typically are limited to particles larger than approximately 1 μm . Devices using the time-of-transition principle can detect particles as small as 0.1 μm . The upper particle size limit usually is determined by the size of the orifice through which particles pass. Manufacturers state particle size limits (lower and upper) for each sensor. See Section 2560B.1c.

2. Apparatus

a. Particle counter and size distribution analyzer.

b. Glassware: See Section 2560A.3a and Section 2560A.7.

3. Reagents

a. Particle-free dilution water: See Section 2560A.4.

b. Calibration particles: See Section 2560A.5.

4. Procedure

a. Preparation: Let instrument warm up according to manufacturer's instructions. Select sensor to measure size and concentration ranges expected; use more than one sensor if size distribution is wider than the range that can be measured with one sensor. Rinse sensor with a volume of particle-free dilution water equivalent to at least three times that used in measuring samples.

Choose settings on the instrument for each type of choice available: including total number of channels and which channels are to be included, absolute or relative counts, cumulative or differential counts, etc. Also choose method of starting and stopping counting based on total count, maximum count in one channel, duration of counting, or analysis of a specified volume of sample. Usually, the known-volume method is preferable because it lends itself to determination of absolute size distribution.

b. Calibration: See Section 2560A.5. Initial calibration of each sensor on an instrument requires several particle sizes to determine the relationship between particle size and channel number. Either this relationship may be controlled by varying the range of millivolt responses that are sorted into each channel or it is pre-set by the manufacturer.

After initial calibration, use at least two sizes of calibration particles for each sensor each time the instrument is used. For instruments in which the sample flows through a stationary sensing zone, measure flow rate of sample and adjust it to that used during initial calibration.

c. Blank sample: See Section 2560B.4c but substitute particle-free dilution water for electrolyte solution.

d. Measurement of samples: Dilute sample, if necessary to keep within manufacturer's guidelines. Mix gently (see Section 2560A.3a). For flow-type instruments with pressure-based

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samplers, insert sample container into the instrument; for vacuum-based samplers, insert sampler tubing into the sample container. Start counting only after sufficient sample has passed through the tubing connecting the sample and sensor to ensure that the sensor is receiving the sample. For instruments that scan a stationary sample, gently transfer sample into the measurement device and insert.

For all instruments, when the count is complete, check that the concentration did not exceed the manufacturer's recommendations. Repeat with a greater dilution if necessary.

Between samples, rinse with dilution water or the upcoming sample. When a series of samples with various concentrations is to be measured and sample order is not critical, measure samples in order of expected increasing particle concentration; preferably, rinse with the next sample.

5. Calculation

See Section 2560A.6.

2560 D. Light-Scattering Method

1. General Discussion

a. Principle: Light-scattering instruments may be either flow or static devices. In flow instruments, the direct path of the light beam through the flow cell is blocked by a particle as it flows through the measurement zone with the fluid, and light scattered over a fixed range of angles is collected and measured. Particle size is determined from the angle and intensity of scattering based on the principles of Fraunhofer diffraction and/or Mie scattering. In static instruments, the particles remain quiescent and a laser light beam scans part of the suspension. Scattered light is collected by a photovoltaic cell and the resulting response from all particles scanned is mathematically deconvoluted to generate the size distribution.

Available particle counters vary in the angle or range of angles at which scattered light is measured and in the number of channels (size classes) into which particles are sorted. For flow-type instruments, different (interchangeable) sensors are available to measure different size ranges and different particle concentrations. The particle sizes determined by these instruments are equivalent spherical diameters, i.e., they are determined from the amount of light that would have been scattered at the angle(s) built in to the instrument by a calibration sphere of that diameter.

b. Interferences: For both types of instruments, interferences are caused by contamination of the sample with particles, the presence of gas bubbles, and electronic noise. For static-type instruments, additional sources of interference can be surface contamination (particles, markings, or scratches) on the sample container and sample color. See Section 2560A.3*a* and *b* for sampling precautions and 2560A.4 for particle-free dilution water preparation. Dilution water is necessary only for samples with particle number concentrations exceeding the capacity of the sensor to distinguish different particles. If dilution water is to be used, analyze it without sample

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and subtract particle counts in each channel from sample counts. To correct for color interference, consult the instrument manual.

In flow-type instruments, some electronic noise can be detected by counting with clean water in the sensor and no flow (that is, with no true particle counts). If noise is present, it is likely to occur as a very large number of counts in the smallest size range (lowest channel). Eliminate this noise by resetting the size range of the smallest channel (increasing the lowest size range to be measured) high enough to avoid significant counts in the lowest channel. For further measures to minimize electronic noise, see Section 2560A.7.

For static-type instruments, use a glass container that is optically clear and has no lettering or markings, at least in the light path. Minimize the effect of specific glassware by using the same sample container for all samples that will be compared and ensuring that the orientation of the sample container in the light beam is the same for every sample.

c. Detectable sizes and concentrations: The lower size limit of measurement depends on both electronic noise and sensor capability. For flow-type instruments, the upper particle size limit usually is determined by the size of the orifice through which particles pass. Manufacturers state particle size limits (lower and upper) for each sensor. See Section 2560B.1c.

Application of the Mie theory to the scattering data can extend the range of light-scattering instruments to as low as 0.1 μm . Alternatively, some instruments extend the lower limit of detectable diameters to 0.1 μm by augmenting Fraunhofer diffraction data with scattering intensity measurements at a fixed angle (often 90°) using two or three different wavelengths of incident light.

2. Apparatus

a. Particle counter and size distribution analyzer.

b. Glassware: See Section 2560A.3a and Section 2560A.7.

3. Reagents

a. Particle-free dilution water: See Section 2560A.4.

b. Calibration particles: See Section 2560A.5.

4. Procedure

a. Preparation: Let instrument warm up according to manufacturer's instructions. For flow-type instruments, select sensor to measure size and concentration ranges expected; use more than one sensor if size distribution is wider than the range that can be measured with one sensor. Preferably use a sensor designed for the concentration range of the undiluted sample rather than diluting samples into the concentration range of the sensor. For both types of instruments, place in or pass through the sensor at least three times a volume of particle-free dilution water equivalent to that used in measuring samples, before the first sample.

Choose settings on the instrument for each type of choice available, including flow rate, total number of channels and which channels are to be included, absolute or relative counts, etc.

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Also choose method of starting and stopping counting: total count, maximum count in one channel, duration of counting, or analysis of a specified volume of sample. For flow-type instruments, the known-volume method is preferable because it lends itself to determination of absolute size distribution. For static-type analyzers, the analyst does not control sample size directly, but can control duration of measurement. Take care not to exceed maximum particle number concentration limits suggested by manufacturer.

Some flow-type instruments permit choice between vacuum or pressure systems for transporting the sample through the sensor. If floc preservation is essential, preferably use a vacuum system but ensure that bubbles do not form before the sensor; otherwise, use a pressure system with a pulseless gear pump.

b. Calibration: See Section 2560A.5. For flow-type instruments, initial calibration of each sensor on an instrument requires several particle sizes to determine the relationship between particle size and channel number. Either this relationship may be controlled by varying the range of millivolt responses that are sorted into each channel, or it is pre-set by the manufacturer. Static-type instruments are pre-set by the manufacturer because sizing is done by a software conversion of the light sensed. After initial calibration, use at least two sizes of calibration particles for each sensor each time the instrument is used. Although the calculation of particle sizes is based on first principles and not on this calibration, calibrate to ensure that the instrument is functioning properly. For flow-type instruments, measure flow rate through the sensor and adjust to that used during initial calibration.

c. Blank sample: See Section 2560B.4c, but substitute particle-free dilution water for electrolyte solution.

d. Measurement of samples: If dilution is necessary, dilute sample to keep within manufacturer's guidelines for the maximum counting rate. Mix gently (see Section 2560A.3a).

For flow-type instruments, initiate the flow from the sample through the sensor. Check that flow rate is the same as that used for sensor calibration. Start counting only after sufficient sample has passed through the tubing connecting the sample and sensor to ensure that the sensor is receiving the sample. When counting is complete, check that the concentration did not exceed the manufacturer's recommendations. Repeat with a greater dilution if necessary. For subsequent samples, rinse with dilution water or the next sample. When a series of samples with varying concentrations is to be measured and sample order is not critical, measure samples in order of expected increasing particle concentration; in this case, preferably rinse with next sample.

For static-type instruments, insert sample beaker or bottle and start particle counting. When counting is complete, check that the concentration did not exceed the manufacturer's recommendations by diluting and measuring again. If the second count is related to the first according to the ratio of the dilution factors of the two measurements, consider the first measurement acceptable. Repeat with a greater dilution if necessary. For subsequent samples, see directions in preceding paragraph.

5. Calculation

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See Section 2560A.6.

2570 ASBESTOS#(52)*

2570 A. Introduction

1. Occurrence and Significance

The term “asbestos” describes a group of naturally occurring, inorganic, highly fibrous silicate minerals that are easily separated into long, thin, flexible fibers when crushed or processed. Included in the definition are the asbestiform (see ¶ 2 below) varieties of serpentine (chrysotile), riebeckite (crocidolite), grunerite (grunerite asbestos), anthophyllite (anthophyllite asbestos), tremolite (tremolite asbestos), and actinolite (actinolite asbestos).

Asbestos has been used widely as a thermal insulator and in filtration. The tiny, almost indestructible fibers penetrate lung tissue and linings of other body cavities, causing asbestosis and cancer in the lungs and mesothelioma in other cavity linings.

2. Definitions

Asbestiform—having a special type of fibrous habit (form) in which the fibers are separable into thinner fibers and ultimately into fibrils. This habit accounts for greater flexibility and higher tensile strength than other habits of the same mineral. More information on asbestiform mineralogy is available.^{1,2}

Aspect ratio—ratio of the length of a fibrous particle to its average width.

Bundle—structure composed of three or more fibers in parallel arrangement with the fibers closer than one fiber diameter to each other.

Cluster—structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group; groupings of fibers must have more than two points touching.

Fiber (AHERA)—structure having a minimum length greater than or equal to 0.5 μm , an aspect ratio of 5:1 or greater, and substantially parallel sides.³

Fibril—single fiber that cannot be separated into smaller components without losing its fibrous properties or appearance.

Fibrous—composed of parallel, radiating, or interlaced aggregates of fibers, from which the fibers are sometimes separable. The crystalline aggregate of a mineral may be referred to as fibrous even if it is not composed of separable fibers, but has that distinct appearance. “Fibrous” is used in a general mineralogical way to describe aggregates of grains that crystallize in a needle-like habit and appear to be composed of fibers; it has a much more general meaning than “asbestos.” While all asbestos minerals are fibrous, not all minerals having fibrous habits are asbestos.

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Matrix—fiber or fibers with one end free and the other end embedded in, or hidden by, a particle. The exposed fiber must meet the fiber definition.

Structures—all the types of asbestos particles, including fibers, bundles, clusters, and matrices.

3. References

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3. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1987. Asbestos-containing materials in schools: Final rule and notice. *Federal Register*, 40 CFR Part 763, Appendix A to Sub-part E, Oct. 30, 1987.

2570 B. Transmission Electron Microscopy Method

1. General Discussion

This method is used to determine the concentration of asbestos structures, expressed as the number of such structures per liter of water. Asbestos identification by transmission electron microscopy (TEM) is based on morphology, selected area electron diffraction (SAED), and energy dispersive X-ray analysis (EDXA). Information about structure size also is generated. Only asbestos structures containing fibers greater than or equal to 0.5 μm in length are counted. The concentrations of both fibrous asbestos structures greater than 10 μm in length and total asbestos structures per liter of water are determined. The fibrous asbestos structures greater than 10 μm in length are of specific interest for meeting the Federal Maximum Contaminant Level Goal (MCLG) for drinking water, but in many cases the total asbestos concentration provides important additional information.

a. Principle: Sample portions are filtered through a membrane filter. A section of the filter is prepared and transferred to a TEM grid using the direct transfer method. The asbestiform structures are identified, sized, and counted by transmission electron microscopy (TEM), using selected area electron diffraction (SAED) and energy dispersive spectroscopy (EDS or EDXA) at a magnification of 15 000 to 20 000 $\times$.

b. Interferences: Certain minerals have properties (i.e., chemical or crystalline structure) that are very similar to those of asbestos minerals and may interfere with the analysis by causing false positives. Maintain references for the following materials in the laboratory for comparison with asbestos minerals, so that they are not misidentified as asbestos minerals: antigorite, attapulgite (palygorskite), halloysite, hornblende, pyroxenes, sepiolite, and vermiculite scrolls.

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High concentrations of iron or other minerals in the water may coat asbestos fibers and prevent their full identification.

2. Sampling

a. Containers: Use new, pre-cleaned, capped bottles of glass or low-density (conventional) polyethylene, capable of holding at least 1 L. Do not use polypropylene bottles. Rinse bottles twice by filling approximately one-third full with fiber-free water and shaking vigorously for 30 s. Discard rinse water, fill bottles with fiber-free water, treat in ultrasonic bath (60 to 100 W) for 15 min, and rinse several times with fiber-free water.

Make blank determinations on the bottles before collecting a sample. Use one bottle in each batch or a minimum of one bottle in each 24 to test for background level when using polyethylene bottles. When sampling waters probably containing very low levels of asbestos, or for additional confidence in the bottle blanks, run additional blank determinations.

b. Collection: Follow general principles for water sampling (see Section 1060). Some specific considerations apply to asbestos fibers, which range in length from 0.1 μm to 20 μm or more. In large bodies of water, because of the range of sizes there may be a vertical distribution of particle sizes that may vary with depth. If a representative sample from a water supply tank or impoundment is required, take a carefully designated set of samples representing vertical as well as horizontal distribution and composite for analysis. When sampling from a distribution system, choose a commonly used faucet, remove all hoses or fittings, and let water run to waste for 1 to 3 min. (Often, the appropriate time to obtain a main's sample can be determined by waiting for a change in water temperature.) Because sediment may build up in valving works, do not adjust faucets or valves until all samples have been collected. Similarly, do not consider samples at hydrants and at dead ends of the distribution systems to be representative of the water in the system. As an additional precaution against contamination, rinse each bottle several times with the source water being sampled. For depth sampling, omit rinsing. Obtain a sample of approximately 800 mL from each sampling site, leaving some air space in each bottle. Using a waterproof marker, label each container with date, time, place, and sampler's initials.

c. Shipment: Ship water samples in a sealed container, but separate from any bulk or air samples intended for asbestos analysis. Preferably, ship in a cooler to retard bacterial or algal growth. Do not freeze the sample. In the laboratory, prepare sample within 48 h of collection.

3. Apparatus

a. High-efficiency particulate air (HEPA) filtered negative-flow hood.

b. Filter funnel assemblies, either 25 mm or 47 mm, of either of the following types:

1) *Disposable plastic units, or*

2) *Glass filtering unit.* With this type of unit, observe the following precautions: Never let unit dry after filtering. Immediately place it in detergent solution, scrub with a test-tube brush, and rinse several times in particle-free water. Periodically treat unit in detergent solution in an ultrasonic bath. Clean unit after each sample is filtered. Run a blank on particle-free water

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filtered through the glass filtering unit at frequent intervals to ensure absence of residual asbestos contamination.

c. Side-arm filter flask, 1000 mL.

d. Either:

1) *Mixed cellulose ester (MCE) membrane filters, 25- or 47-mm diam, $\leq 0.45\text{-}\mu\text{m}$ and $5\text{-}\mu\text{m}$ pore size, or*

2) *Polycarbonate (PC) filters, 25- or 47-mm diam, $\leq 0.4\text{-}\mu\text{m}$ pore size.*

e. Ultrasonic bath, tabletop model, 60 to 100 W.

f. Graduated pipet, disposable glass, 1, 5, and 10 mL.

g. Cabinet-type dessicator or low-temperature drying oven.

h. Cork borer, 7 mm.

i. Glass slides.

j. Petri dishes, glass, approximately 90-mm diam.

k. Mesh screen, stainless steel or aluminum, 30 to 40 mesh.

l. Ashless filter paper filters, 90-mm diam.

m. Exhaust or fume hood.

n. Scalpel blades.

o. Low-temperature plasma asher.

p. High-vacuum carbon evaporator with rotating stage, capable of less than 0.013 Pa pressure. Do not use units that evaporate carbon filaments in a vacuum generated only by oil rotary pump. Use carbon rods sharpened with a carbon rod sharpener to necks about 4 mm long and 1 mm in diam. Install rods in the evaporator so that the points are approximately 100 to 120 mm from surface of microscope slide held in the rotating device.

q. Lens tissue.

r. Copper TEM finder grids, 200 mesh. Use pre-calibrated grids, or determine grid opening area by either of the following methods:

1) Measure at least 20 grid openings in each of 20 random 200-mesh grids (total of 400 grid openings for every 1000 grids used) by placing the 20 grids on a glass slide and examining them under an optical microscope. Use a calibrated reticule to measure average length and width of 20 openings from each of the individual grids. From the accumulated data, calculate the average grid opening area.

2) Measure grid area at the TEM (§§ below) at a calibrated screen magnification of between 15 000 and 20 000 $\times$. Measure one grid opening for each grid examined. Measure grid openings in both the x and y directions and calculate area.

s. Transmission electron microscope (TEM), 80 to 120 kV, equipped with energy dispersive X-ray system (EDXA), capable of performing electron diffraction with a fluorescent screen

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inscribed with calibrated gradations. The TEM must have a scanning transmission electron microscopy (STEM) attachment or be capable of producing a spot size of less than 250 nm diam at crossover. Calibrate routinely for magnification, camera constant, and EDXA settings according to procedures of 5d.

4. Reagents and Materials

- a. *Acetone.*
- b. *Dimethylformamide (DMF).* CAUTION: *Toxic; use only in a fume hood.*
- c. *Glacial acetic acid.* CAUTION: *Use in a fume hood.*
- d. *Chloroform.*
- e. *1-Methyl-2-pyrrolidinone.*
- f. *Particle-free water:* Use glass-distilled water or treat by reverse osmosis; filter through a filter with pore diam 0.45 μm or smaller.
- g. *Non-asbestos standards* for minerals listed in ¶ 1b above.
- h. *Asbestos standards* for minerals listed in Section 2570A.1.

5. Procedure

a. *Sample filtration:* Samples with high levels of organic contaminants may require pretreatment. A process using ultraviolet light and ozone bubbling is described elsewhere.¹ Drinking water samples prepared within 48 h of collection do not require pretreatment.

Under a HEPA hood, carefully wet-wipe exterior of sample bottle to remove any possible contamination before taking bottle into a clean preparation area separated from preparation areas for bulk or air-sample handling.

Prepare specimen in a clean HEPA filtered negative-pressure hood. Measure cleanliness of preparation area hoods by cumulative process blank concentrations (see ¶ b below).

If using a disposable plastic filter funnel unit, remove funnel assembly and discard top filter supplied with the apparatus, but be sure to retain the coarse polypropylene support pad in place. Assemble unit with the adapter and a properly sized neoprene stopper, and attach funnel to the 1000-mL side-arm vacuum flask. Moisten support pad with a few milliliters distilled water, place a 5.0- μm -pore-size MCE backing filter on support pad, and place an MCE or PC filter ($\leq 0.45\text{-}\mu\text{m}$ -pore-size) on top of backing filter. After both filters are completely wet, apply vacuum, ensuring that filters are centered and pulled flat without air bubbles. If there are any irregularities on the filter surface, discard filters and repeat process. Replace funnel assembly. Return flask to atmospheric pressure. Alternatively, use glass filtering unit, following the same procedure to set up filters.

With flask at atmospheric pressure, add 20 mL particle-free water to funnel. Cover funnel with its plastic cover if the disposable filtering unit is used.

Briefly, by hand, shake capped bottle with sample suspension, then place it in tabletop ultrasonic bath and sonicate for 3.0 min. The water level in the bath should be approximately the

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same as that of the sample. After treatment, return sample bottle to work surface of HEPA hood. Carry out all preparation steps, until filters are ready for drying, in this hood.

Shake suspension vigorously by hand for 2 to 3 s. Estimate amount of liquid to be withdrawn to produce an adequate filter preparation. Experience has shown that a light staining of the filter surface will usually yield a suitable preparation. If the sample is relatively clean, use a volumetric cylinder to measure sample. If sample has a high particulate or asbestos content, withdraw a small volume (but at least 1 mL) with disposable glass pipet, inserting pipet halfway into sample.

NOTE: If, after examination in the TEM, the smallest volume measured (1.0 mL) yields an overloaded sample, make additional serial dilutions of the suspension. Shake suspension vigorously by hand for 2 to 3 s before taking serial dilution portion. Do not re-treat in ultrasonic bath either original solution or any dilutions. Mix 10 mL sample with 90 mL particle-free water in a clean sample bottle to obtain a 1:10 serial dilution.

Uncover filter funnel and dispense mixture into the water in the funnel. Recover funnel and agitate but do not swirl the liquid. (One acceptable means of agitation is to inject 5 to 10 mL particle-free water from a squeeze bottle into the funnel.) Apply vacuum and filter. Discard pipet, if used.

Disassemble filtering unit and carefully remove filter with clean forceps. Place filter, particle side up, in a precleaned, labeled, disposable plastic petri dish or similar container.

To obtain an optimally loaded filter, make several filtrations with different sample portions. Use new disposable plastic funnel units or carefully cleaned glass units for each filtration. When additional filters are prepared, shake suspension without additional ultrasonic treatment before removing the sample portion. Each new filtration should represent at least a five-fold loading difference.

Dry MCE filters for at least 12 h (over desiccant) in airtight, cabinet-type desiccator. Alternatively, to shorten drying time for filters prepared using the acetone collapsing method, plug damp filter and attach to a glass slide as described in ¶ c1)a) below. Place the slide with filter plug(s) (up to eight plugs can be attached to one slide) on a bed of desiccant, cover, and place in desiccator for 1 to 2 h.

Place PC filters in a dessicator for at least 30 min before preparation; lengthy drying is not required.

b. Sample blank preparation: Prepare sample blanks that include both a process blank (50 mL particle-free water) for each set of samples analyzed and one unused filter from each new box of sample filters (MCE or PC). Blanks are considered contaminated if, after analysis, they are shown to contain more than 53 asbestos structures/mm². This corresponds to 3 or 4 asbestos structures found in 10 grid openings. Identify source of contamination before making any further analysis. Reject samples that were processed with the contaminated blanks and prepare new samples after source of contamination is found. Take special care with polycarbonate filters, because some have been shown to contain asbestos contamination.

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c. Specimen preparation:

1) Mixed cellulose ester (MCE) filters

a) Filter fusing—Use either the acetone or the DMF-acetic acid method.

(1) Acetone fusing method—Remove a section from any quadrant of the sample and blank filters with a 7-mm cork borer. Place filter section (particle side up) on a clean microscope slide. Affix filter section to slide with a gummed page reinforcement or other suitable means. Label slide with a glass scribing tool or permanent marker.

Prepare a fusing dish as follows: Make a pad from five to six ashless paper filters and place in bottom of a glass petri dish. Place metal screen bridge on top of pad and saturate filter pads with acetone. Place slide on top of bridge and cover the petri dish. Wait approximately 5 min for sample filter to fuse and clear completely.

(2) DMF-acetic acid fusing method—Place drop of clearing solution (35% dimethylformamide [DMF], 15% glacial acetic acid, and 50% particle-free water by volume) on a clean microscope slide. CAUTION: *DMF is a toxic solvent; use only in a fume hood.* Use an amount of clearing solution that just saturates the filter. Using a clean scalpel blade, cut a wedge-shaped section of filter. A one-eighth filter section is sufficient. Carefully lay filter segment, sample surface upward, on top of solution. Bring filter and solution together at an angle of about 20 deg to help exclude air bubbles. Remove excess clearing solution with filter paper. Place slide in oven, on a slide-warmer, or on hot plate, in a fume hood, at 65 to 70°C for 5 to 10 min. The filter section should fuse and clear completely.

b) Plasma etching—Place microscope slide, with attached collapsed filter pieces, in a low-temperature plasma asher. Because plasma ashers vary greatly in their performance, both from unit to unit and between different positions in the asher barrel, it is difficult to specify operating conditions. Insufficient etching will result in a failure to expose embedded fibers; too much etching may result in the loss of particles from the filter surface. Calculate time for ashing on the basis of final sample observations in transmission electron microscope. Additional information on calibration is available.^{2,3}

c) Carbon coating—Using high-vacuum carbon evaporator (¶ 3 p), proceed as follows: Place glass slide holding filters on the rotation device and evacuate evaporator chamber to a pressure of less than 0.013 Pa. Perform evaporation in very short bursts, separated by 3 to 4 s to let electrodes cool. An experienced analyst can judge the thickness of the carbon film. Make initial tests on unused filters. If the carbon film is too thin, large particles will be lost from the TEM specimen, and there will be few complete and undamaged grid openings. A coating that is too thick will lead to a TEM image lacking in contrast and a compromised ability to obtain electron diffraction patterns. The carbon film should be as thin as possible and still remain intact on most of the grid openings of the TEM specimen.

d) Specimen washing—Prepare a Jaffe washer according to any published design.^{1,4} One such washer consists of a simple stainless steel bridge contained in a glass petri dish. Place on the stainless steel bridge several pieces of lens tissue large enough to hang completely over the

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bridge and into the solvent. In a fume hood, fill petri dish with either acetone or DMF to one quarter of the level in the dish.

Place TEM grids, shiny side up, on a piece of lens tissue or filter paper so that individual grids can be easily picked up with forceps. Prepare from each sample three grids. Using a curved scalpel blade, excise three square (3-mm × 3-mm) pieces of carbon-coated MCE filter from random areas on the filter. Place each square filter piece, carbon-side up, on top of a TEM specimen grid (§ 3r).

Place all three assemblies (filter/grid) for each sample on the same piece of saturated lens tissue in Jaffe washer. Place lid on the Jaffe washer and let system stand, preferably overnight.

Alternatively, place grids on a low-level (petri dish is filled only enough to wet paper on screen bridge) DMF Jaffe washer for 60 min. Then add enough solution of equal parts DMF/acetone to fill washer up to screen level. Remove grids after 30 min if they have cleared, i.e., all filter material has been removed from the carbon film, as determined by inspecting in the TEM. Let grids dry before placing in TEM.

2) Polycarbonate (PC) filters—Cover surface of a clean microscope slide with two strips of double-sided cellophane tape. Cut a strip of filter paper slightly narrower than width of slide. Position filter paper strip on center of length of slide. Using a clean, curved scalpel blade, cut a strip of the PC filter approximately 25 × 6 mm. Use a rocking motion of the scalpel blade to avoid tearing filter. Place PC strip, particle side up, on slide perpendicular to long axis of slide, making sure that the ends of the PC strip contact the double-sided cellophane tape. Each slide can hold several PC strips. Label filter paper next to each PC strip with sample number.

Carbon-coat filter strips as directed in § 1c) above. (Etching is not required.) Take special care to avoid overheating filter sections during carbon coating.

Prepare a Jaffe washer as described in § 1d) above, but fill washer with chloroform or 1-methyl-2-pyrrolidinone to the level of the screen. Using a clean curved scalpel blade, excise three 3-mm-square filter pieces from each PC strip. Place filter squares, carbon side up, on shiny side of a TEM grid (§ 3r). Pick up grid and filter section together and place them on lens tissue in the Jaffe washer. Place lid on Jaffe washer and leave grids for at least 4 h. Best results are obtained with longer wicking times, up to 12 h. Carefully remove grids from the Jaffe washer and let dry in the grid box before placing them in a clean, marked grid box.

d. Instrument calibration: Calibrate instrumentation regularly, and keep a calibration record for each TEM in the laboratory, in accordance with the laboratory's quality assurance program. Record all calibrations in a log book along with dates of calibration and attached backup documentation.

Check TEM for both alignment and systems operation. Refer to manufacturer's operational manual for detailed instructions.

Calibrate camera length of TEM in electron diffraction (ED) operating mode before observing ED patterns of unknown samples. Measure camera length by using a carbon-coated grid on which a thin film of gold has been sputtered or evaporated. A thin film of gold may be evaporated directly on to a specimen grid containing asbestos fibers. This yields zone-axis ED

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patterns from the asbestos fibers superimposed on a ring pattern from the polycrystalline gold film. Optimize thickness of gold film so that only one or two sharp rings are obtained. Thicker gold films may mask weaker diffraction spots from the fibrous particles. Because unknown d-spacings of most interest in asbestos analysis are those lying closest to the transmitted beam, multiple gold rings from thicker films are unnecessary. Alternatively, use a gold standard specimen to obtain an average camera constant calculated for that particular instrument, which can then be used for ED patterns of unknowns taken during the corresponding period.

Calibrate magnification at the fluorescent screen at magnification used for structure counting. Use a grating replica (e.g., one containing at least 2160 lines/mm). Define a field of view on the fluorescent screen; the field must be measurable or previously inscribed with a scale or concentric circles (use metric scales). Place grating replica at the same distance from the objective lens as the specimen. For instruments that incorporate a eucentric tilting specimen stage, place all specimens and the grating replica at the eucentric position. Follow the instructions provided with the grating replica to calculate magnification. Frequency of calibration depends on service history of the microscope. Check calibration after any maintenance that involves adjustment of the power supply to the lens, the high-voltage system, or the mechanical disassembly of the electron optical column (apart from filament exchange).

Check smallest spot size of the TEM. At the crossover point, photograph spot size at a magnification of 25 000 $\times$ (screen magnification 20 000 $\times$). An exposure time of 1 s usually is adequate. Measured spot size must be less than or equal to 250 nm.

Verify resolution and calibration of the EDXA as follows: Collect a standard EDXA Cu peak from the Cu grid and compare X-ray energy with channel number for Cu peak to make sure readings are within ± 10 eV. Collect a standard EDXA of crocidolite asbestos; elemental analysis of the crocidolite must resolve the Na peak. Collect a standard EDXA spectrum of chrysotile asbestos; elemental analysis of chrysotile must resolve both Si and Mg on a single chrysotile fiber.

e. Sample analysis: Carefully load TEM grid with grid bars oriented parallel/perpendicular to length of specimen holder. Use a hand lens or eye loupe if necessary. This procedure will line up the grid with the x and y translation directions of the microscope. Insert specimen holder into microscope.

Scan entire grid at low magnification (250 $\times$ to 1000 $\times$) to determine its acceptability for high-magnification analysis. Grids are acceptable if the following conditions are met:

- The fraction of grid openings covered by the replica section is at least 50%.
- Relative to that section of the grid covered by the carbon replica, the fraction of intact grid openings is greater than 50%.
- The fractional area of undissolved filter is less than 10%.
- The fraction of grid squares with overlapping or folded replica film is less than 50%.
- At least 20 grid squares have no overlapping or folded replica, are less than 5% covered with

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holes, and have less than 5% opaque area due to incomplete filter dissolution.

If the grid meets these criteria, choose grid squares for analysis from various areas of the grid so that the entire grid is represented. To be suitable for analysis, an individual grid square must meet the following criteria:

- It must have less than 5% holes over its area.
- It must be less than 25% covered with particulate matter.
- It must be uniformly loaded.

Observe and record orientation of grid at 80 to 150× on a grid map record sheet along with the location of the grid squares examined. If indexed grids are used, a grid map is not needed, but record identifying coordinates of the grid square.

At a screen magnification of 15 000 to 25 000×, evaluate the grids for the most concentrated sample loading. Reject sample if it is estimated to contain more than about 25 asbestos structures per grid opening. Proceed to the next most concentrated sample until a set of grids is obtained that have less than 25 asbestos structures per grid opening.

Analyze a minimum of four grid squares for each sample.

Analyze approximately one-half of the predetermined sample area on one sample grid preparation and the remainder on a second sample grid preparation.

Use structure definitions given in Section 2570A.2 to enumerate asbestos structures. Record all data on count sheet. Record asbestos structures in two size categories: $\geq 0.5 \mu\text{m}$ to $\geq 10.0 \mu\text{m}$ and $>10.0 \mu\text{m}$. For fibers and bundles, record size category of greatest length of the structure. For matrices and clusters, record size category of the visible portion of the longest fiber or bundle involved with the structure, not the greatest overall dimension of the structure. No minimum or maximum width restrictions are applied to the fiber definition, as long as the minimum length and aspect ratio criteria are met. Record “NSD” (no structures detected) when no structures are found in the grid opening.

Record a typical electron diffraction pattern for each type of asbestos observed for each group of samples (or a minimum of every five samples) analyzed. Record micrograph number on count sheet. For chrysotile, record one X-ray spectrum for each tenth structure analyzed. For each type of amphibole, record one X-ray spectrum for each fifth structure analyzed. (More information on identification is available.^{1,4}) Attach the printouts to the back of the count sheet. If the X-ray spectrum is stored, record file and disk number on count sheet.

Analytical sensitivity can be improved by increasing amount of liquid filtered, increasing number of grid openings analyzed, or decreasing size of filter used. Occasionally, because of high particle loadings or high asbestos concentration, the desired analytical sensitivity cannot be achieved in practice.

Unless a specific analytical sensitivity is desired, stop analysis on the 10th grid opening or

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the grid opening that contains the 100th asbestos structure, whichever comes first. If the analysis is stopped at the grid opening that contains the 100th asbestos structure, count entire grid square containing the 100th structure.

After analysis, remove grids from TEM, replace them in grid storage holder, and store for a minimum of one year from the date of the analysis for legal purposes. Sample filters also may be stored in the plastic petri dishes, if necessary. Prolonged storage of the remaining water sample is not recommended, because microbial growth may cause loss of asbestos structures to the sides of the storage container.

Report the following information for each water sample analyzed: asbestos concentration in structures per liter, for total structures and fibrous asbestos structures greater than 10 µm in length; types of asbestos present; number of asbestos structures counted; effective filtration area; average size of TEM grid openings counted; number of grid openings examined; size category for each structure counted. Include a copy of the TEM count sheet, either hand-written or computer-generated.

6. Calculations

Calculate amount of asbestos in a sample as follows:

$$\text{Asbestos concentration, structures/L} = \frac{N \times A_f \times D}{G \times A_G \times V_s}$$

where:

N = number of asbestos structures counted,

A_f = effective filter area of final sampling filter, mm²,

D = dilution factor (if applicable),

G = number of grid openings counted,

A_G = area of grid openings, mm², and

V_s = volume of sample, L.

The same formula may be used to calculate asbestos fibers greater than 10 µm in length per liter based on the total number of fibers and bundles greater than 10 µm in length whether free or associated with matrices and clusters. Express final results as million structures per liter (MSL) and million fibers per liter (MFL).

7. Quality Control/Quality Assurance

Use the laboratory's quality-control checks to verify that system is performing according to accuracy and consistency specifications. Because of the difficulties in preparing known quantitative asbestos samples, routine quality-control testing focuses on reanalysis of samples

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(duplicate recounts). Reanalyze 1 out of every 10 samples, not including laboratory blanks.

In addition, set up quality assurance programs according to the criteria developed by Federal agencies.^{2,3} These documents cover sample custody, sample preparation, blank checks for contamination, calibration, sample analysis, analyst qualifications, and technical facilities.

8. Precision and Bias

Precision measurements for intralaboratory comparisons have been found to have a relative standard deviation (RSD) of 13 to 22% for standard and environmental water samples, with an RSD of 8.4 to 29% for interlaboratory comparisons.¹ An earlier study found an interlaboratory reproducibility of 25 to 50% in standard samples.⁵

Accuracy measurements from inter- and intralaboratory studies have demonstrated an RSD of 17% for standard chrysotile suspensions, and an RSD of 16% for standard crocidolite suspensions.¹

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2580 OXIDATION-REDUCTION POTENTIAL (ORP)#(53)*

2580 A. Introduction

1. Significance

Oxidation and reduction (redox) reactions mediate the behavior of many chemical

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constituents in drinking, process, and wastewaters as well as most aquatic compartments of the environment.¹⁻⁵ The reactivities and mobilities of important elements in biological systems (e.g., Fe, S, N, and C), as well as those of a number of other metallic elements, depend strongly on redox conditions. Reactions involving both electrons and protons are pH- and Eh-dependent; therefore, chemical reactions in aqueous media often can be characterized by pH and Eh together with the activity of dissolved chemical species. Like pH, Eh represents an intensity factor. It does not characterize the capacity (i.e., poise) of the system for oxidation or reduction.

The potential difference measured in a solution between an inert indicator electrode and the standard hydrogen electrode should not be equated to Eh, a thermodynamic property, of the solution. The assumption of a reversible chemical equilibrium, fast electrode kinetics, and the lack of interfering reactions at the electrode surface are essential for such an interpretation. These conditions rarely, if ever, are met in natural water.

Thus, although measurement of Eh in water is relatively straightforward, many factors limit the interpretation of these values. These factors include irreversible reactions, electrode poisoning, the presence of multiple redox couples, very small exchange currents, and inert redox couples. Eh values measured in the field correlate poorly with Eh values calculated from the redox couples present. Nevertheless, measurement of redox potential, when properly performed and interpreted, is useful in developing a more complete understanding of water chemistry.

2. Sampling and Storage

Do not store samples; analyze on collection. Minimize both atmospheric contact and delay in analysis.

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2580 B. Oxidation-Reduction Potential Measurement in Clean Water

1. General Discussion

a. *Principle:* Electrometric measurements are made by potentiometric determination of electron activity (or intensity) with an inert indicator electrode and a suitable reference electrode. Ideally, the indicator electrode will serve as either an electron donor or acceptor with respect to electroactive oxidized or reduced chemical species in solution. At redox equilibrium, the potential difference between the ideal indicator electrode and the reference electrode equals the redox potential of the system. However, inert indicator electrodes that behave ideally in all aqueous systems, particularly in natural waters, do not exist. Electrodes made of platinum are most commonly used for Eh measurements. They have limitations,¹ as do alternative materials such as gold and graphite.

The standard hydrogen reference electrode is fragile and impractical for routine laboratory and field use. Therefore, silver: silver-chloride or calomel reference electrodes are used commonly. The redox potential measurement is corrected for the difference between the potential of the reference electrode and that of the standard hydrogen electrode. See Section 4500-H pH Value.

It is not possible to calibrate Eh electrodes over a range of redox potentials (as is done with pH electrodes). Instead, standard solutions that exhibit both chemical stability and known redox potentials for specific indicator electrodes are used to check electrode response at the temperature of measurement.

The potential of the platinum (Pt) Eh electrode versus the Ag/AgCl reference electrode with KCl electrolyte in ZoBell's solution ($3 \times 10^{-3}M$ potassium ferrocyanide and $3 \times 10^{-3}M$ potassium ferricyanide in $0.1M$ KCl)² has been measured as a function of temperature.³ Good agreement was obtained between Eh values measured with this electrode pair in ZoBell's solution and those calculated from the stability constants at 8 to 85°C. The potential of the ZoBell's solution with this electrode configuration

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as a function of temperature can be calculated:⁴

$$Eh, V = 0.428 - 0.0022 (T - 25)$$

where T = solution temperature, °C. Alternatively, select the value from Table 2580:I.

To determine the Eh of a sample relative to the standard hydrogen electrode, measure Eh of both sample and standard solution at the same temperature (within $\pm 0.1^\circ\text{C}$). Then calculate Eh value of the sample:

$$Eh_{\text{system}} = E_{\text{observed}} + Eh_{\text{ZoBell/reference}} - Eh_{\text{ZoBell observed}}$$

where:

E_{observed} = sample potential relative to reference electrode.

$Eh_{\text{ZoBell/reference}}$ = theoretical Eh of reference electrode and ZoBell's solution, relative to the standard hydrogen electrode (see Table 2580:I), and

$Eh_{\text{ZoBell observed}}$ = observed potential of ZoBell's solution, relative to the reference electrode.

The measurements described above can be applied analogously to other indicator electrode/reference electrode pairs and standard solutions.

b. Interferences: Specific interferences may be due to operation of either indicator or reference electrode, redox capacity or poise of the sample, sample preservation and handling, and temperature equilibration.

1) Sorption and poisoning effects on electrodes—Contamination of the electrode surface, salt bridge, or internal electrolyte in the case of reference electrodes, can lead to excessive drift, poor electrode response, and artifact potentials. Organic matter, sulfide, and bromide may cause these problems, particularly in long-term electrode use.^{1,5-7} If excessive drift occurs or erratic performance of paired electrodes is observed in redox standard solutions after appropriate cleaning, refilling, or regeneration procedures, discard the faulty electrode and use a new one.

2) pH variations—Redox potential is sensitive to pH if hydrogen ion or hydroxide ion is involved in the redox half-cells. Cell potentials tend to increase as proton concentration increases (i.e., pH decreases) and Eh values drop as hydroxide concentrations increase (i.e., pH increase).

3) Sample handling and preservation—The sample poise will govern the resistance of the sample to change in redox potential; this phenomenon is analogous to the resistance to pH change afforded by buffer capacity. Except in concentrated process streams, sludges, leachates, and highly reducing or treated waters, the concentrations of oxidized or reduced species may be fairly low (i.e., $<10^{-4}\text{M}$). Under these conditions, handle reduced samples very carefully to avoid exposure to atmospheric oxygen. A closed cell sampling configuration may be used.^{4,8} Samples cannot be stored or preserved; analyze at sampling.

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4) Temperature equilibration—Obtain Eh standard solution reading for the electrode pair at a temperature as close as possible to that of the sample. Temperature determines the Eh reference potential for a particular solution and electrode pair. It also may affect the reversibility of the redox reaction, the magnitude of the exchange current, and the stability of the apparent redox potential reading, particularly in poorly poised solutions. Hold temperature constant for all measurements and report it with Eh results.

2. Apparatus

a. *pH or millivolt meter*: Use a pH meter or other type of high-impedance potentiometer capable of reading either pH or millivolts (mV). For most applications, a meter scale readable to ± 1400 mV is sufficient.

b. *Reference electrode* consisting of a half-cell providing a constant electrode potential. See Section 4500-H⁺.B.2b.

c. *Oxidation-reduction indicator electrode*: The platinum electrode is used most commonly. A noble metal or graphite electrode may be useful for specific applications.

1) Noble metal electrode—Noble metal (i.e., gold or platinum) foil, wire, or billet types of electrode are inert and resistant to chemical reaction. Clean and polish electrode surface to insure reliable performance. Platinum electrodes may be cleaned by strong acid soaking,^{9,10} hydrogen peroxide and detergent washing,¹¹ and anodic activation.¹⁰ Abrasive polishing with crocus cloth, jeweler's rouge, or 400 to 600 grit wet/dry carborundum paper may be best.⁵

2) Graphite electrode—A wax-impregnated graphite (WIG) electrode may be used, especially in aqueous suspensions or soils.^{12,13} The WIG electrode is more resistant to electrode poisoning than electrodes made of platinum wire.

d. *Beakers*: Preferably use polyethylene, TFE, or glass beakers.

e. *Stirrer*: Use a magnetic TFE-coated stirring-bar-type mixer.

f. *Flow cell*: Use for continuous flow measurements and for poorly buffered solutions.

3. Reagents

a. *Standard redox solutions*: Standardize the electrode system against redox solutions that provide stable known Eh values over a range of temperatures. Although standard solutions are available, they do not cover the anticipated range of Eh values. Commercially prepared solutions may be used, particularly in field testing. The composition and Eh values of standard solutions are shown in Table 2580:II. With reasonable care, these solutions are stable for several months.

b. *Eh electrode cleaners*: Use either:

1) Aqua regia—Mix 1 volume conc nitric acid with 3 volumes conc hydrochloric acid. Prepare fresh and dilute by at least 50% with water. Neutralize prior to discarding.

2) Chromic acid—Dissolve 5 g potassium dichromate, $K_2Cr_2O_7$, in 500 mL conc sulfuric acid. Conduct cleaning procedure in a fume hood.

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4. Procedure

a. Instrument calibration: Follow manufacturer's instructions for using pH/millivolt meter and in preparing electrodes for use. Use a shorting lead to verify the zero point on the meter's millivolt scale. Equilibrate the standard solution to the temperature of the sample. Immerse electrodes in the gently stirred, standard solution in a beaker (or flow cell). Turn on meter, placing the function switch in the millivolt mode.

After several minutes for electrode equilibration, record reading to nearest millivolt. If the reading is more than ± 10 mV from the theoretical redox standard value at that temperature, replace reference electrode fluid and repeat the measurement. If that procedure fails to bring the reading to within ± 10 mV of the theoretical value, polish the sensing element of the indicator electrode with carborundum paper, crocus cloth, or jeweler's rouge. Rinse electrode thoroughly and recheck reading with a fresh portion of the standard solution. If the reading is within ± 10 mV of the theoretical value, record it and the temperature. If the reading is not within ± 10 mV, repeat the cleaning procedure or try another electrode. Then rinse the electrode with distilled water and proceed with the sample measurement. Recalibrate daily and more frequently if turbid, organic-rich, or high-dissolved-solids solutions are being measured.

b. Electrode cleaning procedure: Useful treatments for noble metal electrodes in restoring performance after long periods of use include immersion in warm (70°C) aqua regia for 1 to 2 min or 5 min in 6N HNO_3 after bringing to a boil. Alternatively treat with chromic acid solution followed by 6N HCl and rinse with water.

c. Sample analysis: Check system for performance with the standard solution, rinse electrodes thoroughly with sample water, then immerse them in the gently stirred sample. Let equilibrate, record Eh value to the nearest millivolt, and temperature to $\pm 0.1^{\circ}\text{C}$. Repeat with a second sample portion to confirm successive readings within ± 10 mV. Equilibration times vary and may take many minutes in poorly poised solutions. Successive readings that vary less than ± 10 mV over 10 min are adequate for most purposes. Make continuous flow or pumped sample measurements, particularly of poorly poised solutions, in a closed flow cell after external calibration of the electrode system.

See Table 2580:III for recommended combinations of electrodes, standards, and sample handling.

5. Trouble Shooting

a. Meter: Use a shorting lead to establish meter reading at zero millivolts whenever possible. If the meter cannot be zeroed, follow the manufacturer's instructions for service.

b. Electrodes: If the potentiometer is in good working order, the fault may be in the electrodes. Frequently, renewal of the filling solution for the salt bridge for the reference electrode is sufficient to restore electrode performance. Another useful check is to oppose the emf of a questionable reference electrode with that of the same type known to be in good order. Using an adapter, plug the good reference electrode into the indicator electrode jack of the

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potentiometer. Then plug the questionable electrode into the reference electrode jack. With the meter in the millivolt position, immerse electrodes in an electrolyte (e.g., KCl) solution and then into a redox standard solution. The two millivolt readings should be 0 ± 5 mV for both solutions. If different electrodes are used (e.g., silver:silver chloride versus calomel or vice versa), the reading should be 44 ± 5 mV for a good reference electrode.

Unless an indicator electrode has been poisoned, physically damaged, or shorted out, it usually is possible to restore function by proper cleaning.

6. Calculation

$$Eh_{\text{system}} = E_{\text{observed}} + E_{\text{reference standard}} - E_{\text{reference observed}}$$

Report temperature at which readings were made.

7. Precision and Bias

Standard solution measurements made at stable temperatures with a properly functioning electrode system should be accurate to within ± 10 mV. Calibration precision as reflected by the agreement of dual platinum electrodes versus an Ag:AgCl reference electrode for over a 2-year period has been estimated at ± 15 mV (i.e., one standard deviation) in ZoBell's solution ($N = 78$) at approximately 12°C . Precision on groundwater samples ($N = 234$) over the same period has been estimated at ± 22 mV (i.e., $\pm$ one standard deviation) in a closed flow cell.¹⁵

8. References

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2710 TESTS ON SLUDGES#(54)*

2710 A. Introduction

This section presents a series of tests uniquely applicable to sludges or slurries. The test data are useful in designing facilities for solids separation and concentration and for assessing operational behavior, especially of the activated sludge process.

2710 B. Oxygen-Consumption Rate

1. General Discussion

This test is used to determine the oxygen consumption rate of a sample of a biological suspension such as activated sludge. It is useful in laboratory and pilot-plant studies as well as in the operation of full-scale treatment plants. When used as a routine plant operation test, it often will indicate changes in operating conditions at an early stage. However, because test conditions are not necessarily identical to conditions at the sampling site, the observed measurement may

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not be identical with actual oxygen consumption rate.

2. Apparatus

a. Oxygen-consumption rate device: Either:

- 1) *Probe with an oxygen-sensitive electrode* (polarographic or galvanic), or
- 2) *Manometric or respirometric device* with appropriate readout and sample capacity of at least 300 mL. The device should have an oxygen supply capacity greater than the oxygen consumption rate of the biological suspension, or at least 150 mg/L·h.

b. Stopwatch or other suitable timing device.

c. Thermometer to read to $\pm 0.5^{\circ}\text{C}$.

3. Procedure

a. Calibration of oxygen-consumption rate device: Either:

- 1) Calibrate the oxygen probe and meter according to the method given in Section 4500-O.G, or
- 2) Calibrate the manometric or respirometric device according to manufacturer's instructions.

b. Volatile suspended solids determination: See Section 2540.

c. Preparation of sample: Adjust temperature of a suitable sample portion to that of the basin from which it was collected or to required evaluation temperature, and maintain constant during analysis. Record temperature. Increase DO concentration of sample by shaking it in a partially filled bottle or by bubbling air or oxygen through it.

d. Measurement of oxygen consumption rate:

- 1) Fill sample container to overflowing with an appropriate volume of a representative sample of the biological suspension to be tested.
- 2) If an oxygen-sensing probe is used, immediately insert it into a BOD bottle containing a magnetic stirring bar and the biological suspension. Displace enough suspension with probe to fill flared top of bottle and isolate its contents from the atmosphere. Activate probe stirring mechanism and magnetic stirrer. (NOTE: Adequate mixing is essential. For suspensions with high concentrations of suspended solids, i.e., $>5000\text{ mg/L}$, more vigorous mixing than that provided by the probe stirring mechanism and magnetic stirrer may be required.) If a manometric or respirometric device is used, follow manufacturer's instructions for startup.
- 3) After meter reading has stabilized, record initial DO and manometric or respirometric reading, and start timing device. Record appropriate DO, manometric, or respirometric data at time intervals of less than 1 min, depending on rate of consumption. Record data over a 15-min period or until DO becomes limiting, whichever occurs first. The oxygen probe may not be accurate below 1 mg DO/L. If a manometric or respirometric device is used, refer to manufacturer's instructions for lower limiting DO value. Low DO ($\leq 2\text{ mg/L}$ at the start of the test) may limit oxygen uptake by the biological suspension and will be indicated by a decreasing

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rate of oxygen consumption as the test progresses. Reject such data as being unrepresentative of suspension oxygen consumption rate and repeat test beginning with higher initial DO levels.

The results of this determination are quite sensitive to temperature variations and poor precision is obtained unless replicate determinations are made at the same temperature. When oxygen consumption is used as a plant control test, run periodic (at least monthly) replicate determinations to establish the precision of the technique. This determination also is sensitive to the time lag between sample collection and test initiation.

4. Calculations

If an oxygen probe is used, plot observed readings (DO, milligrams per liter) versus time (minutes) on arithmetic graph paper and determine the slope of the line of best fit. The slope is the oxygen consumption rate in milligrams per liter per minute.

If a manometric or respirometric device is used, refer to manufacturer's instructions for calculating the oxygen consumption rate.

Calculate specific oxygen consumption rate in milligrams per gram per hour as follows:
Specific oxygen consumption rate, (mg/g)/h

$$= \frac{\text{oxygen consumption rate, (mg/L)/min}}{\text{volatile suspended solids, g/L}} \times \frac{60 \text{ min}}{\text{h}}$$

5. Precision and Bias

Bias is not applicable. The precision for this test has not been determined.

6. Bibliography

UMBREIT, W.W., R.H. BURRIS & J.F. STAUFFER. 1964. Manometric Techniques. Burgess Publishing Co., Minneapolis, Minn.

2710 C. Settled Sludge Volume

1. General Discussion

The settled sludge volume of a biological suspension is useful in routine monitoring of biological processes. For activated sludge plant control, a 30-min settled sludge volume or the ratio of the 15-min to the 30-min settled sludge volume has been used to determine the returned-sludge flow rate and when to waste sludge. The 30-min settled sludge volume also is used to determine sludge volume index¹ (Section 2710D).

This method is inappropriate for dilute sludges because of the small volume of settled material. In such cases, use the volumetric test for settleable solids using an Imhoff cone

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(2540F). Results from 2540F are not comparable with those obtained with the procedure herein.

2. Apparatus

a. *Settling column*: Use 1-L graduated cylinder equipped with a stirring mechanism consisting of one or more thin rods extending the length of the column and positioned within two rod diameters of the cylinder wall. Provide a stirrer able to rotate the stirring rods at no greater than 4 rpm (peripheral tip speed of approximately 1.3 cm/s). See Figure 2710:1.

b. *Stopwatch*.

c. *Thermometer*.

3. Procedure

Place 1.0 L sample in settling column and distribute solids by covering the top and inverting cylinder three times. Insert stirring rods, activate stirring mechanism, start the stop watch, and let suspension settle. Continue stirring throughout test. Maintain suspension temperature during test at that in the basin from which the sample was taken.

Determine volume occupied by suspension at measured time intervals, e.g., 5, 10, 15, 20, 30, 45, and 60 min.

Report settled sludge volume of the suspension in milliliters for an indicated time interval.

Variations in suspension temperature, sampling and agitation methods, dimensions of settling column, and time between sampling and start of the determination significantly affect results.

4. Precision and Bias

Bias is not applicable. The precision for this test has not been determined.

5. Reference

1. DICK, R.I. & P.A. VESILIND. 1969. The SVI—What is It? *J. Water Pollut. Control Fed.* 41:1285.

2710 D. Sludge Volume Index

1. General Discussion

The sludge volume index (SVI) is the volume in milliliters occupied by 1 g of a suspension after 30 min settling. SVI typically is used to monitor settling characteristics of activated sludge and other biological suspensions.¹ Although SVI is not supported theoretically,² experience has shown it to be useful in routine process control.

2. Procedure

Determine the suspended solids concentration of a well-mixed sample of the suspension (See Section 2540D).

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Determine the 30 min settled sludge volume (See Section 2710C).

3. Calculations

$$\text{SVI} = \frac{\text{settled sludge volume (mL/L)} \times 1000}{\text{suspended solids (mg/L)}}$$

4. Precision and Bias

Precision is determined by the precision achieved in the suspended solids measurement, the settling characteristics of the suspension, and variables associated with the measurement of the settled sludge volume. Bias is not applicable.

5. References

1. DICK, R.I. & P.A. VESILIND. 1969. The SVI—What is it? *J. Water Pollut. Control Fed.* 41:1285.
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2710 E. Zone Settling Rate

1. General Discussion

At high concentrations of suspended solids, suspensions settle in the zone-settling regime. This type of settling takes place under quiescent conditions and is characterized by a distinct interface between the supernatant liquor and the sludge zone. The height of this distinct sludge interface is measured with time. Zone settling data for suspensions that undergo zone settling, e.g., activated sludge and metal hydroxide suspensions, can be used in the design, operation, and evaluation of settling basins.¹⁻³

2. Apparatus

a. Settling vessel: Use a transparent cylinder at least 1 m high and 10 cm in diameter. To reduce the discrepancy between laboratory and full-scale thickener results, use larger diameters

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and taller cylinders.^{1,3} Attach a calibrated millimeter tape to outside of cylinder. Equip cylinder with a stirring mechanism, e.g., one or more thin rods positioned within two rod diameters of the internal wall of settling vessel. Stir suspension near vessel wall over the entire depth of suspension at a peripheral speed no greater than 1 cm/s. Greater speeds may interfere with the thickening process and yield inaccurate results.⁴ Provide the settling vessel with a port in the bottom plate for filling and draining. See Figure 2710:2.

b. Stopwatch.

c. Thermometer.

3. Procedure

Maintain suspension in a reservoir in a uniformly mixed condition. Adjust temperature of suspension to that of the basin from which it was collected or to required evaluation temperature. Record temperature. Remove a well-mixed sample from reservoir and measure suspended solids concentration (Section 2540D).

Activate stirring mechanism. Fill settling vessel to a fixed height by pumping suspension from reservoir or by gravity flow. Fill at a rate sufficient to maintain a uniform suspended solids concentration throughout settling vessel at end of filling. The suspension should agglomerate, i.e., form a coarse structure with visible fluid channels, within a few minutes. If suspension does not agglomerate, test is invalid and should be repeated.

Record height of solids-liquid interface at intervals of about 1 min. Collect data for sufficient time to assure that suspension is exhibiting a constant zone-settling velocity and that any initial reflocculation period, characterized by an accelerating interfacial settling velocity, has been passed.

Zone settling rate is a function of suspended solids concentration, suspension characteristics, vessel dimensions, and laboratory artifacts. With the filling method described above and a sufficiently large cylinder, these artifacts should be minimized. However, even with careful testing suspensions often may behave erratically. Unpredictable behavior increases for sludges with high solids concentrations and poor settling characteristics, and in small cylinders.

4. Calculations

Plot interface height in centimeters vs. time in minutes.^{1,3} Draw straight line through data points, ignoring initial shoulder or reflocculation period and compression shoulder. Calculate interfacial settling rate as slope of line in centimeters per minute.

5. Precision and Bias

Bias is not applicable. The precision for this test has not been determined.

6. References

1. DICK, R.I. 1972. Sludge treatment. In W.J. Weber, ed., *Physicochemical Processes for Water Quality Control*. Wiley-Interscience, New York, N.Y.

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2. DICK, R.I. & K.W. YOUNG. 1972. Analysis of thickening performance of final settling tanks. *Proc. 27th Ind. Waste Conf.*, Purdue Univ., Eng. Ext. Ser. No. 141, 33.
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2710 F. Specific Gravity

1. General Discussion

The specific gravity of a sludge is the ratio of the masses of equal volumes of a sludge and distilled water. It is determined by comparing the mass of a known volume of a homogeneous sludge sample at a specific temperature to the mass of the same volume of distilled water at 4°C.

2. Apparatus

Container: A marked flask or bottle to hold a known sludge volume during weighing.

3. Procedure

Follow either *a* or *b*.

a. Record sample temperature, *T*. Weigh empty container and record weight, *W*. Fill empty container to mark with sample, weigh, and record weight, *S*. Fill empty container to mark with water, weigh, and record weight, *R*. Measure all masses to the nearest 10 mg.

b. If sample does not flow readily, add as much of it to container as possible without exerting pressure, record volume, weigh, and record mass, *P*. Fill container to mark with distilled water, taking care that air bubbles are not trapped in the sludge or container. Weigh and record mass, *Q*. Measure all masses to nearest 10 mg.

4. Calculation

Use *a* or *b*, matching choice of procedure above.

a. Calculate specific gravity, *SG*, from the formula

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$$SG_{T/4^{\circ}\text{C}} = \frac{\text{weight of sample}}{\text{weight of equal volume of water at } 4^{\circ}\text{C}} = \frac{S - W}{R - W} \times F$$

The values of the temperature correction factor F are given in Table 2710:I.

b. Calculate specific gravity, SG , from the formula

$$SG_{T/4^{\circ}\text{C}} = \frac{\text{weight of sample}}{\text{weight of equal volume of water at } 4^{\circ}\text{C}} = \frac{(P - W)}{(R - W) - (Q - P)} \times F$$

For values of F , see Table 2710:I.

2710 G. Capillary Suction Time

1. General Discussion

The capillary suction time (CST) test determines rate of water release from sludge. It provides a quantitative measure, reported in seconds, of how readily a sludge releases its water. The results can be used to assist in sludge dewatering processes; to evaluate sludge conditioning aids and dosages; or, when used with a jar test and the settleable solids procedure, to evaluate coagulation effects on the rate of water release from sludges.

The test consists of placing a sludge sample in a small cylinder on a sheet of chromatography paper. The paper extracts liquid from the sludge by capillary action. The time required for the liquid to travel a specified distance is recorded automatically by monitoring the conductivity change occurring at two contact points appropriately spaced and in contact with the chromatography paper. The elapsed time is indicative of the water drainage rate. The CST test has been used as a relative indicator to characterize the performance of most sludge dewatering processes.

2. Apparatus

a. *Test materials and apparatus* may be fabricated (see Figure 2710:3) or are commercially available.† The unit includes a paper support block, stainless steel reservoir with 18-mm ID and 25-mm height, and a digital timer.

b. *CST paper*.‡

c. *Thermometer* to read $\pm 0.5^{\circ}\text{C}$.

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d. Pipet, 10-mL, plastic with tip trimmed to allow free passage of sludge flocs.

3. Procedure

Turn on and reset CST meter. Dry CST test block and reservoir. Place a new CST paper on lower test block with rough side up and grain parallel to the 9-cm side. Add upper test block, insert sludge reservoir into test block, and seat it using light pressure and a quarter turn to prevent surface leaks. Measure and record temperature of sludge. Pipet 6.4 mL sludge into test cell reservoir; if pipetting is difficult because of sludge consistency, pour a representative sludge sample into the test cell until it is full. The CST device will begin time measurement as liquid being drawn into the paper reaches the inner pair of electrical contacts. Timing ends when the outer contact is reached. Record CST shown on digital display. Empty remaining sludge from reservoir and remove and discard used CST paper. Rinse and dry test block and reservoir. Repeat for a minimum of five determinations per sample to account for measurement variation and to allow identification of any faulty readings due to leaks or spills.

Variations in sludge temperature and sample volume can affect CST results. Ensure that all analyses are run under similar conditions. Sludge suspended solids concentration has a significant effect on test results. In evaluating sludge conditioners or monitoring operation of a dewatering process, avoid this effect by ensuring homogeneity among sludge samples. Comparison of CST data from different sludge samples from the same source (especially if taken on different days) cannot be made with confidence unless suspended solids concentrations are comparable. Make a rough correction for different solids contents by dividing the sludge's CST value by its corresponding solids concentration.

Characteristics of CST paper may vary between lots. If comparison of CST values for distilled water indicates such variations, subtract times for distilled water blanks from sample times to improve comparisons.

Record CST model used, paper type, sludge type, sludge temperature, and capillary suction time. Measure solids concentration and CST of distilled water using the same paper to provide useful information.

4. Precision and Bias

Ten tests conducted on an anaerobically digested pulp mill sludge resulted in a mean CST of 363.2 s with a standard deviation of 36.2 s. Twenty tests using an anaerobically digested municipal wastewater sludge gave a mean of 85.2 s with a standard deviation of 14.12 s. Triplicate analyses of 30 sample sets of conditioned and unconditioned alum sludge resulted in an average standard deviation of 1.0 s with means between 5 and 80 s. Method bias cannot be determined.

5. Bibliography

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2710 H. Time-to-Filter

1. General Discussion

The time to filter (TTF) correlates with capillary suction time (CST) and is similar to the specific resistance to filtration if sludge solids content and filtrate viscosity do not vary among compared samples. The test requires approximately 200 mL sludge and can be used to assist in the daily operation of sludge dewatering processes or to evaluate sludge-conditioning polymers and dosages.

Testing with a smaller volume is possible in applications to evaluate water drainage rate subsequent to jar tests and settleable solids determination (see Section 2540F). In this case, drain collected sludge from one or more Imhoff cones after decanting as much supernatant as possible; use a small-volume TTF apparatus.

The test consists of placing a sludge sample in a Buchner funnel with a paper support filter, applying vacuum, and measuring the time required for 100 mL filtrate (or, for reduced sample volumes, 50% of original sample) to collect. While similar to the specific resistance to filtration test, the time-to-filter test is superior because of its ease of use and simplicity.

2. Apparatus

- a. Time-to-filter large-volume or small-volume (Figure 2710:4) assembly.
- b. Filter paper#(57)*
- c. Stopwatch.

3. Procedure

Place paper filter in funnel and make a firm seal by pre-wetting with a small volume of water with vacuum on. If using large-volume apparatus, take a 200-mL sample of sludge. With vacuum pump providing a constant vacuum of 51 kPa, pour sample into funnel. Start stopwatch or timer and determine time required for 100 mL of sample to collect in graduated cylinder. This is the time to filter. Make a minimum of three replicate determinations.

For the small-volume test, use 7 to 10 mL sludge. Record time required for 50% of sample to collect in graduated cylinder. Compare this time to filter only to other results using the same sample volume.

Sludge suspended solids concentration has a significant effect on test results. In evaluating sludge-conditioning products, compare results for which initial suspended solids concentrations

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are comparable. Make a rough correction for different solids contents by dividing the time-to-filter value by its corresponding solids concentration. However, variations in solids concentration occur in full-scale applications, and the time-to-filter results may be interpreted as indicating the overall rate of water release from sludges, including the effect of differing solids concentrations.

4. Precision and Bias

Variations in vacuum pressure, support filter type, sludge temperature, and sample volume can affect test results. Triplicate analyses of 18 sample sets of conditioned and unconditioned alum sludge resulted in an average method precision of 19 s (approximately 4% of the average value) for the large-volume TTF test. Triplicate analyses of 9 sample sets of conditioned and unconditioned alum sludge resulted in a method precision of 9 s (approximately 6% of the average value) for the small-volume TTF test. Method bias, which refers to the agreement between the value determined by the test method and the real value, cannot be determined.

5. Bibliography

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2720 ANAEROBIC SLUDGE DIGESTER GAS ANALYSIS*#(58)

2720 A. Introduction

Gas produced during the anaerobic decomposition of wastes contains methane (CH_4) and carbon dioxide (CO_2) as the major components with minor quantities of hydrogen (H_2), hydrogen sulfide (H_2S), nitrogen (N_2), and oxygen (O_2). It is saturated with water vapor. Common practice is to analyze the gases produced to estimate their fuel value and to check on the treatment process. The relative proportions of CO_2 , CH_4 , and N_2 are normally of most concern and the easiest to determine because of the relatively high percentages of these gases.

1. Selection of Method

Two procedures are described for gas analysis, the volumetric method (B), and the gas chromatographic method (C). The volumetric analysis is suitable for the determination of CO_2 , H_2 , CH_4 , and O_2 . Nitrogen is estimated indirectly by difference. Although the method is time-consuming, the equipment is relatively simple. Because no calibration is needed before use, the procedure is particularly appropriate when analyses are conducted infrequently.

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The principal advantage of gas chromatography is speed. Commercial equipment is designed specifically for isothermal or temperature-programmed gas analysis and permits the routine separation and measurement of CO₂, N₂, O₂, and CH₄ in less than 15 to 20 min. The requirements for a recorder, pressure-regulated bottles of carrier gas, and certified standard gas mixtures for calibration raise costs to the point where infrequent analyses by this method may be uneconomical. The advantages of this system are freedom from the cumulative errors found in sequential volumetric measurements, adaptability to other gas component analyses, adaptability to intermittent on-line sampling and analysis, and the use of samples of 1 mL or less.¹

2. Sample Collection

When the source of gas is some distance from the apparatus used for analysis, collect samples in sealed containers and bring to the instrument. Displacement collectors are the most suitable containers. Glass sampling bulbs (or tubes) with three-way glass or TFE stopcocks at each end, as indicated in Figure 2720:1, are particularly useful. These also are available with centrally located ports provided with septa for syringe transfer of samples. Replace septa periodically to prevent contamination by atmospheric gases. Connect one end of collector to gas source and vent three-way stopcock to the atmosphere. Clear line of air by passing 10 to 15 volumes of gas through vent and open stopcock to admit sample. If large quantities of gas are available, sweep air away by passing 10 to 15 volumes of gas through tube. If the gas supply is limited, fill the gas sampling bulb (tube) with an acidic salt solution.² Then completely displace the acidic salt solution in the gas sampling bulb (tube) with the sample gas. Because the acidic salt solution absorbs gases to some extent, fill the gas sampling bulb completely with the gas and seal off from any contact with displacement fluid during temporary storage. When transferring gas to the gas-analyzing apparatus, do not transfer any fluid.

3. References

1. GRUNE, W.N. & C.F. CHUEH. 1962–63. Sludge gas analysis using gas chromatograph. *Water Sewage Works* 109:468; 110:43, 77, 102, 127, 171, 220, and 254.
2. CHIN, K.K. & K.K. WONG. 1983. Thermophilic anaerobic digestion of palm oil mill effluent. *Water Res.* 17:993.

2720 B. Volumetric Method

1. General Discussion

a. Principle: This method may be used for the analysis either of digester gas or of methane in water (see Section 6211, Methane). A measured volume of gas is passed first through a solution of potassium hydroxide (KOH) to remove CO₂, next through a solution of alkaline pyrogallol to remove O₂, and then over heated cupric oxide, which removes H₂ by oxidation to water. After

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each of the above steps, the volume of gas remaining is measured; the decrease that results is a measure of the relative percentage of volume of each component in the mixture. Finally, CH_4 is determined by conversion to CO_2 and H_2O in a slow-combustion pipet or a catalytic oxidation assembly. The volume of CO_2 formed during combustion is measured to determine the fraction of methane originally present. Nitrogen is estimated by assuming that it represents the only gas remaining and equals the difference between 100% and the sum of the measured percentages of the other components.

When only CO_2 is measured, report only CO_2 . No valid assumptions may be made about the remaining gases present without making a complete analysis.

Follow the equipment manufacturers' recommendations with respect to oxidation procedures.

CAUTION: *Do not attempt any slow-combustion procedure on digester gas because of the high probability of exceeding the explosive 5% by volume concentration of CH_4 .*

2. Apparatus

Orsat-type gas-analysis apparatus, consisting of at least: (1) a water-jacketed gas buret with leveling bulb; (2) a CO_2 -absorption pipet; (3) an O_2 -absorption pipet; (4) a cupric oxide-hydrogen oxidation assembly; (5) a shielded catalytic CH_4 -oxidation assembly or slow-combustion pipet assembly; and (6) a leveling bulb. With the slow-combustion pipet use a controlled source of current to heat the platinum filament electrically. Preferably use mercury as the displacement fluid; alternatively use aqueous Na_2SO_4 - H_2SO_4 solution for sample collection. Use any commercially available gas analyzer having these units.

3. Reagents

- a. *Potassium hydroxide solution:* Dissolve 500 g KOH in distilled water and dilute to 1 L.
- b. *Alkaline pyrogallol reagent:* Dissolve 30 g pyrogallol (also called pyrogallic acid) in distilled water and make up to 100 mL. Add 500 mL KOH solution.
- c. *Oxygen gas:* Use approximately 100 mL for each gas sample analyzed.
- d. *Displacement liquid (acidic salt solution):* Dissolve 200 g Na_2SO_4 in 800 mL distilled water; add 30 mL conc H_2SO_4 . Add a few drops of methyl orange indicator. When color fades, replace solution.

4. Procedure

- a. *Sample introduction:* Transfer 5 to 10 mL gas sample into gas buret through a capillary-tube connection to the collector. Expel this sample to the atmosphere to purge the system. Transfer up to 100 mL gas sample to buret. Bring sample in buret to atmospheric or reference pressure by adjusting leveling bulb. Measure volume accurately and record as V_1 .

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b. Carbon dioxide absorption: Remove CO_2 from sample by passing it through the CO_2 -absorption pipet charged with the KOH solution. Pass gas back and forth until sample volume remains constant. Before opening stopcocks between buret and any absorption pipet, make sure that the gas in the buret is under a slight positive pressure to prevent reagent in the pipet from contaminating stopcock or manifold. After absorption of CO_2 , transfer sample to buret and measure volume. Record as V_2 .

c. Oxygen absorption: Remove O_2 by passing sample through O_2 -absorption pipet charged with alkaline pyrogallol reagent until sample volume remains constant. Measure volume and record as V_3 . For digester gas samples, continue as directed in ¶ 4d. For CH_4 in water, store gas in CO_2 pipet and proceed ¶ 4e below.

d. Hydrogen oxidation: Remove H_2 by passing sample through CuO assembly maintained at a temperature in the range 290 to 300°C. When a constant volume has been obtained, transfer sample back to buret, cool, and measure volume. Record as V_4 .

Waste to the atmosphere all but 20 to 25 mL of remaining gas. Measure volume and record as V_5 . Store temporarily in CO_2 -absorption pipet.

e. Methane oxidation: Purge inlet connections to buret with O_2 by drawing 5 to 10 mL into buret and expelling to the atmosphere. Oxidize CH_4 either by the catalytic oxidation process for digester gas and gas phase of water samples or by the slow-combustion process for gas phase of water samples.

1) Catalytic oxidation process—For catalytic oxidation of digester gas and gas phase of water samples, transfer 65 to 70 mL O_2 to buret and record this volume as V_6 . Pass O_2 into CO_2 -absorption pipet so that it will mix with sample stored there. Return this mixture to buret and measure volume. Record as V_7 . This volume should closely approximate V_5 plus V_6 . Pass O_2 -sample mixture through catalytic oxidation assembly, which should be heated in accordance with the manufacturer's directions. Keep rate of gas passage less than 30 mL/min. After first pass, transfer mixture back and forth through the assembly between buret and reservoir at a rate not faster than 60 mL/min until a constant volume is obtained. Record as V_8 .

2) Slow-combustion process—For slow combustion of the gas phase of water samples, transfer 35 to 40 mL O_2 to buret and record volume as V_6 . Transfer O_2 to slow-combustion pipet and then transfer sample from CO_2 -absorption pipet to buret. Heat platinum coil in combustion pipet to yellow heat while controlling temperature by adjusting current. Reduce pressure of O_2 in pipet to somewhat less than atmospheric pressure by means of the leveling bulb attached to the pipet. Pass sample into slow-combustion pipet at rate of approximately 10 mL/min. After the first pass, transfer sample and O_2 mixture back and forth between pipet and buret several times at a faster rate, allowing mercury in pipet to rise to a point just below heated

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coil. Collect sample in combustion pipet, turn off coil, and cool pipet and sample to room temperature with a jet of compressed air. Transfer sample to buret and measure volume. Record as V_8 .

f. Measurement of carbon dioxide produced: Determine amount of CO_2 formed in the reaction by passing sample through CO_2 -absorption pipet until volume remains constant. Record volume as V_9 . Check accuracy of determination by absorbing residual O_2 from sample. After this absorption, record final volume as V_{10} .

5. Calculation

a. CH_4 and H_2 usually are the only combustible gases present in sludge digester gas. When this is the case, determine percentage by volume of each gas as follows:

$$\% \text{CO}_2 = \frac{(V_1 - V_2) \times 100}{V_1}$$

$$\% \text{O}_2 = \frac{(V_2 - V_3) \times 100}{V_1}$$

$$\% \text{H}_2 = \frac{(V_3 - V_4) \times 100}{V_1}$$

$$\% \text{CH}_4 = \frac{V_4 \times (V_8 - V_9) \times 100}{V_1 \times V_5}$$

$$\% \text{N}_2 = 100 - (\% \text{CO}_2 + \% \text{O}_2 + \% \text{H}_2 + \% \text{CH}_4)$$

b. Alternatively, calculate CH_4 by either of the two following equations:

$$\% \text{CH}_4 = \frac{V_4 \times (V_6 + V_{10} - V_9) \times 100}{2 \times V_1 \times V_5}$$

$$\% \text{CH}_4 = \frac{V_4 (V_7 - V_8) \times 100}{2 \times V_1 \times V_5}$$

Results from the calculations for CH_4 by the three equations should be in reasonable agreement.

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If not, repeat analysis after checking apparatus for sources of error, such as leaking stopcocks or connections. Other combustible gases, such as ethane, butane, or pentane, will cause a lack of agreement among the calculations; however, the possibility that digester gas contains a significant amount of any of these is remote.

6. Precision and Bias

A gas buret measures gas volume with a precision of 0.05 mL and a probable accuracy of 0.1 mL. With the large fractions of CO₂ and CH₄ normally present in digester gas, the overall error for their determination can be made less than 1%. The error in the determination of O₂ and H₂, however, can be considerable because of the small concentrations normally present. For a concentration as low as 1%, an error as large as 20% can be expected. When N₂ is present in a similar low-volume percentage, the error in its determination would be even greater, because errors in each of the other determinations would be reflected in the calculation for N₂.

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2720 C. Gas Chromatographic Method

1. General Discussion

See Section 6010C for discussions of gas chromatography.

2. Apparatus

a. Gas chromatograph: Use any instrument system equipped with a thermal conductivity detector (TCD), carrier-gas flow controllers, injector and column temperature setting dials, TCD current controller, attenuator, carrier-gas pressure gauge, injection port, signal output, and power switch. Some columns require temperature programming while others are isothermal. Preferably use a unit with a gas sampling loop and valve that allow automatic injection of a constant sample volume.

b. Sample introduction apparatus: An instrument equipped with gas-sampling valves is designed to permit automatic injection of a specific sample volume into the chromatograph. If such an instrument is not available, introduce samples with a 2-mL syringe fitted with a 27-gauge hypodermic needle. Reduce escape of gas by greasing plunger lightly with mineral oil or preferably by using a special gas-tight syringe. One-step separation of oxygen, nitrogen, methane, and carbon dioxide may be accomplished in concentric columns (column within a column) under isothermal conditions at room temperature instead of performing two column

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analyses. These concentric columns permit the simultaneous use of two different packings for the analysis of gas samples.

c. Chromatographic column: #(59)* Select column on the basis of manufacturer's recommendations. #(60)† Report column and packing specifications and conditions of analyses with results.

A two-column system usually is required. A molecular sieve column separates H_2 , O_2 , N_2 , CO , and CH_4 isothermally, but because CO_2 is adsorbed by the molecular sieve, a second column is needed to complete the analysis. A commonly used two-column system utilizes a Chromosorb 102 column and a Molecular Sieve 5A or 13X column to separate H_2 , O_2 , N_2 , CH_4 , and CO_2 isothermally.^{1,2}

A single-column procedure can be used; however, it requires temperature programming. Columns packed with special porous spherical or granular packing materials effect separation with sharp, well-resolved peaks.³ With temperature programming, columns packed with Chromosorb 102,² Carbosphere,⁴ and Carbosieve³ separate the gases listed.† #(61) Commercial equipment specifically designed for such operations is available.^{2,4-6}

d. Integrator/recorder: Use a 10-mV full-span strip chart recorder with the gas chromatograph. When minor components such as H_2 and H_2S are to be detected, a 1-mV full-span recorder is preferable.

Integrators that can easily detect very minute quantities of gases are available. Computerized data-processing systems, to record and manipulate the chromatographic signal, chromatographic base line, etc., also are available.

3. Reagents

a. Carrier gases: Preferably use helium for separating digester gases. It is impossible to detect less than 1% hydrogen when helium is used as the carrier gas.¹ Obtain linear TCD responses for molar concentrations of hydrogen between 0 and 60% by using an 8.5% hydrogen–91.5% helium carrier gas mixture.⁷ To detect trace quantities of hydrogen use argon or nitrogen as carrier gas.¹

b. Calibration gases: Use samples of CH_4 , CO_2 , and N_2 of known purity, or gas mixtures of certified composition, for calibration. Also use samples of O_2 , H_2 , and H_2S of known purity if these gases are to be measured. Preferably use custom-made gas mixtures to closely approximate digester gas composition.

c. Displacement liquids: See Section 2720B.3d.

4. Procedure

a. Preparation of gas chromatograph: Open main valve of carrier-gas cylinder and adjust carrier-gas flow rate to recommended values. To obtain accurate flow measurements, connect a soap-film flow meter to the TCD vent. Turn power on. Turn on oven heaters, if used, and

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detector current and adjust to desired values.

Set injection port and column temperatures as specified for the column being used. Set TCD current. Turn on recorder or data processor. Check that the injector/detector temperature has risen to the appropriate level and confirm that column temperature is stabilized. Set range and attenuation at appropriate positions.

The instrument is ready for use when the recorder yields a stable base line. Silica gel and molecular sieve columns gradually lose activity because of adsorbed moisture or materials permanently adsorbed at room temperature. If insufficient separations occur, reactivate by heating or repacking.

b. Calibration: For accurate results, prepare a calibration curve for each gas to be measured because different gas components do not give equivalent detector responses on either a weight or a molar basis. Calibrate with synthetic mixtures or with pure gases.

1) Synthetic mixtures—Use purchased gas mixtures of certified composition or prepare in the laboratory. Inject a standard volume of each mixture into the gas chromatograph and note response for each gas. Compute detector response, either as area under a peak or as height of peak, after correcting for attenuation. Read peak heights accurately and correlate with concentration of component in sample. Reproduce operating parameters exactly from one analysis to the next. If sufficient reproducibility cannot be obtained by this procedure, use peak areas for calibration. Preferably use peak areas when peaks are not symmetrical. Prepare calibration curve by plotting either peak area or peak height against volume or mole percent for each component.

Modern integrators and data-processing systems are able to generate calibration tables for certified gas mixtures.

2) Pure gases—Introduce pure gases into chromatograph individually with a syringe. Inject sample volumes of 0.25, 0.5, 1.0 mL, etc., and plot detector response, corrected for attenuation, against gas volume.

When the analysis system yields a linear detector response with increasing gas component concentration from zero to the range of interest, run standard mixtures along with samples. If the same sample size is used, calculate gas concentration by direct proportions.

c. Sample analysis: If samples are to be injected with a syringe, equip sample collection container with a port closed by a rubber or silicone septum. To take a sample for analysis, expel air from barrel of syringe by depressing plunger and force needle through the septum. Withdraw plunger to take gas volume desired, pull needle from collection container, and inject sample rapidly into chromatograph.

When samples are to be injected through a gas-sampling valve, connect sample collection container to inlet tube. Let gas flow from collection tube through the valve to purge dead air space and fill sample tube. About 15 mL normally are sufficient to clear the lines and to provide a sample of 1 to 2 mL. Transfer sample from loop into carrier gas stream by following manufacturer's instructions. Bring samples to atmospheric pressure before injection.

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When calibration curves have been prepared with a synthetic gas mixture of certified composition, use the same sample volume as that used during calibration. When calibration curves are prepared by the procedure using varying volumes of pure gases, inject any convenient gas sample volume up to about 2 mL.

Inject sample and standard gases in sequence to permit calculation of unknown gas concentration in volume (or mole) percent by direct comparison of sample and standard gas peak heights or areas. For more accurate analysis, make duplicate or triplicate injections of sample and standard gases.

5. Calculation

a. When calibration curves have been prepared with synthetic mixtures and the volume of the sample analyzed is the same as that used in calibration, read volume percent of each component directly from calibration curve after detector response for that component is computed.

b. *When calibration curves are prepared with varying volumes of pure gases, calculate the percentage of each gas in the mixture as follows:*

$$\text{Volume \%} = \frac{A}{B} \times 100$$

where:

A = partial volume of component (read from calibration curve) and

B = volume sample injected.

c. Where standard mixtures are run with samples and instrument response is linear from zero to the concentration range of interest:

$$\text{Volume \%} = \text{volume \% (std)} \frac{C}{D}$$

where:

C = recorder value of sample and

D = recorder value of standard.

d. Digester gases usually are saturated with water vapor that is not a digestion product. Therefore, apply corrections to calculate the dry volume percent of each digester gas component as follows:

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$$\text{Dry volume \%} = \frac{\text{volume \% as above}}{1 - P_v}$$

where:

P_v = saturated water vapor pressure at room temperature and pressure, decimal %.

The saturation water vapor pressure can be found in common handbooks. The correction factor

$\frac{1}{1 - P_v}$ is usually small and is often neglected by analysts.

6. Precision and Bias

Precision and bias depend on the instrument, the column, operating conditions, gas concentrations, and techniques of operation. The upper control limits for replicate analyses of a high-methane-content standard gas mixture (65.01% methane, 29.95% carbon dioxide, 0.99% oxygen, and 4.05% nitrogen) by a single operator were as follows: 65.21% for methane, 30.36% for carbon dioxide, 1.03% for oxygen, and 4.15% for nitrogen. The lower control limits for this same standard gas mixture were: 64.67% for methane, 29.88% for carbon dioxide, 0.85% for oxygen, and 3.85% for nitrogen. The observed relative standard deviations (RSD) were: 0.14% for methane, 0.26% for carbon dioxide, 3.2% for oxygen, and 1.25% for nitrogen.

In a similar analysis of another standard gas mixture (55% methane, 35% carbon dioxide, 10% diatomic gases—2% hydrogen, 6% nitrogen, 2% oxygen), typical upper control limits for precision of duplicate determinations were: 55.21% for methane, 35.39% for carbon dioxide, and 10.26% for the diatomic gases. The lower control limits for this standard gas mixture were: 54.78% for methane, 34.62% for carbon dioxide, and 9.73% for the diatomic gases. The observed RSDs for this analysis were: 0.13% for methane, 0.36% for carbon dioxide, and 0.88% for the diatomic gases.

A low-methane-content standard gas mixture (35.70% methane, 47.70% carbon dioxide, 3.07% oxygen, 8.16% nitrogen, and 5.37% hydrogen) was analyzed as above yielding upper control limits as follows: 34.4% for methane, 49.46% for carbon dioxide, 3.07% for oxygen, and 8.2% for nitrogen. The lower control limits were: 34.18% for methane, 49.11% for carbon dioxide, 2.83% for oxygen, and 7.98% for nitrogen. The observed RSDs were: 0.11% for methane, 0.16% for carbon dioxide, 1.37% for oxygen, and 0.46% for nitrogen.

With digester gas the sum of the percent CH_4 , CO_2 , and N_2 should approximate 100%. If it does not, suspect errors in collection, handling, storage, and injection of gas, or in instrumental operation or calibration.

7. References

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2810 DISSOLVED GAS SUPERSATURATION#(62)*

2810 A. Introduction

Water can become supersaturated with atmospheric gases by various means, heating and air entrainment in spilled or pumped water being the most common. The primary sign of gas supersaturation is the formation of bubbles on submerged surfaces or within the vascular systems and tissues of aquatic organisms.

Gas supersaturation can limit aquatic life and interfere with water treatment processes. Levels of supersaturation lethal to aquatic organisms have been found in springs, rivers, wells, lakes, estuaries, and seawater. Gas supersaturation can be produced in pumped or processed water intended for drinking, fish hatchery supply, and laboratory bioassays. Seasonal and other temporal variations in supersaturation may occur in surface waters as a result of solar heating and photosynthesis. Because the rate of equilibration may be slow, supersaturation may persist in flowing water for days and excessive dissolved gas levels thus may persist far from the source of

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supersaturation.

Gas bubbles form only when the total dissolved gas pressure is greater than the sum of compensating pressures. Compensating pressures include water, barometric and, for organisms, tissue or blood pressure. The total dissolved gas pressure is equal to the sum of the partial pressures of all the dissolved gases, including water vapor. Typically, only nitrogen, oxygen, argon, carbon dioxide, and water vapor pressures need to be considered in most natural waters. Gas bubble disease, of fish or other aquatic organisms, is a result of excessive uncompensated gas pressure. A single supersaturated gas such as oxygen or nitrogen may not necessarily result in gas bubble disease because bubble formation depends largely on total dissolved gas pressure. The degree of gas saturation should be described in terms of pressures rather than concentration or volume units.

2810 B. Direct-Sensing Membrane-Diffusion Method

1. General Discussion

a. Principle: This method requires an instrument with a variable length of “gas permeable” tubing, connected to a pressure-measuring device. Dimethyl silicone rubber tubing often is used because it is highly permeable to dissolved gases, including water vapor. At steady state, the gauge pressure inside the tubing is equal to the difference in gas pressure (ΔP) between the total dissolved gas pressure and the ambient barometric pressure. When the water is in equilibrium with the atmosphere, ΔP equals zero.

If ΔP is greater than zero, the water is supersaturated. Conversely, if ΔP is negative the water is undersaturated.

b. Working range: The working range of this method depends on the pressure-sensing device used, but typically will range from -150 to $+600$ mm Hg. Dissolved solids in wastewater will not interfere with this method. The practical depth range for these instruments is 1 to 10 m.

2. Apparatus

Several types of membrane-diffusion instruments are available commercially. #63)* Alternatively, construct a unit from commercially available parts. Several units have been described, including a direct-reading instrument using pressure transducers and a digital readout,¹ an on-line unit that can activate an alarm system,² and an early model of the Weiss satumeter.³ Each of these units has specific advantages and limitations; the instrument of choice will depend on the specific application. All these instruments are portable so that data collection is completed in the field.

Test the instrument for leaks according to the manufacturer’s recommendation. Even a very small leak, difficult to detect and locate, will result in useless data. Calibrate the pressure-measuring device with a mercury manometer or certified pressure gauge. If a manometer is used, include fresh mercury that flows freely in the tubing. An alternative method

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for directly testing membrane-diffusion instruments in a small, closed chamber where induced ΔP levels can be compared against observed ΔP levels is available.²

Van Slyke-Neill⁴ or gas chromatography methods¹ are inappropriate for calibration but they may be used to verify results. These methods measure individual gas concentrations and require further conversion to ΔP or partial pressure and suffer from sampling and sample handling problems.⁵⁻⁷

3. Procedure

At the start of each day, test the instrument for leaks and recalibrate. At a monitoring site, completely submerge the sensing element in the water, preferably below the hydrostatic compensation depth. This is the depth where the hydrostatic and total gas pressures are equal and as a result, bubbles will not form on the tubing. Bubble formation on the silicone rubber tubing seriously reduces accuracy. Compute hydrostatic compensation depth⁵ as follows:

$$Z = \frac{\Delta P}{73.42}$$

where:

Z = hydrostatic compensation depth, m, and

ΔP = pressure difference between total dissolved gas pressure and the ambient barometric pressure, mm Hg.

The factor 73.42 is the hydrostatic pressure of fresh water at 20°C expressed in terms of mm Hg/m water depth. Because the variation of hydrostatic pressure with temperature and salinity is small, this equation can be used for all natural waters.

Dislodge formed bubbles on the tubing by gently striking the instrument or moving the instrument rapidly in the water. Movement of water across the silicone rubber tubing also facilitates establishing the equilibrium between gas pressure in the water and in the tubing.

Operate the instrument “bubble free” until a stable ΔP is observed. This may take from 5 to 30 min, depending on the ΔP , water temperature, water flow, and geometry of the system. The time response of the membrane-diffusion method is shown in Figure 2810:1 for “bubble-free” and “bubble” conditions.

If the instrument is used in heavily contaminated water containing oil or other organic compounds, clean the silicone tubing with a mild detergent according to the manufacturer’s instructions. Silicone rubber tubing has been used in uncontaminated natural water for at least eight years without being adversely affected by attached algal growth.² The tubing can be damaged by abrasive grit, diatoms, biting aquatic organisms, certain organic compounds, and strong acids.²

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Obtain the barometric pressure with each measurement by using a laboratory mercury barometer, a calibrated portable barometer, or pressure transducer. Barometric pressures reported by weather agencies (or airports) are corrected to sea level and are unusable.

4. Calculation

a. Total gas pressure: Preferably report total gas pressure as ΔP .^{2,6,8} Express pressure as millimeters of mercury.

Total gas pressure also has been reported as a percentage of local barometric pressure:

$$\text{TGP } \% = \left[\frac{P_b + \Delta P}{P_b} \right] \times 100$$

where:

P_b = true local barometric pressure, mm Hg.

The reporting of total gas pressure as a percentage is not encouraged.⁸

b. Component gas pressures: When information on component gas supersaturation is needed, express data as partial pressures, differential pressures, or percent saturation.^{5,8} This requires additional measurements of dissolved oxygen, temperature, and salinity^{#(64)†} at the monitoring site. In a mixture of gases in a given volume, the partial pressure of a gas is the pressure that this gas would exert if it were the only gas present.

1) Oxygen partial pressure—Calculate partial pressure of oxygen as follows:

$$P_{O_2} = \frac{DO}{\beta_{O_2}} \times 0.5318$$

where:

P_{O_2} = partial pressure of dissolved oxygen, mm Hg,

β_{O_2} = Bunsen coefficient for oxygen (Table 2810:I), L/(L·atm), and

DO = measured concentration of oxygen, mg/L.

Bunsen coefficients for marine waters are available.⁵ The factor 0.5318 equals $760/(1000K)$, where K is the ratio of molecular weight to molecular volume for oxygen gas.⁵

2) Nitrogen partial pressure—Estimate the partial pressure of nitrogen by subtracting the partial pressures of oxygen and water vapor from the total gas pressure.

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$$P_{N_2} = P_b + \Delta P - P_{O_2} - P_{H_2O}$$

where:

P_{H_2O} = vapor pressure of water in mm Hg from Table 2810:II.

This term includes a small contribution from argon and any other gases present, including carbon dioxide and methane. The partial pressure of carbon dioxide is negligible in natural waters of pH > 7.0.

3) Nitrogen:oxygen partial pressure ratio—The ratio of the partial pressure of nitrogen to the partial pressure of oxygen ($N_2:O_2$) characterizes the relative contribution of the two gases to the total dissolved gas pressure. In water in equilibrium with air, this ratio is 3.77.

c. Differential pressures: The differential pressure of a gas is the difference between the partial pressures of that gas in water and air. The oxygen differential pressure may be calculated as

$$\Delta P_{O_2} = P_{O_2} - 0.20946(P_b - P_{H_2O})$$

and the nitrogen differential pressure as

$$\Delta P_{N_2} = \Delta P - \Delta P_{O_2}$$

d. Percent of saturation: In older literature, supersaturation values have been reported as percent saturation. This method of reporting component gases is discouraged but can be calculated as follows:

$$N_2(\%) = \left[\frac{P_{N_2}}{0.7902 (P_b - P_{H_2O})} \right] \times 100$$

$$O_2(\%) = \left[\frac{P_{O_2}}{0.20946(P_b - P_{H_2O})} \right] \times 100$$

The following relationships are useful conversions:

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$$\text{TGP}(\%) = 0.20946 \text{ O}_2(\%) + 0.7902 \text{ N}_2(\%)$$

$$\Delta P = 0.20946 \left[\frac{\text{O}_2(\%)}{100} - 1 \right] [P_b - P_{H_2O}]$$
$$+ 0.7902 \left[\frac{\text{N}_2(\%)}{100} - 1 \right] [P_b - P_{H_2O}]$$

$$\Delta P = \frac{DO}{\beta_{O_2}} (0.5318)(1 + \text{N}_2:\text{O}_2) - (P_b - P_{H_2O})$$

Use care with these relationships with older data because both TGP(%) and N₂(%) have been differently defined.⁵

5. Quality Control

The precision of the membrane-diffusion method depends primarily on the pressure-sensing instrument. For an experienced operator it is approximately ± 1 to 2 mm Hg with an accuracy of ± 3 to 5 mm Hg.^{3,6} Air leaks, bubble formation, biofilm development, incomplete equilibration, or condensation produce negative errors while direct water leaks can result in positive errors in submersible units.

For accurate work, measure water temperature to the nearest $\pm 0.1^\circ\text{C}$.

6. Reporting of Results

In reporting results, include the following data:

Sensor depth, m,

Barometric pressure, mm Hg,

Water temperature, $^\circ\text{C}$,

Dissolved oxygen, mm Hg or mg/L,

Salinity, g/kg, and

ΔP , mm Hg.

If component gas information is needed add:

Partial pressure of oxygen, mm Hg,

Partial pressure of nitrogen, mm Hg, and

Nitrogen:oxygen partial pressure ratio

or

ΔP_{O_2} , mm Hg, and

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ΔP_{N_2} , mm Hg.

7. Interpretation of Results

The biological effects of dissolved gas supersaturation depend on the species, age, depth in water column, length of exposure, temperature, and nitrogen:oxygen partial pressure ratio.¹² Safe limits generally are segregated into wild/natural circumstances, where behavior and hydrostatic pressure can modify the exposure by horizontal and vertical movements away from dangers, and captive environments such as aquaria, hatcheries, or laboratories, where conditions not only preclude escape but also include other significant stresses. Of these two realms, captive circumstances are more likely to cause illness or mortality from gas bubble disease and will do so sooner and at the lower ΔP levels.

In wild/natural circumstances, the limit of safe levels of gas supersaturation depends on the depth available to the species and/ or species behavior, but this limit usually occurs at a ΔP between 50 and 150 mm Hg. Under captive conditions, the ΔP should be as close to zero as possible. For sensitive species and life stages, sublethal and lethal effects have been observed at ΔP of 10 to 50 mm Hg.¹³

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Figures

Figure 2120:1. Filtration system for color determinations.

Figure 2120:2. Chromaticity diagrams.

Figure 2150:1. Odor-free-water generator.

Figure 2530:1. Floatables sampler with mixer.

Figure 2530:2. Floatables flotation funnel and filter holder.

Figure 2530:3. Flotation funnels and mixing unit.

Figure 2530:4. Floatable oil tube, 1-L capacity.

Figure 2560:1. Schematic of filtration apparatus for preparation of particle-free dilution water or electrolyte solution.

Figure 2710:1. Schematic diagram of settling vessel for settled sludge volume test.

Figure 2710:2. Schematic diagram of settling vessel for zone settling rate test.

Figure 2710:3. Capillary suction time apparatus.

Figure 2710:4. TTF equipment. Large-volume equipment requires a 9-cm- diam Buchner funnel and a 250-mL graduated cylinder. Small-volume equipment requires a 2.5-cm-diam funnel and a 10-mL cylinder.

Figure 2720:1. Gas collection apparatus.

Figure 2810:1. Time response for the membrane-diffusion method.

Tables

TABLE 2120:I. SELECTED ORDINATES FOR SPECTROPHOTOMETRIC COLOR DETERMINATIONS*

Ordinate No.	X	Y	Z
	Wavelength nm		

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Ordinate No.	X	Y	Z
	Wavelength <i>nm</i>		
1	424.4	465.9	414.1
2*	435.5*	489.5*	422.2*
3	443.9	500.4	426.3
4	452.1	508.7	429.4
5*	461.2*	515.2*	432.0*
6	474.0	520.6	434.3
7	531.2	525.4	436.5
8*	544.3*	529.8*	438.6*
9	552.4	533.9	440.6
10	558.7	537.7	442.5
11*	564.1*	541.4*	444.4*
12	568.9	544.9	446.3
13	573.2	548.4	448.2
14*	577.4*	551.8*	450.1*
15	581.3	555.1	452.1
16	585.0	558.5	454.0
17*	588.7*	561.9*	455.9*
18	592.4	565.3	457.9
19	596.0	568.9	459.9
20*	599.6*	572.5*	462.0*
21	603.3	576.4	464.1
22	607.0	580.4	466.3
23*	610.9*	584.8*	468.7*
24	615.0	589.6	471.4
25	619.4	594.8	474.3
26*	624.2*	600.8*	477.7*
27	629.8	607.7	481.8
28	636.6	616.1	487.2
29*	645.9*	627.3*	495.2*
30	663.0	647.4	511.2

Factors When 30 Ordinates Used

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	X	Y	Z
Ordinate No.	Wavelength nm		
38	0.032 69	0.033 33	0.039
Factors When 10 Ordinates Used			
14	0.098 06	0.100 00	0.118

* Insert in each column the transmittance value (%) corresponding to the wavelength shown. Where limited accuracy is sufficient, use only the ordinates marked with an asterisk.

TABLE 2120:II. COLOR HUES FOR DOMINANT WAVELENGTH RANGES

Wavelength Range nm	Hue
400–465	Violet
465–482	Blue
482–497	Blue-green
497–530	Green
530–575	Greenish yellow
575–580	Yellow
580–587	Yellowish orange
587–598	Orange
598–620	Orange-red
620–700	Red
400–530c*	Blue-purple
530c–700*	Red-purple

* See Figure 2120:2 for significance of "c".

TABLE 2150:I. THRESHOLD ODOR NUMBERS CORRESPONDING TO VARIOUS DILUTIONS

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* See Figure 2120:2 for significance of "c".

TABLE 2150:I. THRESHOLD ODOR NUMBERS CORRESPONDING TO VARIOUS DILUTIONS

Sample Volume Diluted to 200 mL <i>mL</i>	Threshold Odor No.	Sample Volume Diluted to 200 mL <i>mL</i>	Threshold Odor No.
200	1	12.0	17
140	1.4	8.3	24
100	2	5.7	35
70	3	4.0	50
50	4	2.8	70
35	6	2.0	100
25	8	1.4	140
17	12	1.0	200

TABLE 2150:II. DILUTIONS FOR VARIOUS ODOR INTENSITIES

Sample Volume in Which Odor Is First Noted <i>mL</i>	Volumes to Be Diluted to 200 mL <i>mL</i>
200	200, 140, 100, 70, 50
50	50, 35, 25, 17, 12
12	12, 8.3, 5.7, 4.0, 2.8
2.8	Intermediate dilution

TABLE 2160:I. FLAVOR THRESHOLD NUMBERS CORRESPONDING TO VARIOUS DILUTIONS

Sample Volume <i>mL</i>	Diluent Volume <i>mL</i>	Flavor Threshold No. <i>FTN</i>
200	0	1
100	100	2
70	130	3

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Sample Volume <i>mL</i>	Diluent Volume <i>mL</i>	Flavor Threshold No. <i>FTN</i>
50	150	4
35	165	6
25	175	8
17	183	12
12	188	17
8	192	25
6	194	33
4	196	50
3	197	67
2	198	100
1	199	200

TABLE 2160:II. DILUTIONS FOR DETERMINING THE FTN

Sample Volume in Which Taste Is First Noted <i>mL</i>	Volumes to be Diluted to 200 mL <i>mL</i>
200	200, 100, 70, 50, 35, 25, 17
50	50, 35, 25, 17, 12, 8, 6
12	12, 8, 6, 4, 3, 2, 1
4	Intermediate dilution

TABLE 2170:I. QUANTITATIVE ODOR REFERENCES*

Compound	Odor Characteristics	Stock Solution Concentration <i>mg/L</i>	Amount Placed In 200 mL Pure Water 25°C for Presentation
Geosmin	Earthy, red beets	0.2†	300 μ L for 300 ng
2-Methylisoborneol	Earthy, peat-like, Brazil nut, soil	0.2†	200 μ L for 200 ng
Free chlorine	Chlorinous	1.0	0.1 mL for 0.5 mg

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Compound	Odor Characteristics	Stock Solution Concentration mg/L	Amount Placed In 200 mL Pure Water 25°C for Presentation
		pH = 7	
Dichloramine	Swimming pool chlorine	pH = 7	
Monochloramine	Chlorinous	pH = 7	
<i>trans</i> ,2- <i>cis</i> ,6-Nonadienal	Cucumber, green vegetation	1000	1 µL for 5 µg/L
Styrene	Model airplane glue	1000	100 µL for 500 µg
Toluene	Glue, sweet solventy	1000	100 µL for 500 µg
<i>cis</i> -3-Hexenyl-1-acetate	Grassy	1000	100 µL for 500 µg
<i>cis</i> -3-Hexene-1-ol	Grassy, green apple	1000	100 µL for 500 µg
Cumene	Paste shoe polish, solventy	1000	20 µL for 100 µg/l
<i>m</i> -Xylene	Sweet solventy	1000	40 µL for 200 µg/l
Methylisobutyl ketone	Paint solventy	1000	200 µL for 1.0 mg
1,2,4-Trimethylbenzene	Shoe polish, coal tar	1000	50 µL for 250 µg/l
Indene	Glue, moth balls	1000	1 µL for 5 µg/L
Indan	Varnish, coal tar	1000	5 µL for 25 µg/L
Naphthalene	Sweet solventy	1000	1 µL for 5 µg/L
Benzofuran	Shoe polish, moth balls	1000	2 µL for 10 µg/L
2-Methyl-benzofuran	Moth balls, sweet solventy	1000	50 µL for 250 µg/l

NOTE: These compounds have actually been identified as causes of odors in raw and finished drinking water.

* Adapted from AMERICAN WATER WORKS ASSOCIATION. 1993. Flavor Profile Analysis: Screening and Training of Panelists. AWWA Manual, American Water Works Assoc., Denver, Colo.

† Compounds available only in solid form and must be dissolved in methanol.

TABLE 2170:II. REPRESENTATIVE ODOR REFERENCE STANDARDS*

Compound	Odor Characteristics	Stock Solution Concentration mg/L	Amount mL Pure
2,3,6-Trichloroanisole	Leather, earthy	1000	4 µL
2,3-Diethylpyrazine	Mildew, damp basement	1000	2 µL
2-Isopropyl-3-methoxypyrazine	Potato bin, musty	1000	40 µ
Nonanal	Hay, sweet	1000	40 µ
Dimethyl sulfide	Decaying vegetation, canned corn	1000	1 µL

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Compound	Odor Characteristics	Stock Solution Concentration <i>mg/L</i>	Amount mL or μ L
Dimethyl disulfide	Septic	1000	2 μ L
Dimethyltrisulfide	Garlicky, oniony, septic	0.2	50 μ L
Butyric acid	Putrid, sickening	1000	200
<i>trans</i> ,2-Nonenal	Cucumber with skin	1000	40 μ L
Diphenyl ether	Geranium	1000†	20 μ L
D-Limonene	Citrusy	1000	400
Hexanal	Lettuce heart, pumpkin, green pistachio	1000	40 μ L
Benzaldehyde	Sweet almond	1000	200
Ethyl-2-methyl-butyrate	Fruity, pineapple	1000	100
2-Heptanone	Banana-like, sweet solventy	1000	100
Hexachloro-1,3-butadiene	Sweet, minty, Vapo Rub	1000	800
2-Isobutyl-3-methoxypyrazine	Green/bell pepper, musty	1000	40 μ L
<i>trans</i> ,2- <i>trans</i> ,4-Decadienal	Rancid oily	1000	100
Butanol	Alcohol, solventy	1000	200
Eucalyptol (cinole)	Topical ointment for chest colds	1000	40 μ L
Pyridine	Sweet, alcohol, organic	1000	400

NOTE: Possible causes of odors or chemical substitutes for causes of odors.

* Adapted from AMERICAN WATER WORKS ASSOCIATION. 1993. FLAVOR PROFILE ANALYSIS: SCREENING AND TRAINING OF PANELISTS. AWWA Manual, American Water Works Assoc., Denver, Colo.

* Compounds available only in solid form and must be dissolved in methanol.

TABLE 2170:III. SUBSTITUTE ODOR REFERENCE STANDARDS*

Compound	Odor Characteristic	Preparation
Cloves	Spicy like cloves	Use supermarket brand of dried clove buds (spice). Add 3 clove buds to 200 mL pure water and swirl 1–2 min. Allow to stand overnight at room temperature, then discard the buds.

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Compound	Odor Characteristic	Preparation
Dried grass	Hay	Place dried cut grass in erlenmeyer flask until half full.
Grass	Decaying vegetation	Weigh 2 g of fresh grass and mix into 200 mL pure water and let stand at room temperature. In 1–3 d, the odor will appear.
Grass	Septic	Allow the solution above for decaying vegetation to stand for an additional 1–2 weeks.
Rubber hose	Rubber hose	Boil a short section of rubber hose in 200 mL pure water for 5 min. Allow to cool and remove the hose.
Soap	Soapy	Place 5 g of chipped nonscented bar soap in 200 mL pure water.
Pencil shavings	Woody	Instruct panel member to sharpen a wood pencil and sniff the freshly exposed wood.

NOTE: Standards made from materials rather than chemicals.* Adapted from AMERICAN WATER WORKS ASSOCIATION. 1993. Flavor Profile Analysis: Screening and Training of Panelists. AWWA Manual. American Water WorksAssoc., Denver, Colo.

TABLE 2170:IV. BASIC TASTE STANDARDS

Chemical for Basic Tastes	Food or Beverage Corresponding to Intensity	Concentration %	Intensity Scale (1 to 12)*
Sweet: sugar	Canned fruit or vegetables	5.00	4 W
	Carbonated soda	10.00	8 M
	Syrup, jelly	15.00	12 S
Sour: citric acid	Fresh fruit jelly	0.05	4 W
	Some carbonated sodas	0.10	8 M
	Lemon juice	0.20	12 S

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Chemical for Basic Tastes	Food or Beverage Corresponding to Intensity	Concentration %	Intensity Scale (1 to 12)*
Salt: sodium chloride	Level in bread	0.40	4 W
	Dehydrated soup mix	0.70	8 M
	Soy sauce	1.00	12 S
Bitter: caffeine	Strong coffee	0.05	4 W
		0.10	8 M
		0.20	12 S
	or quinine hydrochloride dihydrate	0.001	4 W
		0.002	8 M
		0.004	12 S

* W = weak; M = moderate; S = strong.

TABLE 2320:I. END-POINT pH VALUES

Test Condition	End Point pH	
	Total Alkalinity	Phenolphthalein Alkalinity
Alkalinity, mg CaCO ₃ /L:		
30	4.9	8.3
150	4.6	8.3
500	4.3	8.3
Silicates, phosphates known or suspected	4.5	8.3
Routine or automated analyses	4.5	8.3
Industrial waste or complex system	4.5	8.3

TABLE 2320:II. ALKALINITY RELATIONSHIPS*

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TABLE 2320:II. ALKALINITY RELATIONSHIPS*

Result of Titration	Hydroxide Alkalinity as CaCO_3	Carbonate Alkalinity as CaCO_3	Bicarbonate Concentration as CaCO_3
$P = 0$	0	0	T
$P < \frac{1}{2}T$	0	2P	$T - 2P$
$P = \frac{1}{2}T$	0	2P	0
$P > \frac{1}{2}T$	$2P - T$	$2(T - P)$	0
$P = T$	T	0	0

*Key: P=phenolphthalein alkalinity; T=total alkalinity.

Table 2330:I. Estimating Equilibrium Constants and Activity Coefficients

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TABLE 2330:I. ESTIMATING EQUILIBRIUM CONSTANTS AND ACTIVITY COEFFICIENTS

Equation	Temperature Range	References
When complete mineral analysis is available:		
$I = \frac{1}{2} \sum_{i=1}^i [X_i] Z_i^2$	—	3
When only conductivity is available:		
$I = 1.6 \times 10^{-5} C$	—	4
When only TDS is available:		
$I = TDS/40\,000$	—	5
$p f_m = A \left[\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3I \right]$ (valid to $I < 0.5$)	—	3
$A = 1.82 \times 10^6 (ET)^{-1.5}$	—	3
$E = \frac{60\,954}{T + 116} - 68.937$	—	6
$pK_2 = 107.8871 + 0.032\,528\,49T - 5151.79/T - 38.925\,61 \log_{10} T + 563\,713.9/T^2$	273–373	7
$pK_w = 4471/T + 0.017\,06T - 6.0875$	280–338	8
$pK_{sc} = 171.9065 + 0.077\,993T - 2839.319/T - 71.595 \log_{10} T$	273–363	7
$pK_{sa} = 171.9773 + 0.077\,993T - 2903.293/T - 71.595 \log_{10} T$	273–363	7
$pK_{sv} = 172.1295 + 0.077\,993T - 3074.688/T - 71.595 \log_{10} T$	273–363	7
$*I$ = ionic strength $[X_i]$ = concentration of component i, g-moles/L Z_i = charge of species i C = conductivity, $\mu\text{mhos/cm}$ TDS = total dissolved solids, mg/L pY = $-\log_{10}$ of the value of any factor Y f_m = activity coefficient for monovalent species E = dielectric constant T = temperature, $^{\circ}\text{K}$ ($^{\circ}\text{C} + 273.2$) K_2 = second dissociation constant for carbonic acid K_w = dissociation constant for water K_{sc} = solubility product constant for calcite K_{sa} = solubility product constant for aragonite K_{sv} = solubility product constant for vaterite		

TABLE 2330:II. PRECALCULATED VALUES FOR pK AND A AT SELECTED TEMPERATURES

Temperature $^{\circ}\text{C}$	pK_s					
	pK_2	Calcite	Aragonite	Vaterite	pK_w	A

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Temperature °C	pK_s					
	pK_2	Calcite	Aragonite	Vaterite	pK_w	A
5	10.55	8.39	8.24	7.77	14.73	0.494
10	10.49	8.41	8.26	7.80	14.53	0.498
15	10.43	8.43	8.28	7.84	14.34	0.502
20	10.38	8.45	8.31	7.87	14.16	0.506
25*	10.33	8.48	8.34	7.91	13.99	0.511
30	10.29	8.51	8.37	7.96	13.83	0.515
35	10.25	8.54	8.41	8.00	13.68	0.520
40	10.22	8.58	8.45	8.05	13.53	0.526
45	10.20	8.62	8.49	8.10	13.39	0.531
50	10.17	8.66	8.54	8.16	13.26	0.537
60	10.14	8.76	8.64	8.28	13.02	0.549
70	10.13	8.87	8.75	8.40	—	0.562
80	10.13	8.99	8.88	8.55	—	0.576
90	10.14	9.12	9.02	8.70	—	0.591

NOTE: All values determined from the equations of Table 2330:I.

A is used to calculate pt_m (see Table 2330:I).

* pt_m estimated from TDS values at 25°C are as follows:

TDS	pt_m
100	0.024
200	0.033
400	0.044
800	0.060
1000	0.066

TABLE 2330:III. GRAPHS AND COMPUTER SOFTWARE THAT CAN BE USED TO CALCULATE CaCO_3 SATURATION INDICES*

Item†	CaCO_3 Indices		Approximate Temperature Range °C	Approximate Limit of Ionic Strength	Ion Pairs Considered?	C
	Basis for Calculation of SI	CCPP				
1. Caldwell Lawrence diagrams ⁴	pH_{sa}	P, D	2–25	0.030	No	

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Item†	CaCO ₃ Indices		Approximate Temperature Range °C	Approximate Limit of Ionic Strength	Ion Pairs Considered?	C
	Basis for Calculation of SI	CCPP				
2. ACAPP	RS	P, D	–10–110	6+	Yes	
3. DRIVER	RS	P	7–65	2.5	Yes	
4. INDEX C	pH _{sa} pH _{sb}	P, D	0–50	0.5	No	
5. LEQUIL	RS	No	5–90	0.5	Yes	
6. MINTEQA1	RS	P, D	0–100	0.5	Yes	

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Item†	CaCO ₃ Indices		Approximate Temperature Range °C	Approximate Limit of Ionic Strength	Ion Pairs Considered?	C
	Basis for Calculation of SI	CCPP				
7. PHREEQE Standard	RS	P, D	0–100	0.5	Yes	
For high- salinity waters	RS	P, D	0–80	7–8	Yes	
8. SEQUIL	RS	P, D	7–65	2.5	Yes	
9. SOLMINEQ.88	RS	P, D	0–350	6	Yes	
10. WTRCHEM	pH _{sa}	P, D	0–100	0.5	No	

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Item†	CaCO ₃ Indices		Approximate Temperature Range °C	Approximate Limit of Ionic Strength	Ion Pairs Considered?	C
	Basis for Calculation of SI	CCPP				
11. WATEQ4F	RS	No	0–100	0.5	Yes	

*SI = saturation index

CCPP = CaCO₃ precipitation potential

pH_{sa} = alkalinity-based pH

pH_{sb} = bicarbonate-based pH

P = calculates amount of CaCO₃ theoretically precipitated

D = calculates amount of CaCO₃ theoretically dissolved

RS = relative saturation

PC = personal computer

RAM = random access memory

†1. Loewenthal and Marais³ provide 10.2- by 11.4-cm diagrams, with documentation; Merrill⁵ provides 10.2- by 16.5-cm diagrams, with documentation.

2. Radian Corp., 8501 MoPac Blvd., P.O. Box 201088, Austin, TX 78720-1088 Attn: J.G. Noblett (software and documentation).

3. Power Computing Co., 1930 Hi Line Dr., Dallas, TX, 74207 (software and documentation⁶).

4. Brown and Caldwell, P.O. Box 8045, Walnut Creek, CA 94596-1220 Attn: D.T. Merrill (software and documentation).

5. Illinois State Water Survey, Aquatic Chemistry Section, 2204 Griffith Dr., Champaign, IL 61820-7495 Attn: T.R. Holm (software and documentation).

6. Center for Exposure Assessment Modeling, Environmental Research Laboratory, Office of Research and Development. U.S. Environmental Protection Agency, Athens, GA 30613 (software and documentation⁷).

7. U.S. Geological Survey, National Center, MS 437, Reston, VA 22902, Chief of WATSTORE Program (provides software for mainframe version of standard code); U.S. Geological Survey, Water Resources Division, MS 420, 345 Middlefield Rd., Menlo Park, CA 94025 Attn: K. Nordstrom (provides software for personal computer version of standard code); National Water Research Institute, Canada Centre for Inland Waters, 867 Lakeshore Rd., Burlington, Ont., Canada L7R 4A6 Attn: A.S. Crowe (provides software and documentation^{8,9} for personal computer versions of both standard and high-salinity codes); U.S. Geological Survey, Books and Open File Report Section, Box 25425, Federal Center, Denver, CO 80225 (provides documentation^{8,10} for mainframe and personal computer versions of standard code).

8. Power Computing Company, 1930 Hi Line Dr., Dallas, TX 74207 (software and documentation¹¹).

9. U.S. Geological Survey, Water Resources Division, MS 427, 345 Middlefield Rd., Menlo Park, CA 94025 Attn: Y.K. Kharaka (software and documentation¹²).

10. D.T. Merrill, Brown and Caldwell, P.O. Box 8045, Walnut Creek, CA 94596-1220 (code listing and documentation).

11. U.S. Geological Survey, Water Resources Division, MS 420, 345 Middlefield Rd., Menlo Park, CA 94025 Attn: K. Nordstrom (software), Books and Open File Report Section, Box 25425, Federal Center, Denver, CO 80225 (documentation¹³).

‡Codes differ in the species included in Alk₀.

TABLE 2340:I. MAXIMUM CONCENTRATIONS OF INTERFERENCES PERMISSIBLE WITH VARIOUS INHIBITORS*

**Max. Interference
Concentration
mg/L**

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TABLE 2340:I. MAXIMUM CONCENTRATIONS OF INTERFERENCES PERMISSIBLE WITH VARIOUS INHIBITORS*

Interfering Substance	Max. Interference Concentration <i>mg/L</i>	
	Inhibitor I	Inhibitor II
Aluminum	20	20
Barium	†	†
Cadmium	†	20
Cobalt	over 20	0.3
Copper	over 30	20
Iron	over 30	5
Lead	†	20
Manganese (Mn ²⁺)	†	1
Nickel	over 20	0.3
Strontium	†	†
Zinc	†	200
Polyphosphate		10

* Based on 25-mL sample diluted to 50 mL.

† Titrates as hardness.

TABLE 2510:I. EQUIVALENT CONDUCTIVITY, Λ , AND CONDUCTIVITY, k , OF POTASSIUM CHLORIDE AT 25.0°C.*2-4

KCl Concentration <i>M or equivalent/L</i>	Equivalent Conductivity, Λ <i>mho-cm²/equivalent</i>	Conductivity, k_s <i>μmho/cm</i>
0	149.9	
0.0001	148.9	14.9
0.0005	147.7	73.9
0.001	146.9	146.9
0.005	143.6	717.5
0.01	141.2	1 412
0.02	138.2	2 765

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KCl Concentration <i>M or equivalent/L</i>	Equivalent Conductivity, Λ <i>mho-cm²/equivalent</i>	Conductivity, k_s <i>μmho/cm</i>
0.05	133.3	6 667
0.1	128.9	12 890
0.2	124.0	24 800
0.5	117.3	58 670
1	111.9	111 900

* Based on the absolute ohm, the 1968 temperature standard, and the dm³ volumestandard.² Values are accurate to $\pm 0.1\%$ or 0.1 μ mho/cm, whichever is greater.

TABLE 2510:II. SAMPLE ANALYSIS ILLUSTRATING CALCULATION OF CONDUCTIVITY, k_{calc} , FOR NATURAL WATERS.⁷

Ions	mg/L	mM	$ z \lambda^\circ \pm mM$	$z^2 mM$
Ca	55	1.38	164.2	5.52
Mg	12	0.49	52.0	1.96
Na	28	1.22	61.1	1.22
K	3.2	0.08	5.9	0.08
HCO ₃	170	2.79	124.2	2.79
SO ₄	77	0.80	128.0	3.20
Cl	20	0.56	<u>42.8</u>	<u>0.56</u>
			578.2	15.33

TABLE 2510:III. EQUIVALENT CONDUCTANCES, $\lambda^\circ+$ AND $\lambda^\circ-$, (MHO-CM²/EQUIVALENT) FOR IONS IN WATER AT 25.0 C.⁸

Cation	$\lambda^\circ+$	Anion	$\lambda^\circ-$
H ⁺	350	OH ⁻	198.6
1/2Ca ²⁺	59.5	HCO ₃ ⁻	44.5
1/2Mg ²⁺	53.1	1/2CO ₃ ²⁻	72
Na ⁺	50.1	1/2SO ₄ ²⁻	80.0

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Cation	$\lambda^{\circ+}$	Anion	$\lambda^{\circ-}$
K ⁺	73.5	Cl ⁻	76.4
NH ₄ ⁺	73.5	Ac ⁻	40.9
1/2Fe ²⁺	54	F ⁻	54.4
1/3Fe ³⁺	68	NO ₃ ⁻	71.4
		H ₂ PO ₄ ⁻	33
		1/2HPO ₄ ²⁻	57

TABLE 2530:I. COEFFICIENT OF VARIATION AND RECOVERY FOR PARTICULATE FLOATABLES TEST

Type of Wastewater	Average Floatables Concentration mg/L	No. of Samples	Coefficient of Variation %	Recovery %
Raw*	49	5	5.7	96
Raw	1.0	5	20	92
Primary effluent	2.7	5	15	91

* Additional floatable material added from skimmings of a primary sedimentation basin.

TABLE 2560:I. EXAMPLE CALCULATIONS FOR PARTICLE SIZE DISTRIBUTION ANALYSIS

Channel No. (A)	Lower Limit d_p μm (B)	Upper Limit d_p μm (C)	Mean Diam. d_{pi} μm (D)	Δd_{pi} μm (E)	$\log d_{pi}$ d_p in μm (F)	$\Delta \log d_{pi}$ (G)	Corrected Count (H)	Number Conc. ΔN_i No./mL (I)
6	2.95	3.39	3.16	0.44	0.50	0.06	5125	102 500
7	3.39	3.89	3.63	0.50	0.56	0.06	4568	91 360
8	3.89	4.47	4.17	0.58	0.62	0.06	3888	77 760
9	4.47	5.13	4.79	0.66	0.68	0.06	3088	61 760
10	5.13	5.89	5.50	0.76	0.74	0.06	2289	45 780
11	5.89	6.76	6.31	0.87	0.80	0.06	1584	31 680
12	6.76	7.76	7.24	1.00	0.86	0.06	1023	20 460

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Channel No. (A)	Lower Limit d_p μm (B)	Upper Limit d_p μm (C)	Mean Diam. d_{pi} μm (D)	Δd_{pi} μm (E)	$\log d_{pi}$ d_p in μm (F)	$\Delta \log d_{pi}$ (G)	Corrected Count (H)	Number Conc. ΔN_i No./mL (I)
13	7.76	8.91	8.32	1.15	0.92	0.06	631	12 620
14	8.91	10.23	9.55	1.32	0.98	0.06	363	7 260
15	10.23	11.75	10.96	1.52	1.04	0.06	199	3 980

TABLE 2580:I. POTENTIAL OF ZOBELL'S SOLUTION AS FUNCTION OF TEMPERATURE

T °C	E V	T °C	E V
1	0.481	16	0.448
2	0.479	17	0.446
3	0.476	18	0.443
4	0.474	19	0.441
5	0.472	20	0.439
6	0.470	21	0.437
7	0.468	22	0.435
8	0.465	23	0.432
9	0.463	24	0.430
10	0.461	25	0.428
11	0.459	26	0.426
12	0.457	27	0.424
13	0.454	28	0.421
14	0.452	29	0.419
15	0.450	30	0.417

TABLE 2580:II. PREPARATION OF REDOX STANDARD SOLUTIONS

Standard Solution	Potentials of Pt Electrode vs. Selected Reference Electrodes at 25°C in Standard Solution			Weight of Aqueous Solution
	Calomel	Silver:Silver Chloride Ag/AgCl		
			Standard Hydrogen	

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TABLE 2580:II. PREPARATION OF REDOX STANDARD SOLUTIONS

Standard Solution	Potentials of Pt Electrode vs. Selected Reference Electrodes at 25°C in Standard Solution					Weight of Aq
	Calomel	Silver:Silver Chloride Ag/AgCl			Standard Hydrogen	
	Hg/Hg ₂ Cl ₂ saturated KCl	KCl 1.00M	KCl 4.00M	KCl saturated		
Light's solution ¹⁴	+430	+439	+475	+476	+675	39.21 g ferr Fe(NH ₄) 48.22 g fe Fe(NH, 56.2 mL s 1.84
ZoBell's solution* ²	+183	+192	+228	+229	+428	1.4080 g pr K ₄ Fe(C 1.0975 g K ₃ Fe(C 7.4555 g

* Store in dark plastic bottle in a refrigerator.

TABLE 2580:III. RECOMMENDED COMBINATIONS FOR SELECTED SAMPLE TYPES

Sample Type	Indicator Electrode(s)	Reference Electrode	Type of Sample Cell
Process stream (low Br ⁻) (S ²⁻)	Pt or Au	Calomel or silver: silver chloride	Closed continuous flow (dual indicator electrode)
(high Br ⁻)	Pt or Au	Calomel or silver: silver chloride with salt bridge (double junction reference electrode)	
Natural waters			
Surface waters	Pt or Au	Calomel or silver: silver chloride	Closed continuous flow (dual indicator electrode) or beaker
Groundwater	Pt or Au	Calomel or silver: silver chloride	Closed continuous flow (dual indicator electrode)

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Sample Type	Indicator Electrode(s)	Reference Electrode	Type of Sample Cell
Soils, sludges	WIG, Pt wire	Calomel or silver: silver chloride	Beaker or soil core

TABLE 2710:I. TEMPERATURE CORRECTION FACTOR

Temperature °C	Temperature Correction Factor
15	0.9991
20	0.9982
25	0.9975
30	0.9957
35	0.9941
40	0.9922
45	0.9903

TABLE 2810:I. BUNSEN COEFFICIENT FOR OXYGEN IN FRESH WATER

Temperature °C	Bunsen Coefficient at Given Temperature (to nearest 0.1°C) <i>L</i> real gas at STP/(L · atmosphere)							
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
0	0.04914	0.04901	0.04887	0.04873	0.04860	0.04847	0.04833	0.04820
1	0.04780	0.04767	0.04754	0.04741	0.04728	0.04716	0.04703	0.04689
2	0.04653	0.04640	0.04628	0.04615	0.04603	0.04591	0.04579	0.04567
3	0.04531	0.04519	0.04507	0.04495	0.04484	0.04472	0.04460	0.04449
4	0.04414	0.04403	0.04392	0.04381	0.04369	0.04358	0.04347	0.04336
5	0.04303	0.04292	0.04282	0.04271	0.04260	0.04250	0.04239	0.04229
6	0.04197	0.04187	0.04177	0.04166	0.04156	0.04146	0.04136	0.04126
7	0.04096	0.04086	0.04076	0.04066	0.04056	0.04047	0.04037	0.04027
8	0.03999	0.03989	0.03980	0.03971	0.03961	0.03952	0.03943	0.03933
9	0.03906	0.03897	0.03888	0.03879	0.03870	0.03861	0.03852	0.03843
10	0.03817	0.03809	0.03800	0.03791	0.03783	0.03774	0.03766	0.03757
11	0.03732	0.03724	0.03716	0.03707	0.03699	0.03691	0.03683	0.03675
12	0.03651	0.03643	0.03635	0.03627	0.03619	0.03611	0.03604	0.03596

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Temperature °C	Bunsen Coefficient at Given Temperature (to nearest 0.1°C) <i>L real gas at STP/(L · atmosphere)</i>							
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
13	0.03573	0.03565	0.03558	0.03550	0.03543	0.03535	0.03528	0.03520
14	0.03498	0.03491	0.03448	0.03476	0.03469	0.03462	0.03455	0.03448
15	0.03426	0.03419	0.03412	0.03406	0.03399	0.03392	0.03385	0.03378
16	0.03358	0.03351	0.03344	0.03338	0.03331	0.03324	0.03318	0.03311
17	0.03292	0.03285	0.03279	0.03272	0.03266	0.03260	0.03253	0.03247
18	0.03228	0.03222	0.03216	0.03210	0.03204	0.03198	0.03192	0.03186
19	0.03168	0.03162	0.03156	0.03150	0.03144	0.03138	0.03132	0.03126
20	0.03109	0.03103	0.03098	0.03092	0.03086	0.03081	0.03075	0.03070
21	0.03053	0.03048	0.03042	0.03037	0.03031	0.03026	0.03020	0.03015
22	0.02999	0.02994	0.02989	0.02983	0.02978	0.02973	0.02968	0.02963
23	0.02947	0.02942	0.02937	0.02932	0.02927	0.02922	0.02917	0.02912
24	0.02897	0.02893	0.02888	0.02883	0.02878	0.02873	0.02868	0.02864
25	0.02850	0.02845	0.02840	0.02835	0.02831	0.02826	0.02822	0.02817
26	0.02803	0.02799	0.02794	0.02790	0.02785	0.02781	0.02777	0.02772
27	0.02759	0.02755	0.02750	0.02746	0.02742	0.02737	0.02733	0.02729
28	0.02716	0.02712	0.02708	0.02704	0.02700	0.02695	0.02691	0.02687
29	0.02675	0.02671	0.02667	0.02663	0.02659	0.02655	0.02651	0.02647
30	0.02635	0.02632	0.02628	0.02624	0.02620	0.02616	0.02612	0.02609
31	0.02597	0.02594	0.02590	0.02586	0.02582	0.02579	0.02575	0.02571
32	0.02561	0.02557	0.02553	0.02550	0.02546	0.02543	0.02539	0.02536
33	0.02525	0.02522	0.02518	0.02515	0.02511	0.02508	0.02504	0.02501
34	0.02491	0.02488	0.02484	0.02481	0.02478	0.02474	0.02471	0.02468
35	0.02458	0.02455	0.02452	0.02448	0.02445	0.02442	0.02439	0.02436
36	0.02426	0.02423	0.02420	0.02417	0.02414	0.02411	0.02408	0.02405
37	0.02396	0.02393	0.02390	0.02387	0.02384	0.02381	0.02378	0.02375
38	0.02366	0.02363	0.02360	0.02358	0.02355	0.02352	0.02349	0.02346
39	0.02338	0.02335	0.02332	0.02329	0.02327	0.02324	0.02321	0.02318
40	0.02310	0.02308	0.02305	0.02302	0.02300	0.02297	0.02294	0.02292

Based on Benson and Krause.^{9,10} $\beta = 9.9902 \times 10^{-4} \exp(9.7265 - 5.26895 \times 10^3/T + 1.00417 \times 10^6/T^2)$, where $T = 273.15 + ^\circ\text{C}$.

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TABLE 2810:II. VAPOR PRESSURE OF FRESH WATER

Temperature °C	Vapor Pressure at Given Temperature (to nearest 0.1°C)mm Hg									
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	4.58	4.61	4.64	4.68	4.71	4.75	4.78	4.82	4.85	4.89
1	4.92	4.96	4.99	5.03	5.07	5.10	5.14	5.18	5.21	5.25
2	5.29	5.33	5.36	5.40	5.44	5.48	5.52	5.56	5.60	5.64
3	5.68	5.72	5.76	5.80	5.84	5.88	5.92	5.97	6.01	6.05
4	6.09	6.14	6.18	6.22	6.27	6.31	6.36	6.40	6.44	6.49
5	6.54	6.58	6.63	6.67	6.72	6.77	6.81	6.86	6.91	6.96
6	7.01	7.05	7.10	7.15	7.20	7.25	7.30	7.35	7.40	7.45
7	7.51	7.56	7.61	7.66	7.71	7.77	7.82	7.87	7.93	7.98
8	8.04	8.09	8.15	8.20	8.26	8.31	8.37	8.43	8.48	8.54
9	8.60	8.66	8.72	8.87	8.84	8.89	8.95	9.02	9.08	9.14
10	9.20	9.26	9.32	9.39	9.45	9.51	9.58	9.64	9.70	9.77
11	9.83	9.90	9.97	10.03	10.10	10.17	10.23	10.30	10.37	10.44
12	10.51	10.58	10.65	10.72	10.76	10.86	10.93	11.00	11.07	11.15
13	11.22	11.29	11.37	11.44	11.52	11.59	11.67	11.74	11.82	11.90
14	11.98	12.05	12.13	12.21	12.29	12.37	12.45	12.53	12.61	12.69
15	12.78	12.86	12.94	13.05	13.11	13.19	13.28	13.36	13.45	13.54
16	13.62	13.71	13.80	13.89	13.97	14.06	14.15	14.24	14.33	14.43
17	14.52	14.61	14.70	14.80	14.89	14.98	15.08	15.17	15.27	15.37
18	15.46	15.56	15.66	15.76	15.86	15.96	16.06	16.16	16.26	16.36
19	16.46	16.57	16.67	16.77	16.88	16.98	17.09	17.20	17.30	17.41
20	17.52	17.63	17.74	17.85	17.96	18.07	18.18	18.29	18.41	18.52
21	18.64	18.75	18.87	18.98	19.10	19.22	19.33	19.45	19.57	19.69
22	19.81	19.93	20.05	20.48	20.60	20.42	20.55	20.67	20.80	20.93
23	21.05	21.18	21.31	21.44	21.57	21.70	21.83	21.96	22.09	22.23
24	22.36	22.50	22.63	22.77	22.90	23.04	23.18	23.32	23.46	23.60
25	23.74	23.88	24.03	24.17	24.31	24.46	24.60	24.75	24.90	25.04
26	25.19	25.34	25.49	25.64	25.80	25.95	26.10	26.26	26.41	26.57
27	26.72	26.88	27.04	27.20	27.36	27.52	27.68	27.84	28.00	28.17
28	28.33	28.50	28.66	28.83	29.00	29.17	29.34	29.51	29.68	29.85

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Temperature °C	Vapor Pressure at Given Temperature (to nearest 0.1°C)mm Hg									
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
29	30.03	30.80	30.37	30.55	30.73	30.91	31.08	31.26	31.44	31.62
30	31.81	31.99	32.17	32.36	32.54	32.73	32.92	33.11	33.30	33.49
31	33.68	33.87	34.06	34.26	34.45	34.65	34.85	35.05	35.24	35.44
32	35.65	35.85	36.05	36.25	36.46	36.67	36.87	37.08	37.29	37.50
33	37.71	37.92	38.14	38.35	38.57	38.78	39.00	39.22	39.44	39.66
34	39.88	40.10	40.33	40.55	40.78	41.01	41.23	41.46	41.69	41.92
35	42.16	42.39	42.63	42.86	43.10	43.34	43.58	43.82	44.06	44.30
36	44.55	44.79	45.04	45.28	45.53	45.78	46.03	46.29	46.54	46.79
37	47.05	47.31	47.56	47.82	48.08	48.35	48.61	48.87	49.14	49.41
38	49.67	49.94	50.21	50.49	50.76	51.03	51.31	51.59	51.87	52.14
39	52.43	52.71	52.99	53.28	53.56	53.85	54.14	54.43	54.72	55.01
40	55.31	55.60	55.90	56.20	56.50	56.80	57.10	57.41	57.71	58.02

Based on an equation presented by Green and Carritt.¹¹ This equation is cumbersome to use. The following equation⁹ is adequate for most applications:

$$P_{H_2O} = 760\{\exp(11.8571 - 3,840.70/T - 216,961/T^2)\}, \text{ where } T = 273.15 + ^\circ\text{C}.^5$$

Part 3000 METALS

3010 INTRODUCTION

3010 A. General Discussion

1. Significance

The effects of metals in water and wastewater range from beneficial through troublesome to dangerously toxic. Some metals are essential to plant and animal growth while others may adversely affect water consumers, wastewater treatment systems, and receiving waters. The benefits versus toxicity of some metals depend on their concentrations in waters.

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2. Types of Methods

Preliminary treatment is often required to present the metals to the analytical methodology in an appropriate form. Alternative methods for pretreatment of samples are presented in Section 3030.

Metals may be determined satisfactorily by a variety of methods, with the choice often depending on the precision and sensitivity required. Part 3000 describes colorimetric methods as well as instrumental methods, i.e., atomic absorption spectrometry, including flame, electrothermal (furnace), hydride, and cold vapor techniques; flame photometry; inductively coupled plasma emission spectrometry; inductively coupled plasma mass spectrometry, and anodic stripping voltammetry. Flame atomic absorption methods generally are applicable at moderate (0.1- to 10-mg/L) concentrations in clean and complex-matrix samples. Electrothermal methods generally can increase sensitivity if matrix problems do not interfere. Inductively coupled plasma emission techniques are applicable over a broad linear range and are especially sensitive for refractory elements. Inductively coupled plasma mass spectrometry offers significantly increased sensitivity for some elements (as low as 0.01 µg/L) in a variety of environmental matrices. Flame photometry gives good results at higher concentrations for several Group I and II elements. Anodic stripping offers high sensitivity for several elements in relatively clean matrices. Colorimetric methods are applicable to specific metal determinations where interferences are known not to compromise method accuracy; these methods may provide speciation information for some metals. Table 3010:I lists the methods available in Part 3000 for each metal.

3. Definition of Terms

a. *Dissolved metals*: Those metals in an unacidified sample that pass through a 0.45-µm membrane filter.

b. *Suspended metals*: Those metals in an unacidified sample that are retained by a 0.45-µm membrane filter.

c. *Total metals*: The concentration of metals determined in an unfiltered sample after vigorous digestion, or the sum of the concentrations of metals in the dissolved and suspended fractions. Note that total metals are defined operationally by the digestion procedure.

d. *Acid-extractable metals*: The concentration of metals in solution after treatment of an unfiltered sample with hot dilute mineral acid. To determine either dissolved or suspended metals, filter sample immediately after collection. Do not preserve with acid until after filtration.

3010 B. Sampling and Sample Preservation

Before collecting a sample, decide what fraction is to be analyzed (dissolved, suspended, total, or acid-extractable). This decision will determine in part whether the sample is acidified with or without filtration and the type of digestion required.

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Serious errors may be introduced during sampling and storage because of contamination from sampling device, failure to remove residues of previous samples from sample container, and loss of metals by adsorption on and/or precipitation in sample container caused by failure to acidify the sample properly.

1. Sample Containers

The best sample containers are made of quartz or TFE. Because these containers are expensive, the preferred sample container is made of polypropylene or linear polyethylene with a polyethylene cap. Borosilicate glass containers also may be used, but avoid soft glass containers for samples containing metals in the microgram-per-liter range. Store samples for determination of silver in light-absorbing containers. Use only containers and filters that have been acid rinsed.

2. Preservation

Preserve samples immediately after sampling by acidifying with concentrated nitric acid (HNO_3) to pH <2. Filter samples for dissolved metals before preserving (see Section 3030). Usually 1.5 mL conc HNO_3 /L sample (or 3 mL 1 + 1 HNO_3 /L sample) is sufficient for short-term preservation. For samples with high buffer capacity, increase amount of acid (5 mL may be required for some alkaline or highly buffered samples). Use commercially available high-purity acid#(65)* or prepare high-purity acid by sub-boiling distillation of acid.

After acidifying sample, preferably store it in a refrigerator at approximately 4°C to prevent change in volume due to evaporation. Under these conditions, samples with metal concentrations of several milligrams per liter are stable for up to 6 months (except mercury, for which the limit is 5 weeks). For microgram-per-liter metal levels, analyze samples as soon as possible after sample collection.

Alternatively, preserve samples for mercury analysis by adding 2 mL/L 20% (w/v) $\text{K}_2\text{Cr}_2\text{O}_7$ solution (prepared in 1 + 1 HNO_3). Store in a refrigerator not contaminated with mercury. (CAUTION: Mercury concentrations may increase in samples stored in plastic bottles in mercury-contaminated laboratories.)

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3010 C. General Precautions

1. Sources of Contamination

Avoid introducing contaminating metals from containers, distilled water, or membrane filters. Some plastic caps or cap liners may introduce metal contamination; for example, zinc has been found in black bakelite-type screw caps as well as in many rubber and plastic products, and cadmium has been found in plastic pipet tips. Lead is a ubiquitous contaminant in urban air and dust.

2. Contaminant Removal

Thoroughly clean sample containers with a metal-free nonionic detergent solution, rinse with tap water, soak in acid, and then rinse with metal-free water. For quartz, TFE, or glass materials, use 1 + 1 HNO₃, 1 + 1 HCl, or aqua regia (3 parts conc HCl + 1 part conc HNO₃) for soaking. For plastic material, use 1 + 1 HNO₃ or 1 + 1 HCl. Reliable soaking conditions are 24 h at 70°C. Chromic acid or chromium-free substitutes⁶⁶ may be used to remove organic deposits from containers, but rinse containers thoroughly with water to remove traces of chromium. Do not use chromic acid for plastic containers or if chromium is to be determined. Always use metal-free water in analysis and reagent preparation (see Section 3111B.3c). In these methods, the word “water” means metal-free water.

3. Airborne Contaminants

For analysis of microgram-per-liter concentrations of metals, airborne contaminants in the form of volatile compounds, dust, soot, and aerosols present in laboratory air may become significant. To avoid contamination use “clean laboratory” facilities such as commercially available laminar-flow clean-air benches or custom-designed work stations and analyze blanks that reflect the complete procedure.

4. Bibliography

MITCHELL, J.W. 1973. Ultrapurity in trace analysis. *Anal. Chem.* 45:492A.

GARDNER, M., D. HUNT & G. TOPPING. 1986. Analytical quality control (AQC) for monitoring trace metals in the coastal and marine environment. *Water Sci. Technol.* 18:35.

3020 QUALITY ASSURANCE/QUALITY CONTROL

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3020 A. Introduction

General information and recommendations for quality assurance (QA) and quality control (QC) are provided in Section 1020 Quality Assurance, 1030 Data Quality, and 1040 Method Development and Evaluation. This section discusses QA/QC requirements that are common to the analytical methods presented in Part 3000. The requirements are recommended minimum QA/QC activities; refer to individual methods and regulatory program requirements for method-specific QA/QC requirements. Always consider the overall purpose of analyses. QA/QC measures and substantiation for operational-control determinations may differ significantly from those for determinations of trace metals at water quality criteria levels. Levels of trace metals in environmental samples may be orders of magnitude lower than in potential sources of contamination.

Use replicates of measurable concentration to establish precision and known-additions recovery to determine bias. Use blanks, calibrations, control charts, known additions, standards, and other ancillary measurement tools as appropriate. Provide adequate documentation and record keeping to satisfy client requirements and performance criteria established by the laboratory.

3020 B. Quality Control Practices

1. Initial Quality Control

a. Initial demonstration of capability: Verify analyst capability before analyzing any samples and repeat periodically to demonstrate proficiency with the analytical method. Verify that the method being used provides sufficient sensitivity for the purpose of the measurement. Test analyst capability by analyzing at least four reagent water portions containing known additions of the analyte of interest. Confirm proficiency by generating analytical results that demonstrate precision and bias within acceptable limits representative of the analytical method.

b. Method detection limit (MDL): Before samples are analyzed, determine the MDL for each analyte by the procedures of Section 1030, or other applicable procedure.¹ Determine MDL at least annually for each method and major matrix category. Verify MDL for a new analyst or whenever instrument hardware or method operating conditions are modified. Analyze samples for MDL determinations over a 3- to 5-d period to generate a realistic value. Preferably use pooled data from several analysts rather than data from a single analyst.

c. Dynamic range (DR): Before using a new method, determine the dynamic range, i.e., the concentration range over which a method has an increasing response (linear or second-order), for each analyte by analyzing several standard solutions that bracket the range of interest. Each standard measurement should be within 10% of the true value for acceptance into the DR determination. Take measurements at both the low and high end of the calibration range to

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determine method suitability. Analytical instrumentation with curve-fitting features may allow utilization of nonlinear instrument response.

2. Calibration

a. *Initial calibration:* Calibrate initially with a minimum of a blank and three calibration standards of the analyte(s) of interest. Select calibration standards that bracket the expected concentration of the sample and that are within the method's dynamic range. The number of calibration points depends on the width of the dynamic range and the shape of the calibration curve. One calibration standard should be below the reporting limit for the method. As a general rule, differences between calibration standard concentrations should not be greater than one order of magnitude (i.e., 1, 10, 100, 1000). Apply linear or polynomial curve-fitting statistics, as appropriate, for analysis of the concentration-instrument response relationship. The appropriate linear or nonlinear correlation coefficient for standard concentration to instrument response should be ≥ 0.995 . Use initial calibration for quantitation of analyte concentration in samples. Use calibration verification, ¶ b below, only for checks on the initial calibration and not for sample quantitation. Repeat initial calibration daily and whenever calibration verification acceptance criteria are not satisfied.

b. *Calibration verification:* Calibration verification is the periodic confirmation that instrument response has not changed significantly from the initial calibration. Verify calibration by analyzing a midpoint calibration standard (check standard) and calibration blank at the beginning and end of a sample run, periodically during a run (normally after each set of ten samples). A check standard determination outside 90 to 110% of the expected concentration indicates a potential problem. If a check standard determination is outside 80 to 120% of the expected concentration, immediately cease sample analyses and initiate corrective action. Repeat initial calibration and sample determinations since the last acceptable calibration verification. Use calculated control limits (Section 1020B) to provide better indications of system performance and to provide tighter control limits.

c. *Quality control sample:* Analyze an externally generated quality control sample of known concentration at least quarterly and whenever new calibration stock solutions are prepared. Obtain this sample from a source external to the laboratory or prepare it from a source different from those used to prepare working standards. Use to validate the laboratory's working standards both qualitatively and quantitatively.

3. Batch Quality Control

a. *Method blank (MB):* A method blank (also known as reagent blank) is a portion of reagent water treated exactly as a sample, including exposure to all equipment, glassware, procedures, and reagents. The MB is used to assess whether analytes or interference are present within the analytical process or system. No analyte of interest should be present in the MB at a warning level based on the end user's requirements. Undertake immediate corrective action for MB measurements above the MDL. Include a minimum of one MB with each set of 20 or fewer samples.

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b. *Laboratory-fortified blank (LFB)*: The laboratory-fortified blank (also known as blank spike) is a method blank that has been fortified with a known concentration of analyte. It is used to evaluate ongoing laboratory performance and analyte recovery in a clean matrix. Prepare fortified concentrations approximating the midpoint of the calibration curve or lower with stock solutions prepared from a source different from those used to develop working standards. Calculate percent recovery, plot control charts, and determine control limits (Section 1020B) for these measurements. Ensure that the LFB meets performance criteria for the method. Establish corrective actions to be taken in the event that LFB does not satisfy acceptance criteria. Include a minimum of one LFB with each set of 20 or fewer samples.

c. *Duplicates*: Use duplicate samples of measurable concentration to measure precision of the analytical process. Randomly select routine samples to be analyzed twice. Process duplicate sample independently through entire sample preparation and analytical process. Include a minimum of one duplicate for each matrix type with each set of 20 or fewer samples.

d. *Laboratory-fortified matrix (LFM)/laboratory-fortified matrix duplicate*: Use LFM (also known as matrix spike) and LFM duplicate to evaluate the bias and precision, respectively, of the method as influenced by a specific matrix. Prepare by adding a known concentration of analytes to a randomly selected routine sample. Prepare addition concentrations to approximately double the concentration present in the original sample. If necessary, dilute sample to bring the measurement within the established calibration curve. Limit addition volume to 5% or less of sample volume. Calculate percent recovery and relative percent difference, plot control charts, and determine control limits (Section 1020B). Ensure that performance criteria for the method are satisfied. Process fortified samples independently through entire sample preparation and analytical process. Include a minimum of one LFM/LFM duplicate with each set of 20 or fewer samples.

e. *Method of known additions*: To analyze a new or unfamiliar matrix, use the method of known additions (Section 1020B) to demonstrate freedom from interference before reporting concentration data for the analyte. Verify absence of interferences by analyzing such samples undiluted and in a 1:10 dilution; results should be within 10% of each other. Limit known-addition volume to 10% or less of the sample volume.

4. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1995. Definition and procedure for the determination of the method detection limit, revision 1.11. 40 CFR Part 136, Appendix B. *Federal Register* 5:23703.

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3030 A. Introduction

Samples containing particulates or organic material generally require pretreatment before spectroscopic analysis. “Total metals” includes all metals, inorganically and organically bound, both dissolved and particulate. Colorless, transparent samples (primarily drinking water) having a turbidity of <1 NTU, no odor, and single phase may be analyzed directly by atomic absorption spectroscopy (flame or electrothermal vaporization) or inductively coupled plasma spectroscopy (atomic emission or mass spectrometry) for total metals without digestion. For further verification or if changes in existing matrices are encountered, compare digested and undigested samples to ensure comparable results. On collection, acidify such samples to pH <2 with conc nitric acid (1.5 mL HNO₃/L is usually adequate for drinking water) and analyze directly. Digest all other samples before determining total metals. To analyze for dissolved metals, filter sample, acidify filtrate, and store until analyses can be performed. To determine suspended metals, filter sample, digest filter and the material on it, and analyze. To determine acid-extractable metals, extract metals as indicated in Section 3030E through K and analyze extract.

This section describes general pretreatment for samples in which metals are to be determined according to Section 3110 through 3500-Zn with several exceptions. The special digestion techniques for mercury are given in Section 3112B.4*b* and *c*, and those for arsenic and selenium in Section 3114 and Section 3500-Se.

Take care not to introduce metals into samples during preliminary treatment. During pretreatment avoid contact with rubber, metal-based paints, cigarette smoke, paper tissues, and all metal products including those made of stainless steel, galvanized metal, and brass. Conventional fume hoods can contribute significantly to sample contamination, particularly during acid digestion in open containers. Keep vessels covered with watch glasses and turn spouts away from incoming air to reduce airborne contamination. Plastic pipet tips often are contaminated with copper, iron, zinc, and cadmium; before use soak in 2*N* HCl or HNO₃ for several days and rinse with deionized water. Avoid using colored plastics, which can contain metals. Use certified metal-free plastic containers and pipet tips when possible. Avoid using glass if analyzing for aluminum or silica.

Use metal-free water (see Section 3111B.3*c*) for all operations. Check reagent-grade acids used for preservation, extraction, and digestion for purity. If excessive metal concentrations are found, purify the acids by distillation or use ultra-pure acids. Inductively coupled plasma mass spectrometry (ICP-MS) may require use of ultra-pure acids and reagents to avoid measurable contamination. Process blanks through all digestion and filtration steps and evaluate blank results relative to corresponding sample results. Either apply corrections to sample results or take other corrective actions as necessary or appropriate.

3030 B. Filtration for Dissolved and Suspended Metals

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1. Filtration Procedures

If dissolved or suspended metals (see Section 3010A) are to be determined, filter sample at time of collection using a preconditioned plastic filtering device with either vacuum or pressure, containing a filter support of plastic or fluorocarbon, through a prewashed ungridded 0.4- to 0.45- μm -pore-diam membrane filter (polycarbonate or cellulose esters). Before use filter a blank consisting of metal-free (deionized) water to insure freedom from contamination. Precondition filter and filter device by rinsing with 50 mL deionized water. If the filter blank contains significant metals concentrations, soak membrane filters in approximately 0.5N HCl or 1N HNO₃ (recommended for electrothermal and ICP-MS analyses) and rinse with deionized water before use.

Before filtering, centrifuge highly turbid samples in acid-washed fluorocarbon or high-density plastic tubes to reduce loading on filters. Stirred, pressure filter units foul less readily than vacuum filters; filter at a pressure of 70 to 130 kPa. After filtration acidify filtrate to pH 2 with conc HNO₃ and store until analyses can be performed. If a precipitate forms on acidification, digest acidified filtrate before analysis as directed (see Section 3030E). Retain filter and digest it for direct determination of suspended metals.

If it is not possible to field-filter the sample without contaminating it, obtain sample in an “unpreserved” bottle as above and promptly cool to 4°C. Do not acid-preserve the sample. Then, without delay, filter sample under cleaner conditions in the laboratory.

Test pH of a portion of aqueous sample upon receipt in the laboratory to ensure that the sample has been properly filtered and acid-preserved.¹

NOTE: Different filters display different sorption and filtration characteristics²; for trace analysis, test filter and filtration system to verify complete recovery of metals.

If suspended metals (see Section 3010A) are to be determined, filter sample as above for dissolved metals, but do not centrifuge before filtration. Retain filter and digest it for direct determination of suspended metals. Record sample volume filtered and include a filter in determination of the blank.

CAUTION: *Do not use perchloric acid to digest membrane filters.* (See Section 3030H for more information on handling HClO₄).

2. References

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1994. Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements, Method 200.2. Environmental Monitoring Systems Lab., Cincinnati, Ohio.
2. HOROWITZ, A.J., K.R. LUM, J.R. GARBARINO, G.E.M. HALL, C. LEMIEUX & C.R. DEMAS. 1996. Problems with using filtration to define dissolved trace element concentrations in natural water samples. *Environ. Sci. Technol.* 30, 954.

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3030 C. Treatment for Acid-Extractable Metals

Extractable metals (see Section 3010A) are lightly adsorbed on particulate material. Because some sample digestion may be unavoidable use rigidly controlled conditions to obtain meaningful and reproducible results. Maintain constant sample volume, acid volume, and contact time. Express results as extractable metals and specify extraction conditions.

At collection, acidify entire sample with 5 mL conc HNO_3 /L sample. To prepare sample, mix well, transfer 100 mL to a beaker or flask, and add 5 mL 1 + 1 high-purity HCl. Heat 15 min on a steam bath. Filter through a membrane filter (preconditioned as in Section 3030B) and carefully transfer filtrate to a tared volumetric flask. Adjust volume to 100 mL with metal-free water, mix, and analyze. If volume is greater than 100 mL, determine volume to nearest 0.1 mL by weight, analyze, and correct final concentration measurement by multiplying by the dilution factor (final volume $\div$ 100).

3030 D. Digestion for Metals

To reduce interference by organic matter and to convert metals associated with particulates to a form (usually the free metal) that can be determined by atomic absorption spectrometry or inductively-coupled plasma spectroscopy, use one of the digestion techniques presented below. Use the least rigorous digestion method required to provide acceptable and consistent recovery compatible with the analytical method and the metal being analyzed.¹⁻³

1. Selection of Acid

Nitric acid will digest most samples adequately (Section 3030E). Nitrate is an acceptable matrix for both flame and electrothermal atomic absorption and the preferred matrix for ICP-MS.⁴ Some samples may require addition of perchloric, hydrochloric, hydrofluoric, or sulfuric acid for complete digestion. These acids may interfere in the analysis of some metals and all provide a poorer matrix for both electrothermal and ICP-MS analysis. Confirm metal recovery for each digestion and analytical procedure used. Use Table 3030:I as a guide in determining which acids (in addition to HNO_3) to use for complete digestion. As a general rule, HNO_3 alone is adequate for clean samples or easily oxidized materials; HNO_3 - H_2SO_4 or HNO_3 -HCl digestion is adequate for readily oxidizable organic matter; HNO_3 - HClO_4 or HNO_3 - HClO_4 -HF digestion is necessary for difficult-to-oxidize organic matter or minerals containing silicates. Although dry ashing is not generally recommended because of the loss of many volatile elements, it may be helpful if large amounts of organic matter are present.

2. Digestion Procedures

Dilute samples with Ag concentrations greater than 1 mg/L to contain less than 1 mg Ag/L for flame atomic absorption methods and 25 $\mu\text{g/L}$ or less for electrothermal analysis.^{2,5,6} To

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address problems with silver halide solubility in HNO_3 , digest using method 3030F.3b.

Report digestion technique used.

Acid digestion techniques (Section 3030E through I) generally yield comparable precision and bias for most sample types that are totally digested by the technique. Because acids used in digestion will add metals to the samples and blanks, minimize the volume of acids used.

Because the acid digestion techniques (Section 3030E and F) normally are not total digestions, the microwave digestion procedure (Section 3030K) may be used as an alternative. The microwave method is a closed-vessel procedure and thus is expected to provide improved precision when compared with hot-plate techniques. Microwave digestion is recommended for samples being analyzed by ICP-MS. The microwave digestion method is recommended for the analysis of Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Tl, V, and Zn. Microwave digestion may be acceptable for additional analytes provided its performance for those elements is validated.

Suggested sample volumes are indicated below for flame atomic absorption spectrometry. Lesser volumes, to a minimum of 5 mL, are appropriate for graphite furnace, ICP, and ICP-MS. Do not subsample volumes less than 5 mL, especially when particulates are present. Instead dilute samples with elevated analyte concentrations after digestion. If the recommended volume exceeds digestion vessel capacity, add sample as evaporation proceeds. For samples containing particulates, wide-bore pipets may be useful for volume measurement and transfer.

When samples are concentrated during digestion (e.g., >100mL sample used) determine metal recovery for each matrix digested, to verify method validity. Using larger samples will require additional acid, which also would increase the concentration of impurities.

Estimated Metal Concentration <i>mg/L</i>	Sample Volume* <i>mL</i>
<0.1	1000
0.1–10	100
10–100+	10

*For flame atomic absorption spectrometry.

Report results as follows:

$$\text{Metal concentration, mg/L} = A \times \frac{B}{C}$$

where:

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A = concentration of metal in digested solution, mg/L,

B = final volume of digested solution, mL, and

C = sample size, mL.

Prepare solid samples or liquid sludges with high solids contents on a weight basis. Mix sample and transfer a suitable amount (typically 1 g of a sludge with 15% total solids) directly into a preweighed digestion vessel. Reweigh and calculate weight of sample. Proceed with one of the digestion techniques presented below. However, as these digestion methods are predominantly for dissolved and extractable metals in aqueous samples, other approaches may be more appropriate for solid samples. For complete mineralization of solid samples, consult methods available elsewhere.^{1,4,6,7} Report results on wet- or dry-weight basis as follows:

$$\text{Metal concentration, mg/kg (wet-weight basis)} = \frac{A \times B}{\text{g sample}}$$

$$\text{Metal concentration, mg/kg (dry-weight basis)} = \frac{A \times B}{\text{g sample}} \times \frac{100}{D}$$

where:

A = concentration of metal in digested solution, mg/L,

B = final volume of digested solution, mL, and

D = total solids, % (see Section 2540G).

Always prepare acid blanks for each type of digestion performed. Although it is always best to eliminate all relevant sources of contamination, a reagent blank prepared with the same acids and subjected to the same digestion procedure as the sample can correct for impurities present in acids and reagent water. However, blank correction is not recommended for any other sources of contamination such as impurities adsorbed on glassware.

3. References

1. BOUMANS, P.W.J.M., ed. 1987. Inductively Coupled Plasma Emission Spectroscopy, Part II: Applications and Fundamentals. John Wiley & Sons, New York, N.Y.
2. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1992. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846, 3rd ed. Update 1, Methods 3005A, 3010A, 3020A & 3050A. Off. Solid Waste & Emergency Response, Washington, D.C.
3. HOENIG, M. & A.M. DE KERSABIEC. 1996. Sample preparation steps for analysis by atomic spectroscopy methods: Present status. *Spectrochim. Acta*. B51:1297.
4. JARVIS, K.E., A.L. GRAY & R.S. HOUK, eds. 1992. Sample preparation for ICP-MS.

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Chapter 7 in Handbook of Inductively Coupled Plasma Mass Spectrometry. Blackie, Glasgow & London, U.K.

5. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1994. Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements, Method 200.2. Environmental Monitoring Systems Lab., Cincinnati, Ohio.
6. KINGSTON, H.M. & S. HASWELL, eds. 1997. Microwave Enhanced Chemistry: Fundamentals, Sample Preparation and Applications. American Chemical Soc., Washington, D.C.
7. BOCK, R. 1979. A Handbook of Decomposition Methods in Analytical Chemistry. Blackie, Glasgow, U.K.

3030 E. Nitric Acid Digestion

Because of the wide variation in concentration levels detected by various instrumental techniques and the need to deal adequately with sources of contamination at trace levels, this method presents one approach for high-level analytes (>0.1 mg/L) and another for trace levels (≤ 0.1 mg/L).

1. Digestion for Flame Atomic Absorption and High-Level Concentrations

a. Apparatus:

- 1) Hot plate.
- 2) Conical (erlenmeyer) flasks, 125-mL, or Griffin beakers, 150-mL, acid-washed and rinsed with water.
- 3) Volumetric flasks, 100-mL.
- 4) Watch glasses, ribbed and unribbed.

b. Reagent:

Nitric acid, HNO_3 , conc, analytical or trace-metals grade.

c. Procedure: Transfer a measured volume (100 mL recommended) of well-mixed, acid-preserved sample appropriate for the expected metals concentrations to a flask or beaker (see Section 3030D for sample volume). In a hood, add 5 mL conc HNO_3 . If a beaker is used, cover with a ribbed watch glass to minimize contamination. Boiling chips, glass beads, or Hengar granules may be added to aid boiling and minimize spatter when high concentration levels (>10 mg/L) are being determined. Bring to a slow boil and evaporate on a hot plate to the lowest volume possible (about 10 to 20 mL) before precipitation occurs. Continue heating and adding conc HNO_3 as necessary until digestion is complete as shown by a light-colored, clear solution. Do not let sample dry during digestion.

Wash down flask or beaker walls and watch glass cover (if used) with metal-free water and

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then filter if necessary (see Section 3030B). Transfer filtrate to a 100-mL volumetric flask with two 5-mL portions of water, adding these rinsings to the volumetric flask. Cool, dilute to mark, and mix thoroughly. Take portions of this solution for required metal determinations.

2. Digestion for Trace-Level (≤ 0.1 mg/L) Concentrations for ICP and ICP-MS¹

a. Apparatus:

- 1) *Block heater*, dry, with temperature control.
- 2) *Polypropylene tubes**(68), graduated, round-bottom tubes with caps, 17 × 100 mm, acid-washed and rinsed with metal-free water. Preferably use tubes that simultaneously match the analysis instrument autosampler and the block digester. A fit with the centrifuge is secondary but also desirable.

3) *Pipetters*, assorted sizes or adjustable.

4) *Pipet tips*.

5) *Centrifuge*.

b. Reagent:

Nitric acid, HNO₃, conc, double distilled.†(69)

c. *Procedure*: Soak new polypropylene tubes and caps overnight or for several days in 2N HNO₃. Triple rinse with metal-free water, and preferably dry in poly racks or baskets in a low-temperature oven overnight. Store cleaned tubes in plastic bags before use. Pipet tips also may need to be cleaned; evaluate before use.

Pipet 10 mL well-mixed, acid-preserved sample into a precleaned, labeled tube with a macropipet. With a minimum volume change (<0.5 mL), add appropriate amount of analyte for matrix fortified samples. With a pipet, add 0.5 mL conc HNO₃ (or 1.0 mL 1 + 1 HNO₃) to all samples, blanks, standards, and quality control samples.

Place tubes in block heater in a hood and adjust temperature to 105°C. Drape caps over each tube to allow escape of acid vapors while preventing contamination. NOTE: Do not screw on caps at this time. Digest samples for a minimum of 2 h. Do not let samples boil. Add more conc nitric acid as necessary until digestion is complete by observation of a clear solution.

Remove tubes from heat and cool. Dilute back to original 10 mL volume with metal-free water. Adjust over-volume samples to next convenient gradation for calculations and note volume. (Apply concentration correction from Section 3030D.) If tubes contain particulates, centrifuge and decant clear portion into another precleaned tube. Tighten screw caps and store at 4°C until ready for analysis.

3. Reference

1. JARVIS, K.E., A.L. GRAY & R.S. HOUK, eds. 1992. Sample preparation for ICP-MS. Chapter 7 in *Handbook of Inductively Coupled Plasma Mass Spectrometry*. Blackie & Son, Ltd., Glasgow & London, U.K.

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3030 F. Nitric Acid-Hydrochloric Acid Digestion

1. Apparatus

See Section 3030E.1a. The following also may be needed:

Steam bath.

2. Reagents

a. *Nitric acid*, HNO_3 , conc, analytical grade or better (see Section 3030E).

b. *Hydrochloric acid*, HCl , 1 + 1.

c. *Nitric acid*, HNO_3 , 1 + 1.

3. Procedure

a. *Total HNO_3/HCl* : Transfer a measured volume of well-mixed, acid-preserved sample appropriate for the expected metals concentrations to a flask or beaker (see Section 3030D for sample volume). In a hood add 3 mL conc HNO_3 and cover with a ribbed watch glass. Place flask or beaker on a hot plate and cautiously evaporate to less than 5 mL, making certain that sample does not boil and that no area of the bottom of the container is allowed to go dry. Cool. Rinse down walls of beaker and watch glass with a minimum of metal-free water and add 5 mL conc HNO_3 . Cover container with a nonribbed watch glass and return to hot plate. Increase temperature of hot plate so that a gentle reflux action occurs. Continue heating, adding additional acid as necessary, until digestion is complete (generally indicated when the digestate is light in color or does not change in appearance with continued refluxing). Cool. Add 10 mL 1 + 1 HCl and 15 mL water per 100 mL anticipated final volume. Heat for an additional 15 min to dissolve any precipitate or residue. Cool, wash down beaker walls and watch glass with water, filter to remove insoluble material that could clog the nebulizer (see Section 3030B), and transfer filtrate to a 100-mL volumetric flask with rinsings. Alternatively centrifuge or let settle overnight. Adjust to volume and mix thoroughly.

b. *Recoverable HNO_3/HCl* : For this less rigorous digestion procedure, transfer a measured volume of well-mixed, acid-preserved sample to a flask or beaker. Add 2 mL 1 + 1 HNO_3 and 10 mL 1 + 1 HCl and cover with a ribbed watch glass. Heat on a steam bath or hot plate until volume has been reduced to near 25 mL, making certain sample does not boil. Cool and filter to remove insoluble material or alternatively centrifuge or let settle overnight. Quantitatively transfer sample to volumetric flask, adjust volume to 100 mL, and mix.

For trace-level digestion, use precautionary measures similar to those detailed in Section 3030E.

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3030 G. Nitric Acid-Sulfuric Acid Digestion

1. Apparatus

See Section 3030E.1a.

2. Reagents

a. *Nitric acid*, HNO_3 , conc. (See Section 3030E for acid grades.)

b. *Sulfuric acid*, H_2SO_4 , conc.

3. Procedure

Transfer a measured volume of well-mixed, acid-preserved sample appropriate for the expected metals concentrations to a flask or beaker (see Section 3030D for sample volume). Add 5 mL conc HNO_3 and cover with a ribbed watch glass. Bring to slow boil on hot plate and evaporate to 15 to 20 mL. Add 5 mL conc HNO_3 and 10 mL conc H_2SO_4 , cooling flask or beaker between additions. Evaporate on a hot plate until dense white fumes of SO_3 just appear. If solution does not clear, add 10 mL conc HNO_3 and repeat evaporation to fumes of SO_3 . Heat to remove all HNO_3 before continuing treatment. All HNO_3 will be removed when the solution is clear and no brownish fumes are evident. Do not let sample dry during digestion.

Cool and dilute to about 50 mL with water. Heat to almost boiling to dissolve slowly soluble salts. Filter if necessary, then complete procedure as directed in Section 3030E.1c beginning with, "Transfer filtrate . . ."

3030 H. Nitric Acid-Perchloric Acid Digestion

1. Apparatus

See Section 3030E.1a. The following also are needed:

a. *Safety shield*.

b. *Safety goggles*.

c. *Watch glasses*.

2. Reagents

a. *Nitric acid*, HNO_3 , conc.

b. *Perchloric acid*, HClO_4 .

c. *Ammonium acetate solution*: Dissolve 500 g $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ in 600 mL water.

3. Procedure

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CAUTION: *Heated mixtures of HClO_4 and organic matter may explode violently. Avoid this hazard by taking the following precautions:* (a) do not add HClO_4 to a hot solution containing organic matter; (b) always pretreat samples containing organic matter with HNO_3 before adding HClO_4 ; (c) avoid repeated fuming with HClO_4 in ordinary hoods (For routine operations, use a water pump attached to a glass fume eradicator. Stainless steel fume hoods with adequate water washdown facilities are available commercially and are acceptable for use with HClO_4); and (d) never let samples being digested with HClO_4 evaporate to dryness.

Transfer a measured volume of well-mixed, acid-preserved sample appropriate for the expected metals concentrations to a flask or beaker (see Section 3030D for sample volume). In a hood add 5 mL conc HNO_3 and cover with a ribbed watch glass. Evaporate sample to 15 to 20 mL on a hot plate. Add 10 mL each of conc HNO_3 and HClO_4 , cooling flask or beaker between additions. Evaporate gently on a hot plate until dense white fumes of HClO_4 just appear. If solution is not clear, keep solution just boiling until it clears. If necessary, add 10 mL conc HNO_3 to complete digestion. Cool, dilute to about 50 mL with water, and boil to expel any chlorine or oxides of nitrogen. Filter, then complete procedure as directed in 3030E.1c beginning with, "Transfer filtrate . . ."

If lead is to be determined in the presence of high amounts of sulfate (e.g., determination of Pb in power plant fly ash samples), dissolve PbSO_4 precipitate as follows: Add 50 mL ammonium acetate solution to flask or beaker in which digestion was carried out and heat to incipient boiling. Rotate container occasionally to wet all interior surfaces and dissolve any deposited residue. Reconnect filter and slowly draw solution through it. Transfer filtrate to a 100-mL volumetric flask, cool, dilute to mark, mix thoroughly, and set aside for determination of lead.

3030 I. Nitric Acid-Perchloric Acid-Hydrofluoric Acid Digestion

1. Apparatus

- Hot plate.*
- TFE beakers, 250-mL, acid-washed and rinsed with water.*
- Volumetric flasks, 100-mL, polypropylene or other suitable plastic.*

2. Reagents

- Nitric acid, HNO_3 , conc and 1 + 1.*
- Perchloric acid, HClO_4 .*
- Hydrofluoric acid, HF, 48 to 51%.*

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3. Procedure

CAUTION: See precautions for using HClO_4 in 3030H; handle HF with extreme care and provide adequate ventilation, especially for the heated solution. Avoid all contact with exposed skin. Provide medical attention for HF burns.

Transfer a measured volume of well-mixed, acid-preserved sample appropriate for the expected metals concentrations into a 250-mL TFE beaker (see Section 3030D for sample volume). Evaporate on a hot plate to 15 to 20 mL. Add 12 mL conc HNO_3 and evaporate to near dryness. Repeat HNO_3 addition and evaporation. Let solution cool, add 20 mL HClO_4 and 1 mL HF, and boil until solution is clear and white fumes of HClO_4 have appeared. Cool, add about 50 mL water, filter, and proceed as directed in Section 3030E.1c beginning with, “Transfer filtrate . . .”

3030 J. Dry Ashing

The procedure appears in the Eighteenth Edition of *Standard Methods*. It has been deleted from subsequent editions.

3030 K. Microwave-Assisted Digestion

1. Apparatus

a. *Microwave unit* with programmable power (minimum 545 W) to within ± 10 W of required power, having a corrosion-resistant, well-ventilated cavity and having all electronics protected against corrosion for safe operation. Use a unit having a rotating turntable with a minimum speed of 3 rpm to insure homogeneous distribution of microwave radiation. Use only laboratory-grade microwave equipment and closed digestion containers with pressure relief that are specifically designed for hot acid.¹

b. *Vessels*: Construction requires an inner liner of perfluoroalkoxy (PFA) Teflon™, or other TFE, or composite fluorinated polymers, capable of withstanding pressures of at least 760 ± 70 kPa (110 ± 10 psi), and capable of controlled pressure relief at the manufacturer's maximum pressure rating.

Acid wash all digestion vessels and rinse with water (§ 2a). For new vessels or when changing between high- and low-concentration samples, clean by leaching with hot hydrochloric acid (1:1) for a minimum of 2 h and then with hot nitric acid (1:1) for a minimum of 2 h; rinse with water and dry in a clean environment. Use this procedure whenever the previous use of digestion vessels is unknown or cross-contamination from vessels is suspected.

c. *Temperature feedback control system*, using shielded thermocouple, fiber-optic probe, or infrared detector.

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- d. *Bottles*, polyethylene, 125-mL, with caps.
- e. *Thermometer*, accurate to $\pm 0.1^{\circ}\text{C}$.
- f. *Balance*, large-capacity (1500 g), accurate to 0.1 g.
- g. *Filtration or centrifuge equipment* (optional).
- h. *Plastic container* with cover, 1-L, preferably made of PFA TeflonTM.#(73)

2. Reagents

- a. *Metal-free water*: See Section 3111B.3c.
- b. *Nitric acid*, HNO_3 , conc, sub-boiling distilled. Non-sub-boiling acids can be used if they are shown not to contribute blanks.

3. Calibration of Microwave Unit

NOTE: For microwave units equipped with temperature feedback electronic controls, calibration of the microwave unit is not required provided performance specifications can be duplicated.

For cavity-type microwave equipment, evaluate absolute power (watts) by measuring the temperature rise in 1 kg water exposed to microwave radiation for a fixed time. With this measurement, the relationship between available power (W) and the partial power setting (%) of the unit can be estimated, and any absolute power in watts may be transferred from one unit to another. The calibration format required depends on type of electronic system used by manufacturer to provide partial microwave power. Few units have an accurate and precise linear relationship between percent power settings and absorbed power. Where linear circuits have been used, determine calibration curve by a three-point calibration method; otherwise, use the multiple-point calibration method.

a. *Three-point calibration method*: Measure power at 100% and 50% power using the procedure described in ¶ 3c and calculate power setting corresponding to required power in watts as specified in the procedure from the two-point line. Measure absorbed power at the calculated partial power setting. If the measured absorbed power does not correspond to the calculated power within ± 10 W, use the multiple-point calibration method, ¶ 3b. Use this point periodically to verify integrity of calibration.

b. *Multiple-point calibration method*: For each microwave unit, measure the following power settings: 100, 99, 98, 97, 95, 90, 80, 70, 60, 50, and 40% using the procedure described in ¶ 3c. These data are clustered about the customary working power ranges. Nonlinearity commonly is encountered at the upper end of the calibration curve. If the unit's electronics are known to have nonlinear deviations in any region of proportional power control, make a set of measurements that bracket the power to be used. The final calibration point should be at the partial power setting that will be used in the test. Check this setting periodically to evaluate the integrity of the calibration. If a significant change (± 10 W) is detected, re-evaluate entire calibration.

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c. Equilibrate a large volume of water to room temperature ($23 \pm 2^\circ\text{C}$). Weigh 1 kg water ($1000 \text{ g} \pm 1 \text{ g}$) or measure ($1000 \text{ mL} \pm 1 \text{ mL}$) into a plastic, not glass, container, and measure the temperature to $\pm 0.1^\circ\text{C}$. Condition microwave unit by heating a glass beaker with 500 to 1000 mL tap water at full power for 5 min with the exhaust fan on. Loosely cover plastic container to reduce heat loss and place in normal sample path (at outer edge of rotating turntable); circulate continuously through the microwave field for 120 s at desired power setting with exhaust fan on as it will be during normal operation. Remove plastic container and stir water vigorously. Use a magnetic stirring bar inserted immediately after microwave irradiation; record maximum temperature within the first 30 s to $\pm 0.1^\circ\text{C}$. Use a new sample for each additional measurement. If the water is reused, return both water and beaker to $23 \pm 2^\circ\text{C}$. Make three measurements at each power setting. When any part of the high-voltage circuit, power source, or control components in the unit have been serviced or replaced, recheck calibration power. If power output has changed by more than $\pm 10 \text{ W}$, re-evaluate entire calibration.

Compute absorbed power by the following relationship:

$$P = \frac{(K) (Cp) (m) (\Delta T)}{t}$$

where:

P = apparent power absorbed by sample, W,

K = conversion factor for thermochemical calories sec^{-1} to watts (4.184),

Cp = heat capacity, thermal capacity, or specific heat ($\text{cal g}^{-1} ^\circ\text{C}^{-1}$) of water,

m = mass of water sample, g,

ΔT = final temperature minus initial temperature, $^\circ\text{C}$, and

t = time, s.

For the experimental conditions of 120 s and 1 kg water (Cp at $25^\circ\text{C} = 0.9997$), the calibration equation simplifies to:

$$P = (\Delta T) (34.85)$$

Stable line voltage within the manufacturer's specification is necessary for accurate and reproducible calibration and operation. During measurement and operation it must not vary by more than $\pm 2 \text{ V}$. A constant power supply may be necessary if line voltage is unstable.

4. Procedure

CAUTION: This method is designed for microwave digestion of waters only. It is not intended for the digestion of solids, for which high concentrations of organic compounds may result in high pressures and possibly unsafe conditions.

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CAUTION: As a safety measure, never mix different manufacturers' vessels in the same procedure. Vessels constructed differently will retain heat at different rates; control of heating conditions assumes that all vessels have the same heat-transfer characteristics. Inspect casements for cracks and chemical corrosion. Failure to maintain the vessels' integrity may result in catastrophic failure.

Both prescription controls and performance controls are provided for this procedure. Performance controls are the most general and most accurate. When equipment capability permits, use the performance criterion.

a. Performance criterion: The following procedure is based on heating acidified samples in two stages where the first stage is to reach $160 \pm 4^\circ$ in 10 min and the second stage is to permit a slow rise to 165 to 170°C during the second 10 min. This performance criterion is based on temperature feedback control system capability that is implemented in various ways by different manufacturers. Because the temperature of the acid controls the reaction, this is the essential condition that will reproduce results in this preparation method. Verification of temperature conditions inside the vessel at these specific times is sufficient to verify the critical procedural requirements.

b. Prescription criterion: For all PFA vessels without liners, a verified program that meets the performance-based temperature-time profile is 545 W for 10 min followed by 344 W for 10 min using five single-wall PFA TeflonTM (74) digestion vessels.² Any verified program for a given microwave unit depends on unit power and operational power settings, heating times, number, type, and placement of digestion vessels within the unit, and sample and acid volumes. The change in power, time, and temperature profile is not directly proportional to the change in the number of sample vessels. Any deviations from the verified program conditions will require verification of the time-temperature profile to conform to the given two-stage profile. This may be done by laboratory personnel if suitable test equipment is available, or by the manufacturer of the microwave equipment.

c. General conditions: Weigh entire digestion vessel assembly to 0.1 g before use and record (A). Accurately transfer 45 mL of well-shaken sample into the digestion vessel. Pipet 5 mL conc HNO_3 into each vessel. Attach all safety equipment required for appropriate and safe vessel operation following manufacturer's specifications. Tighten cap to manufacturer's specifications. Weigh each capped vessel to the nearest 0.1 g (B).

Place appropriate number of vessels evenly distributed in the carousel. Treat sample blanks, known additions, and duplicates in the same manner as samples. For prescription control only, when fewer samples than the appropriate number are digested, fill remaining vessels with 45 mL water and 5 mL conc HNO_3 to obtain full complement of vessels for the particular program being used.

Place carousel in unit and seat it carefully on turntable. Program microwave unit to heat samples to $160 \pm 4^\circ\text{C}$ in 10 min and then, for the second stage, to permit a slow rise to 165 to 170°C for 10 min. Start microwave generator, making sure that turntable is turning and that

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exhaust fan is on.

At completion of the microwave program, let vessels cool for at least 5 min in the unit before removal. Cool samples further outside the unit by removing the carousel and letting them cool on a bench or in a water bath. When cooled to room temperature, weigh each vessel (to 0.1 g) and record weight (C).

If the net weight of sample plus acid decreased by more than 10%, discard sample.

Complete sample preparation by carefully uncapping and venting each vessel in a fume hood. Follow individual manufacturer's specifications for relieving pressure in individual vessel types. Transfer to acid-cleaned noncontaminating plastic bottles. If the digested sample contains particulates, filter, centrifuge, or settle overnight and decant.

5. Calculations

a. *Dilution correction:* Multiply results by 50/45 or 1.11 to account for the dilution caused by the addition of 5 mL acid to 45 mL sample.

b. *Discarding of sample:* To determine if the net weight of sample plus acid decreased by more than 10% during the digestion process, use the following calculation

$$\frac{[(B - A) - (C - A)]}{(B - A)} \times 100 > 10\% \text{ (1\% for multilayer vessels)}$$

6. Quality Control

NOTE: When nitric acid digestion is used, recoveries of silver and antimony in some matrices may be unacceptably low. Verify recoveries using appropriate known additions.

Preferably include a quality-control sample in each loaded carousel. Prepare samples in batches including preparation blanks, sample duplicates, and pre-digestion known additions. Determine size of batch and frequency of quality-control samples by method of analysis and laboratory practice. The power of the microwave unit and batch size may prevent including one or more of the quality-control samples in each carousel. Do not group quality-control samples together but distribute them throughout the various carousels to give the best monitoring of digestion.

7. References

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3110 METALS BY ATOMIC ABSORPTION SPECTROMETRY

Because requirements for determining metals by atomic absorption spectrometry vary with metal and/or concentration to be determined, the method is presented as follows:

Section 3111, Metals by Flame Atomic Absorption Spectrometry, encompasses:

- Determination of antimony, bismuth, cadmium, calcium, cesium, chromium, cobalt, copper, gold, iridium, iron, lead, lithium, magnesium, manganese, nickel, palladium, platinum, potassium, rhodium, ruthenium, silver, sodium, strontium, thallium, tin, and zinc by direct aspiration into an air-acetylene flame (Section 3111B),
- Determination of low concentrations of cadmium, chromium, cobalt, copper, iron, lead, manganese, nickel, silver, and zinc by chelation with ammonium pyrrolidine dithiocarbamate (APDC), extraction into methyl isobutyl ketone (MIBK), and aspiration into an air-acetylene flame (Section 3111C),
- Determination of aluminum, barium, beryllium, calcium, molybdenum, osmium, rhenium, silicon, thorium, titanium, and vanadium by direct aspiration into a nitrous oxide-acetylene flame (Section 3111D), and
- Determination of low concentrations of aluminum and beryllium by chelation with 8-hydroxyquinoline, extraction into MIBK, and aspiration into a nitrous oxide-acetylene flame (Section 3111E).

Section 3112 covers determination of mercury by the cold vapor technique.

Section 3113 concerns determination of micro quantities of aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, iron, lead, manganese, molybdenum, nickel, selenium, silver, and tin by electrothermal atomic absorption spectrometry.

Section 3114 covers determination of arsenic and selenium by conversion to their hydrides and aspiration into an argon-hydrogen or nitrogen-hydrogen flame.

3111 METALS BY FLAME ATOMIC ABSORPTION SPECTROMETRY*(75)

3111 A. Introduction

1. Principle

In flame atomic absorption spectrometry, a sample is aspirated into a flame and atomized. A light beam is directed through the flame, into a monochromator, and onto a detector that

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measures the amount of light absorbed by the atomized element in the flame. For some metals, atomic absorption exhibits superior sensitivity over flame emission. Because each metal has its own characteristic absorption wavelength, a source lamp composed of that element is used; this makes the method relatively free from spectral or radiation interferences. The amount of energy at the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample over a limited concentration range. Most atomic absorption instruments also are equipped for operation in an emission mode, which may provide better linearity for some elements.

2. Selection of Method

See Section 3110.

3. Interferences

a. Chemical interference: Many metals can be determined by direct aspiration of sample into an air-acetylene flame. The most troublesome type of interference is termed “chemical” and results from the lack of absorption by atoms bound in molecular combination in the flame. This can occur when the flame is not hot enough to dissociate the molecules or when the dissociated atom is oxidized immediately to a compound that will not dissociate further at the flame temperature. Such interferences may be reduced or eliminated by adding specific elements or compounds to the sample solution. For example, the interference of phosphate in the magnesium determination can be overcome by adding lanthanum. Similarly, introduction of calcium eliminates silica interference in the determination of manganese. However, silicon and metals such as aluminum, barium, beryllium, and vanadium require the higher-temperature, nitrous oxide-acetylene flame to dissociate their molecules. The nitrous oxide-acetylene flame also can be useful in minimizing certain types of chemical interferences encountered in the air-acetylene flame. For example, the interference caused by high concentrations of phosphate in the determination of calcium in the air-acetylene flame is reduced in the nitrous oxide-acetylene flame.

MIBK extractions with APDC (see Section 3111C) are particularly useful where a salt matrix interferes, for example, in seawater. This procedure also concentrates the sample so that the detection limits are extended.

Brines and seawater can be analyzed by direct aspiration but sample dilution is recommended. Aspiration of solutions containing high concentrations of dissolved solids often results in solids buildup on the burner head. This requires frequent shutdown of the flame and cleaning of the burner head. Preferably use background correction when analyzing waters that contain in excess of 1% solids, especially when the primary resonance line of the element of interest is below 240 nm. Make more frequent recovery checks when analyzing brines and seawaters to insure accurate results in these concentrated and complex matrices.

Barium and other metals ionize in the flame, thereby reducing the ground state (potentially absorbing) population. The addition of an excess of a cation (sodium, potassium, or lithium) having a similar or lower ionization potential will overcome this problem. The wavelength of

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maximum absorption for arsenic is 193.7 nm and for selenium 196.0 nm—wavelengths at which the air-acetylene flame absorbs intensely. The sensitivity for arsenic and selenium can be improved by conversion to their gaseous hydrides and analyzing them in either a nitrogen-hydrogen or an argon-hydrogen flame with a quartz tube (see Section 3114).

b. Background correction: Molecular absorption and light scattering caused by solid particles in the flame can cause erroneously high absorption values resulting in positive errors. When such phenomena occur, use background correction to obtain accurate values. Use any one of three types of background correction: continuum-source, Zeeman, or Smith-Hieftje correction.

1) Continuum-source background correction—A continuum-source background corrector utilizes either a hydrogen-filled hollow cathode lamp with a metal cathode or a deuterium arc lamp. When both the line source hollow-cathode lamp and the continuum source are placed in the same optical path and are time-shared, the broadband background from the elemental signal is subtracted electronically, and the resultant signal will be background-compensated.

Both the hydrogen-filled hollow-cathode lamp and deuterium arc lamp have lower intensities than either the line source hollow-cathode lamp or electrodeless discharge lamps. To obtain a valid correction, match the intensities of the continuum source with the line source hollow-cathode or electrodeless discharge lamp. The matching may result in lowering the intensity of the line source or increasing the slit width; these measures have the disadvantage of raising the detection limit and possibly causing nonlinearity of the calibration curve. Background correction using a continuum source corrector is susceptible to interference from other absorbing lines in the spectral bandwidth. Mis correction occurs from significant atomic absorption of the continuum source radiation by elements other than that being determined. When a line source hollow-cathode lamp is used without background correction, the presence of an absorbing line from another element in the spectral bandwidth will not cause an interference unless it overlaps the line of interest.

Continuum-source background correction will not remove direct absorption spectral overlap, where an element other than that being determined is capable of absorbing the line radiation of the element under study.

2) Zeeman background correction—This correction is based on the principle that a magnetic field splits the spectral line into two linearly polarized light beams parallel and perpendicular to the magnetic field. One is called the pi (π) component and the other the sigma (σ) component. These two light beams have exactly the same wavelength and differ only in the plane of polarization. The π line will be absorbed by both the atoms of the element of interest and by the background caused by broadband absorption and light scattering of the sample matrix. The σ line will be absorbed only by the background.

Zeeman background correction provides accurate background correction at much higher absorption levels than is possible with continuum source background correction systems. It also virtually eliminates the possibility of error from structured background. Because no additional light sources are required, the alignment and intensity limitations encountered using continuum sources are eliminated.

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Disadvantages of the Zeeman method include reduced sensitivity for some elements, reduced linear range, and a “rollover” effect whereby the absorbance of some elements begins to decrease at high concentrations, resulting in a two-sided calibration curve.

3) Smith-Hieftje background correction—This correction is based on the principle that absorbance measured for a specific element is reduced as the current to the hollow cathode lamp is increased while absorption of nonspecific absorbing substances remains identical at all current levels. When this method is applied, the absorbance at a high-current mode is subtracted from the absorbance at a low-current mode. Under these conditions, any absorbance due to nonspecific background is subtracted out and corrected for.

Smith-Hieftje background correction provides a number of advantages over continuum-source correction. Accurate correction at higher absorbance levels is possible and error from structured background is virtually eliminated. In some cases, spectral interferences also can be eliminated. The usefulness of Smith-Hieftje background correction with electrodeless discharge lamps has not yet been established.

4. Sensitivity, Detection Limits, and Optimum Concentration Ranges

The sensitivity of flame atomic absorption spectrometry is defined as the metal concentration that produces an absorption of 1% (an absorbance of approximately 0.0044). The instrument detection limit is defined here as the concentration that produces absorption equivalent to twice the magnitude of the background fluctuation. Sensitivity and detection limits vary with the instrument, the element determined, the complexity of the matrix, and the technique selected. The optimum concentration range usually starts from the concentration of several times the detection limit and extends to the concentration at which the calibration curve starts to flatten. To achieve best results, use concentrations of samples and standards within the optimum concentration range of the spectrometer. See Table 3111:I for indication of concentration ranges measurable with conventional atomization. In many instances the concentration range shown in Table 3111:I may be extended downward either by scale expansion or by integrating the absorption signal over a long time. The range may be extended upward by dilution, using a less sensitive wavelength, rotating the burner head, or utilizing a microprocessor to linearize the calibration curve at high concentrations.

5. Preparation of Standards

Prepare standard solutions of known metal concentrations in water with a matrix similar to the sample. Use standards that bracket expected sample concentration and are within the method’s working range. Very dilute standards should be prepared daily from stock solutions in concentrations greater than 500 mg/L. Stock standard solutions can be obtained from several commercial sources. They also can be prepared from National Institute of Standards and Technology (NIST) reference materials or by procedures outlined in the following sections.

For samples containing high and variable concentrations of matrix materials, make the major ions in the sample and the dilute standard similar. If the sample matrix is complex and components cannot be matched accurately with standards, use the method of standard additions,

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Section 3113B.4d2), to correct for matrix effects. If digestion is used, carry standards through the same digestion procedure used for samples.

6. Apparatus

a. *Atomic absorption spectrometer*, consisting of a light source emitting the line spectrum of an element (hollow-cathode lamp or electrodeless discharge lamp), a device for vaporizing the sample (usually a flame), a means of isolating an absorption line (monochromator or filter and adjustable slit), and a photoelectric detector with its associated electronic amplifying and measuring equipment.

b. *Burner*: The most common type of burner is a premix, which introduces the spray into a condensing chamber for removal of large droplets. The burner may be fitted with a conventional head containing a single slot; a three-slot Belling head, which may be preferred for direct aspiration with an air-acetylene flame; or a special head for use with nitrous oxide and acetylene.

c. *Readout*: Most instruments are equipped with either a digital or null meter readout mechanism. Most modern instruments are equipped with microprocessors or stand-alone control computers capable of integrating absorption signals over time and linearizing the calibration curve at high concentrations.

d. *Lamps*: Use either a hollow-cathode lamp or an electrodeless discharge lamp (EDL). Use one lamp for each element being measured. Multi-element hollow-cathode lamps generally provide lower sensitivity than single-element lamps. EDLs take a longer time to warm up and stabilize.

e. *Pressure-reducing valves*: Maintain supplies of fuel and oxidant at pressures somewhat higher than the controlled operating pressure of the instrument by using suitable reducing valves. Use a separate reducing valve for each gas.

f. *Vent*: Place a vent about 15 to 30 cm above the burner to remove fumes and vapors from the flame. This precaution protects laboratory personnel from toxic vapors, protects the instrument from corrosive vapors, and prevents flame stability from being affected by room drafts. A damper or variable-speed blower is desirable for modulating air flow and preventing flame disturbance. Select blower size to provide the air flow recommended by the instrument manufacturer. In laboratory locations with heavy particulate air pollution, use clean laboratory facilities (Section 3010C).

7. Quality Assurance/Quality Control

Some data typical of the precision and bias obtainable with the methods discussed are presented in Table 3111:II and Table 3111:III.

Analyze a blank between sample or standard readings to verify baseline stability. Rezero when necessary.

To one sample out of every ten (or one sample from each group of samples if less than ten are being analyzed) add a known amount of the metal of interest and reanalyze to confirm recovery. The amount of metal added should be approximately equal to the amount found. If

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little metal is present add an amount close to the middle of the linear range of the test. Recovery of added metal should be between 85 and 115%.

Analyze an additional standard solution after every ten samples or with each batch of samples, whichever is less, to confirm that the test is in control. Recommended concentrations of standards to be run, limits of acceptability, and reported single-operator precision data are listed in Table 3111:III.

See Section 3020 for additional recommended quality control procedures.

8. References

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3111 B. Direct Air-Acetylene Flame Method

1. General Discussion

This method is applicable to the determination of antimony, bismuth, cadmium, calcium,

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cesium, chromium, cobalt, copper, gold, iridium, iron, lead, lithium, magnesium, manganese, nickel, palladium, platinum, potassium, rhodium, ruthenium, silver, sodium, strontium, thallium, tin, and zinc.

2. Apparatus

Atomic absorption spectrometer and associated equipment: See Section 3111A.6. Use burner head recommended by the manufacturer.

3. Reagents

a. *Air*, cleaned and dried through a suitable filter to remove oil, water, and other foreign substances. The source may be a compressor or commercially bottled gas.

b. *Acetylene*, standard commercial grade. Acetone, which always is present in acetylene cylinders, can be prevented from entering and damaging the burner head by replacing a cylinder when its pressure has fallen to 689 kPa (100 psi) acetylene.

CAUTION: *Acetylene gas represents an explosive hazard in the laboratory. Follow instrument manufacturer's directions in plumbing and using this gas. Do not allow gas contact with copper, brass with >65% copper, silver, or liquid mercury; do not use copper or brass tubing, regulators, or fittings.*

c. *Metal-free water:* Use metal-free water for preparing all reagents and calibration standards and as dilution water. Prepare metal-free water by deionizing tap water and/or by using one of the following processes, depending on the metal concentration in the sample: single distillation, redistillation, or sub-boiling. Always check deionized or distilled water to determine whether the element of interest is present in trace amounts. (NOTE: *If the source water contains Hg or other volatile metals, single- or redistilled water may not be suitable for trace analysis because these metals distill over with the distilled water. In such cases, use sub-boiling to prepare metal-free water.*)

d. *Calcium solution:* Dissolve 630 mg calcium carbonate, CaCO_3 , in 50 mL of 1 + 5 HCl. If necessary, boil gently to obtain complete solution. Cool and dilute to 1000 mL with water.

e. *Hydrochloric acid*, HCl, 1%, 10%, 20% (all v/v), 1 + 5, 1 + 1, and conc.

f. *Lanthanum solution:* Dissolve 58.65 g lanthanum oxide, La_2O_3 , in 250 mL conc HCl. Add acid slowly until the material is dissolved and dilute to 1000 mL with water.

g. *Hydrogen peroxide*, 30%.

h. *Nitric acid*, HNO_3 , 2% (v/v), 1 + 1, and conc.

i. *Aqua regia:* Add 3 volumes conc HCl to 1 volume conc HNO_3 .

j. *Standard metal solutions:* Prepare a series of standard metal solutions in the optimum concentration range by appropriate dilution of the following stock metal solutions with water containing 1.5 mL conc HNO_3 /L. Stock standard solutions are available from a number of commercial suppliers. Alternatively, prepare as described below. Thoroughly dry reagents before

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use. In general, use reagents of the highest purity. For hydrates, use fresh reagents.

- 1) *Antimony*: Dissolve 0.2669 g $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6$ in water, add 10 mL 1 + 1 HCl and dilute to 1000 mL with water; 1.00 mL = 100 μg Sb.
- 2) *Bismuth*: Dissolve 0.100 g bismuth metal in a minimum volume of 1 + 1 HNO_3 . Dilute to 1000 mL with 2% (v/v) HNO_3 ; 1.00 mL = 100 μg Bi.
- 3) *Cadmium*: Dissolve 0.100 g cadmium metal in 4 mL conc HNO_3 . Add 8.0 mL conc HNO_3 and dilute to 1000 mL with water; 1.00 mL = 100 μg Cd.
- 4) *Calcium*: Suspend 0.2497 g CaCO_3 (dried at 180° for 1 h before weighing) in water and dissolve cautiously with a minimum amount of 1 + 1 HNO_3 . Add 10.0 mL conc HNO_3 and dilute to 1000 mL with water; 1.00 mL = 100 μg Ca.
- 5) *Cesium*: Dissolve 0.1267 g cesium chloride, CsCl, in 1000 mL water; 1.00 mL = 100 μg Cs.
- 6) *Chromium*: Dissolve 0.1923 g CrO_3 in water. When solution is complete, acidify with 10 mL conc HNO_3 and dilute to 1000 mL with water; 1.00 mL = 100 μg Cr.
- 7) *Cobalt*: Dissolve 0.1000 g cobalt metal in a minimum amount of 1 + 1 HNO_3 . Add 10.0 mL 1 + 1 HCl and dilute to 1000 mL with water; 1.00 mL = 100 μg Co.
- 8) *Copper*: Dissolve 0.100 g copper metal in 2 mL conc HNO_3 , add 10.0 mL conc HNO_3 and dilute to 1000 mL with water; 1.00 mL = 100 μg Cu.
- 9) *Gold*: Dissolve 0.100 g gold metal in a minimum volume of aqua regia. Evaporate to dryness, dissolve residue in 5 mL conc HCl, cool, and dilute to 1000 mL with water; 1.00 mL = 100 μg Au.
- 10) *Iridium*: Dissolve 0.1147 g ammonium chloroiridate, $(\text{NH}_4)_2\text{IrCl}_6$, in a minimum volume of 1% (v/v) HCl and dilute to 100 mL with 1% (v/v) HCl; 1.00 mL = 500 μg Ir.
- 11) *Iron*: Dissolve 0.100 g iron wire in a mixture of 10 mL 1 + 1 HCl and 3 mL conc HNO_3 . Add 5 mL conc HNO_3 and dilute to 1000 mL with water; 1.00 mL = 100 μg Fe.
- 12) *Lead*: Dissolve 0.1598 g lead nitrate, $\text{Pb}(\text{NO}_3)_2$, in a minimum amount of 1 + 1 HNO_3 , add 10 mL conc HNO_3 , and dilute to 1000 mL with water; 1.00 mL = 100 μg Pb.
- 13) *Lithium*: Dissolve 0.5323 g lithium carbonate, Li_2CO_3 , in a minimum volume of 1 + 1 HNO_3 . Add 10.0 mL conc HNO_3 and dilute to 1000 mL with water; 1.00 mL = 100 μg Li.
- 14) *Magnesium*: Dissolve 0.1658 g MgO in a minimum amount of 1 + 1 HNO_3 . Add 10.0 mL conc HNO_3 and dilute to 1000 mL with water; 1.00 mL = 100 μg Mg.
- 15) *Manganese*: Dissolve 0.1000 g manganese metal in 10 mL conc HCl mixed with 1 mL

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conc HNO_3 . Dilute to 1000 mL with water; 1.00 mL = 100 μg Mn.

16) *Nickel*: Dissolve 0.1000 g nickel metal in 10 mL hot conc HNO_3 , cool, and dilute to 1000 mL with water; 1.00 mL = 100 μg Ni.

17) *Palladium*: Dissolve 0.100 g palladium wire in a minimum volume of aqua regia and evaporate just to dryness. Add 5 mL conc HCl and 25 mL water and warm until dissolution is complete. Dilute to 1000 mL with water; 1.00 mL = 100 μg Pd.

18) *Platinum*: Dissolve 0.100 g platinum metal in a minimum volume of aqua regia and evaporate just to dryness. Add 5 mL conc HCl and 0.1 g NaCl and again evaporate just to dryness. Dissolve residue in 20 mL of 1 + 1 HCl and dilute to 1000 mL with water; 1.00 mL = 100 μg Pt.

19) *Potassium*: Dissolve 0.1907 g potassium chloride, KCl, (dried at 110°C) in water and make up to 1000 mL; 1.00 mL = 100 μg K.

20) *Rhodium*: Dissolve 0.386 g ammonium hexachlororhodate, $(\text{NH}_4)_3\text{RhCl}_6 \cdot 1.5\text{H}_2\text{O}$, in a minimum volume of 10% (v/v) HCl and dilute to 1000 mL with 10% (v/v) HCl; 1.00 mL = 100 μg Rh.

21) *Ruthenium*: Dissolve 0.205 g ruthenium chloride, RuCl_3 , in a minimum volume of 20% (v/v) HCl and dilute to 1000 mL with 20% (v/v) HCl; 1.00 mL = 100 μg Ru.

22) *Silver*: Dissolve 0.1575 g silver nitrate, AgNO_3 , in 100 mL water, add 10 mL conc HNO_3 , and make up to 1000 mL; 1.00 mL = 100 μg Ag.

23) *Sodium*: Dissolve 0.2542 g sodium chloride, NaCl, dried at 140°C, in water, add 10 mL conc HNO_3 and make up to 1000 mL; 1.00 mL = 100 μg Na.

24) *Strontium*: Suspend 0.1685 g SrCO_3 in water and dissolve cautiously with a minimum amount of 1 + 1 HNO_3 . Add 10.0 mL conc HNO_3 and dilute to 1000 mL with water; 1 mL = 100 μg Sr.

25) *Thallium*: Dissolve 0.1303 g thallium nitrate, TlNO_3 , in water. Add 10 mL conc HNO_3 and dilute to 1000 mL with water; 1.00 mL = 100 μg Tl.

27) *Zinc*: Dissolve 0.100 g zinc metal in 20 mL 1 + 1 HCl and dilute to 1000 mL with water; 1.00 mL = 100 μg Zn.

4. Procedure

a. *Sample preparation*: Required sample preparation depends on the metal form being measured.

If dissolved metals are to be determined, see Section 3030B for sample preparation. If total or acid-extractable metals are to be determined, see Section 3030C through K. For all samples, make certain that the concentrations of acid and matrix modifiers are the same in both samples and standards.

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When determining Ca or Mg, dilute and mix 100 mL sample or standard with 10 mL lanthanum solution (§ 3 f) before aspirating. When determining Fe or Mn, mix 100 mL with 25 mL of Ca solution (§ 3d) before aspirating. When determining Cr, mix 1 mL 30% H₂O₂ with each 100 mL before aspirating. Alternatively use proportionally smaller volumes.

b. Instrument operation: Because of differences between makes and models of atomic absorption spectrometers, it is not possible to formulate instructions applicable to every instrument. See manufacturer's operating manual. In general, proceed according to the following: Install a hollow-cathode lamp for the desired metal in the instrument and roughly set the wavelength dial according to Table 3111:I. Set slit width according to manufacturer's suggested setting for the element being measured. Turn on instrument, apply to the hollow-cathode lamp the current suggested by the manufacturer, and let instrument warm up until energy source stabilizes, generally about 10 to 20 min. Readjust current as necessary after warmup. Optimize wavelength by adjusting wavelength dial until optimum energy gain is obtained. Align lamp in accordance with manufacturer's instructions.

Install suitable burner head and adjust burner head position. Turn on air and adjust flow rate to that specified by manufacturer to give maximum sensitivity for the metal being measured. Turn on acetylene, adjust flow rate to value specified, and ignite flame. Let flame stabilize for a few minutes. Aspirate a blank consisting of deionized water containing the same concentration of acid in standards and samples. Zero the instrument. Aspirate a standard solution and adjust aspiration rate of the nebulizer to obtain maximum sensitivity. Adjust burner both vertically and horizontally to obtain maximum response. Aspirate blank again and rezero the instrument. Aspirate a standard near the middle of the linear range. Record absorbance of this standard when freshly prepared and with a new hollow-cathode lamp. Refer to these data on subsequent determinations of the same element to check consistency of instrument setup and aging of hollow-cathode lamp and standard.

The instrument now is ready to operate. When analyses are finished, extinguish flame by turning off first acetylene and then air.

c. Standardization: Select at least three concentrations of each standard metal solution (prepared as in § 3 j above) to bracket the expected metal concentration of a sample. Aspirate blank and zero the instrument. Then aspirate each standard in turn into flame and record absorbance.

Prepare a calibration curve by plotting on linear graph paper absorbance of standards versus their concentrations. For instruments equipped with direct concentration readout, this step is unnecessary. With some instruments it may be necessary to convert percent absorption to absorbance by using a table generally provided by the manufacturer. Plot calibration curves for Ca and Mg based on original concentration of standards before dilution with lanthanum solution. Plot calibration curves for Fe and Mn based on original concentration of standards before dilution with Ca solution. Plot calibration curve for Cr based on original concentration of standard before addition of H₂O₂.

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d. *Analysis of samples:* Rinse nebulizer by aspirating water containing 1.5 mL conc HNO_3 /L. Aspirate blank and zero instrument. Aspirate sample and determine its absorbance.

5. Calculations

Calculate concentration of each metal ion, in micrograms per liter for trace elements, and in milligrams per liter for more common metals, by referring to the appropriate calibration curve prepared according to ¶ 4c. Alternatively, read concentration directly from the instrument readout if the instrument is so equipped. If the sample has been diluted, multiply by the appropriate dilution factor.

6. Bibliography

WILLIS, J.B. 1962. Determination of lead and other heavy metals in urine by atomic absorption spectrophotometry. *Anal. Chem.* 34:614.

Also see Section 3111A.8 and Section 3111A.9.

3111 C. Extraction/Air-Acetylene Flame Method

1. General Discussion

This method is suitable for the determination of low concentrations of cadmium, chromium, cobalt, copper, iron, lead, manganese, nickel, silver, and zinc. The method consists of chelation with ammonium pyrrolidine dithiocarbamate (APDC) and extraction into methyl isobutyl ketone (MIBK), followed by aspiration into an air-acetylene flame.

2. Apparatus

- a. *Atomic absorption spectrometer and associated equipment:* See Section 3111A.6.
- b. *Burner head,* conventional. Consult manufacturer's operating manual for suggested burner head.

3. Reagents

- a. *Air:* See Section 3111B.3a.
- b. *Acetylene:* See Section 3111B.3b.
- c. *Metal-free water:* See Section 3111B.3c.
- d. *Methyl isobutyl ketone (MIBK),* reagent grade. For trace analysis, purify MIBK by redistillation or by sub-boiling distillation.
- e. *Ammonium pyrrolidine dithiocarbamate (APDC) solution:* Dissolve 4 g APDC in 100 mL water. If necessary, purify APDC with an equal volume of MIBK. Shake 30 s in a separatory funnel, let separate, and withdraw lower portion. Discard MIBK layer.
- f. *Nitric acid,* HNO_3 , conc, ultrapure.

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- g. Standard metal solutions:* See Section 3111B.3 j.
- h. Potassium permanganate solution, KMnO_4 , 5% (w/v) aqueous.*
- i. Sodium sulfate, Na_2SO_4 , anhydrous.*
- j. Water-saturated MIBK:* Mix one part purified MIBK with one part water in a separatory funnel. Shake 30 s and let separate. Discard aqueous layer. Save MIBK layer.
- k. Hydroxylamine hydrochloride solution, 10% (w/v).* This solution can be purchased commercially.

4. Procedure

a. Instrument operation: See Section 3111B.4b. After final adjusting of burner position, aspirate water-saturated MIBK into flame and gradually reduce fuel flow until flame is similar to that before aspiration of solvent.

b. Standardization: Select at least three concentrations of standard metal solutions (prepared as in Section 3111B.3 j) to bracket expected sample metal concentration and to be, after extraction, in the optimum concentration range of the instrument. Adjust 100mL of each standard and 100 mL of a metal-free water blank to pH 3 by adding 1N HNO_3 or 1N NaOH. For individual element extraction, use the following pH ranges to obtain optimum extraction efficiency:

Element	pH Range for Optimum Extraction
Ag	2–5 (complex unstable)
Cd	1–6
Co	2–10
Cr	3–9
Cu	0.1–8
Fe	2–5
Mn	2–4 (complex unstable)
Ni	2–4
Pb	0.1–6
Zn	2–6

NOTE: For Ag and Pb extraction the optimum pH value is 2.3 ± 0.2 . The Mn complex deteriorates rapidly at room temperature, resulting in decreased instrument response. Chilling the extract to 0°C may preserve the complex for a few hours. If this is not possible and Mn cannot be analyzed immediately after extraction, use another analytical procedure.

Transfer each standard solution and blank to individual 200-mL volumetric flasks, add 1 mL

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APDC solution, and shake to mix. Add 10 mL MIBK and shake vigorously for 30 s. (The maximum volume ratio of sample to MIBK is 40.) Let contents of each flask separate into aqueous and organic layers, then carefully add water (adjusted to the same pH at which the extraction was carried out) down the side of each flask to bring the organic layer into the neck and accessible to the aspirating tube.

Aspirate organic extracts directly into the flame (zeroing instrument on a water-saturated MIBK blank) and record absorbance.

Prepare a calibration curve by plotting on linear graph paper absorbances of extracted standards against their concentrations before extraction.

c. Analysis of samples: Prepare samples in the same manner as the standards. Rinse atomizer by aspirating water-saturated MIBK. Aspirate organic extracts treated as above directly into the flame and record absorbances.

With the above extraction procedure only hexavalent chromium is measured. To determine total chromium, oxidize trivalent chromium to hexavalent chromium by bringing sample to a boil and adding sufficient KMnO_4 solution dropwise to give a persistent pink color while the solution is boiled for 10 min. Destroy excess KMnO_4 by adding 1 to 2 drops hydroxylamine hydrochloride solution to the boiling solution, allowing 2 min for the reaction to proceed. If pink color persists, add 1 to 2 more drops hydroxylamine hydrochloride solution and wait 2 min. Heat an additional 5 min. Cool, extract with MIBK, and aspirate.

During extraction, if an emulsion forms at the water-MIBK interface, add anhydrous Na_2SO_4 to obtain a homogeneous organic phase. In that case, also add Na_2SO_4 to all standards and blanks.

To avoid problems associated with instability of extracted metal complexes, determine metals immediately after extraction.

5. Calculations

Calculate the concentration of each metal ion in micrograms per liter by referring to the appropriate calibration curve.

6. Bibliography

ALLAN, J.E. 1961. The use of organic solvents in atomic absorption spectrophotometry. *Spectrochim. Acta* 17:467.

SACHDEV, S.L. & P.W. WEST. 1970. Concentration of trace metals by solvent extraction and their determination by atomic absorption spectrophotometry. *Environ. Sci. Technol.* 4:749.

3111 D. Direct Nitrous Oxide-Acetylene Flame Method

1. General Discussion

This method is applicable to the determination of aluminum, barium, beryllium, calcium,

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molybdenum, osmium, rhenium, silicon, thorium, titanium, and vanadium.

2. Apparatus

- a. *Atomic absorption spectrometer and associated equipment:* See Section 3111A.6.
- b. *Nitrous oxide burner head:* Use special burner head as suggested in manufacturer's manual. At roughly 20-min intervals of operation it may be necessary to dislodge the carbon crust that forms along the slit surface with a carbon rod or appropriate alternative.
- c. *T-junction valve* or other switching valve for rapidly changing from nitrous oxide to air, so that flame can be turned on or off with air as oxidant to prevent flashbacks.

3. Reagents

- a. *Air:* See Section 3111B.3a.
- b. *Acetylene:* See Section 3111B.3b.
- c. *Metal-free water:* See Section 3111B.3c.
- d. *Hydrochloric acid*, HCl, 1N, 1+1, and conc.
- e. *Nitric acid*, HNO₃, conc.
- f. *Sulfuric acid*, H₂SO₄, 1% (v/v).
- g. *Hydrofluoric acid*, HF, 1N.
- h. *Nitrous oxide*, commercially available cylinders. Fit nitrous oxide cylinder with a special nonfreezable regulator or wrap a heating coil around an ordinary regulator to prevent flashback at the burner caused by reduction in nitrous oxide flow through a frozen regulator. (Most modern atomic absorption instruments have automatic gas control systems that will shut down a nitrous oxide-acetylene flame safely in the event of a reduction in nitrous oxide flow rate.)

CAUTION: Use nitrous oxide with strict adherence to manufacturer's directions. Improper sequencing of gas flows at startup and shutdown of instrument can produce explosions from flashback.
- i. *Potassium chloride solution:* Dissolve 250 g KCl in water and dilute to 1000 mL.
- j. *Aluminum nitrate solution:* Dissolve 139 g Al(NO₃)₃·9H₂O in 150 mL water. Acidify slightly with conc HNO₃ to preclude possible hydrolysis and precipitation. Warm to dissolve completely. Cool and dilute to 200 mL.
- k. *Standard metal solutions:* Prepare a series of standard metal solutions in the optimum concentration ranges by appropriate dilution of stock metal solutions with water containing 1.5 mL conc HNO₃/L. Stock standard solutions are available from a number of commercial suppliers. Alternatively, prepare as described below.
 - 1) *Aluminum:* Dissolve 0.100 g aluminum metal in an acid mixture of 4 mL 1 + 1 HCl and 1 mL conc HNO₃ in a beaker. Warm gently to effect solution. Transfer to a 1-L flask, add 10 mL 1 + 1 HCl, and dilute to 1000 mL with water; 1.00 mL = 100 µg Al.

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2) *Barium*: Dissolve 0.1516 g BaCl_2 (dried at 250° for 2 h), in about 10 mL water with 1 mL 1+1 HCl. Add 10.0 mL 1+1 HCl and dilute to 1000 mL with water; 1.00 mL = 100 μg Ba.

3) *Beryllium*: *Do not dry*. Dissolve 1.966 g $\text{BeSO}_4 \cdot 4\text{H}_2\text{O}$ in water, add 10.0 mL conc HNO_3 , and dilute to 1000 mL with water; 1.00 mL = 100 μg Be.

4) *Calcium*: See Section 3111B.3 j4).

5) *Molybdenum*: Dissolve 0.2043 g $(\text{NH}_4)_2 \text{MoO}_4$ in water and dilute to 1000 mL; 1.00 mL = 100 μg Mo.

6) *Osmium*: Obtain standard 0.1M osmium tetroxide solution*#(76) and store in glass bottle; 1.00 mL = 19.02 mg Os. Make dilutions daily as needed using 1% (v/v) H_2SO_4 . CAUTION: OsO_4 is extremely toxic and highly volatile.

7) *Rhenium*: Dissolve 0.1554 g potassium perrhenate, KReO_4 , in 200 mL water. Dilute to 1000 mL with 1% (v/v) H_2SO_4 ; 1.00 mL = 100 μg Re.

8) *Silica*: *Do not dry*. Dissolve 0.4730 g $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ in water. Add 10.0 mL conc HNO_3 and dilute to 1000 mL with water. 1.00 mL = 100 μg SiO_2 . Store in polyethylene.

9) *Thorium*: Dissolve 0.238 g thorium nitrate, $\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$ in 1000 mL water; 1.00 mL = 100 μg Th.

10) *Titanium*: Dissolve 0.3960 g pure (99.8 or 99.9%) titanium chloride, TiCl_4 ,†#(77) in a mixture of equal volumes of 1N HCl and 1N HF. Make up to 1000 mL with this acid mixture; 1.00 mL = 100 μg Ti.

11) *Vanadium*: Dissolve 0.2297 g ammonium metavanadate, NH_4VO_3 , in a minimum amount of conc HNO_3 . Heat to dissolve. Add 10 mL conc HNO_3 , and dilute to 1000 mL with water; 1.00 mL = 100 μg V.

4. Procedure

a. *Sample preparation*: See Section 3111B.4a.

When determining Al, Ba, or Ti, mix 2 mL KCl solution into 100 mL sample or standard before aspiration. When determining Mo and V, mix 2 mL $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ into 100 mL sample or standard before aspiration.

b. *Instrument operation*: See Section 3111B.4b. After adjusting wavelength, install a nitrous oxide burner head. Turn on acetylene (without igniting flame) and adjust flow rate to value specified by manufacturer for a nitrous oxide-acetylene flame. Turn off acetylene. With both air and nitrous oxide supplies turned on, set T-junction valve to nitrous oxide and adjust flow rate according to manufacturer's specifications. Turn switching valve to the air position and verify that flow rate is the same. Turn acetylene on and ignite to a bright yellow flame. With a rapid motion, turn switching valve to nitrous oxide. The flame should have a red cone above the

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burner. If it does not, adjust fuel flow to obtain red cone. After nitrous oxide flame has been ignited, let burner come to thermal equilibrium before beginning analysis.

Aspirate a blank consisting of deionized water containing 1.5 mL conc HNO_3/L and check aspiration rate. Adjust if necessary to a rate between 3 and 5 mL/min. Zero the instrument. Aspirate a standard of the desired metal with a concentration near the midpoint of the optimum concentration range and adjust burner (both horizontally and vertically) in the light path to obtain maximum response. Aspirate blank again and re-zero the instrument. The instrument now is ready to run standards and samples.

To extinguish flame, turn switching valve from nitrous oxide to air and turn off acetylene. This procedure eliminates the danger of flashback that may occur on direct ignition or shutdown of nitrous oxide and acetylene. (See also discussion in Section 3111B.4b.)

c. *Standardization:* Select at least three concentrations of standard metal solutions (prepared as in ¶ 3k) to bracket the expected metal concentration of a sample. Aspirate each in turn into the flame and record absorbances.

Most modern instruments are equipped with microprocessors and digital readout which permit calibration in direct concentration terms. If instrument is not so equipped, prepare a calibration curve by plotting on linear graph paper absorbance of standards versus concentration. Plot calibration curves for Al, Ba, and Ti based on original concentration of standard before adding KCl solution. Plot calibration curves for Mo and V based on original concentration of standard before adding $\text{Al}(\text{NO}_3)_3$ solution.

d. *Analysis of samples:* Rinse atomizer by aspirating water containing 1.5 mL conc HNO_3/L and zero instrument. Aspirate a sample and determine its absorbance.

5. Calculations

Calculate concentration of each metal ion in micrograms per liter by referring to the appropriate calibration curve prepared according to ¶ 4c.

Alternatively, read the concentration directly from the instrument readout if the instrument is so equipped. If sample has been diluted, multiply by the appropriate dilution factor.

6. Bibliography

WILLIS, J.B. 1965. Nitrous oxide-acetylene flame in atomic absorption spectroscopy. *Nature* 207:715.

Also see Section 3111A.8 and Section 3111A.9.

3111 E. Extraction/Nitrous Oxide-Acetylene Flame Method

1. General Discussion

a. *Application:* This method is suitable for the determination of aluminum at concentrations

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less than 900 µg/L and beryllium at concentrations less than 30 µg/L. The method consists of chelation with 8-hydroxyquinoline, extraction with methyl isobutyl ketone (MIBK), and aspiration into a nitrous oxide-acetylene flame.

b. Interferences: Concentrations of Fe greater than 10 mg/L interfere by suppressing Al absorption. Iron interference can be masked by addition of hydroxylamine hydrochloride/1,10-phenanthroline. Mn concentrations up to 80 mg/L do not interfere if turbidity in the extract is allowed to settle. Mg forms an insoluble chelate with 8-hydroxyquinoline at pH 8.0 and tends to remove Al complex as a coprecipitate. However, the Mg complex forms slowly over 4 to 6 min; its interference can be avoided if the solution is extracted immediately after adding buffer.

2. Apparatus

Atomic absorption spectrometer and associated equipment: See Section 3111A.6.

3. Reagents

- a. Air:* See Section 3111B.3a.
- b. Acetylene:* See Section 3111B.3b.
- c. Ammonium hydroxide, NH₄OH, conc.*
- d. Buffer:* Dissolve 300 g ammonium acetate, NH₄C₂H₃O₂, in water, add 105 mL conc NH₄OH, and dilute to 1 L.
- e. Metal-free water:* See Section 3111B.3c.
- f. Hydrochloric acid, HCl, conc.*
- g. 8-Hydroxyquinoline solution:* Dissolve 20 g 8-hydroxyquinoline in about 200 mL water, add 60 mL glacial acetic acid, and dilute to 1 L with water.
- h. Methyl isobutyl ketone:* See Section 3111C.3d.
- i. Nitric acid, HNO₃, conc.*
- j. Nitrous oxide:* See Section 3111D.3h.
- k. Standard metal solutions:* Prepare a series of standard metal solutions containing 5 to 1000 µg/L by appropriate dilution of the stock metal solutions prepared according to Section 3111D.3k.
- l. Iron masking solution:* Dissolve 1.3 g hydroxylamine hydrochloride and 6.58 g 1,10-phenanthroline monohydrate in about 500 mL water and dilute to 1 L with water.

4. Procedure

a. Instrument operation: See Section 3111B.4b, Section 3111C.4a, and Section 3111D.4b. After final adjusting of burner position, aspirate MIBK into flame and gradually reduce fuel flow until flame is similar to that before aspiration of solvent. Adjust wavelength setting according to Table 3111:I.

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b. Standardization: Select at least three concentrations of standard metal solutions (prepared as in ¶ 3k) to bracket the expected metal concentration of a sample and transfer 100 mL of each (and 100 mL water blank) to four different 200-mL volumetric flasks. Add 2 mL 8-hydroxyquinoline solution, 2 mL masking solution (if required), and 10 mL buffer to one flask, immediately add 10 mL MIBK, and shake vigorously. The duration of shaking affects the forms of aluminum complexed. A fast, 10-s shaking time favors monomeric Al, whereas 5 to 10 min of shaking also will complex polymeric species. Adjustment of the 8-hydroxyquinoline to sample ratio can improve recoveries of extremely high or low concentrations of aluminum. Treat each blank, standard, and sample in similar fashion. Continue as in Section 3111C.4b.

c. Analysis of samples: Rinse atomizer by aspirating water-saturated MIBK. Aspirate extracts of samples treated as above, and record absorbances.

5. Calculations

Calculate concentration of each metal in micrograms per liter by referring to the appropriate calibration curve prepared according to ¶ 4b.

3112 METALS BY COLD-VAPOR ATOMIC ABSORPTION SPECTROMETRY*#(78)

3112 A. Introduction

For general introductory material on atomic absorption spectrometric methods, see Section 3111A.

3112 B. Cold-Vapor Atomic Absorption Spectrometric Method

1. General Discussion

This method is applicable to the determination of mercury.

2. Apparatus

When possible, dedicate glassware for use in Hg analysis. Avoid using glassware previously exposed to high levels of Hg, such as those used in COD, TKN, or Cl⁻ analysis.

a. Atomic absorption spectrometer and associated equipment: See Section 3111A.6. Instruments and accessories specifically designed for measurement of mercury by the cold vapor technique are available commercially and may be substituted.

b. Absorption cell, a glass or plastic tube approximately 2.5 cm in diameter. An 11.4-cm-long tube has been found satisfactory but a 15-cm-long tube is preferred. Grind tube ends perpendicular to the longitudinal axis and cement quartz windows in place. Attach gas inlet and

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outlet ports (6.4 mm diam) 1.3 cm from each end.

c. Cell support: Strap cell to the flat nitrous-oxide burner head or other suitable support and align in light beam to give maximum transmittance.

d. Air pumps: Use any peristaltic pump with electronic speed control capable of delivering 2 L air/min. Any other regulated compressed air system or air cylinder also is satisfactory.

e. Flowmeter, capable of measuring an air flow of 2 L/min.

f. Aeration tubing, a straight glass frit having a coarse porosity for use in reaction flask.

g. Reaction flask, 250-mL erlenmeyer flask or a BOD bottle, fitted with a rubber stopper to hold aeration tube.

h. Drying tube, 150-mm × 18-mm-diam, containing 20 g $\text{Mg}(\text{ClO}_4)_2$. A 60-W light bulb with a suitable shade may be substituted to prevent condensation of moisture inside the absorption cell. Position bulb to maintain cell temperature at 10°C above ambient.

i. Connecting tubing, glass tubing to pass mercury vapor from reaction flask to absorption cell and to interconnect all other components. Clear vinyl plastic*#(79) tubing may be substituted for glass.

3. Reagents†#(80)

a. Metal-free water: See Section 3111B.3c.

b. Stock mercury solution: Dissolve 0.1354 g mercuric chloride, HgCl_2 , in about 70 mL water, add 1 mL conc HNO_3 , and dilute to 100 mL with water; 1.00 mL = 1.00 mg Hg.

c. Standard mercury solutions: Prepare a series of standard mercury solutions containing 0 to 5 µg/L by appropriate dilution of stock mercury solution with water containing 10 mL conc HNO_3 /L. Prepare standards daily.

d. Nitric acid, HNO_3 , conc.

e. Potassium permanganate solution: Dissolve 50 g KMnO_4 in water and dilute to 1 L.

f. Potassium persulfate solution: Dissolve 50 g $\text{K}_2\text{S}_2\text{O}_8$ in water and dilute to 1 L.

g. Sodium chloride-hydroxylamine sulfate solution: Dissolve 120 g NaCl and 120 g $(\text{NH}_2\text{OH})_2\cdot\text{H}_2\text{SO}_4$ in water and dilute to 1 L. A 10% hydroxylamine hydrochloride solution may be substituted for the hydroxylamine sulfate.

h. Stannous ion (Sn^{2+}) solution: Use either stannous chloride, ¶ 1), or stannous sulfate, ¶ 2), to prepare this solution containing about 7.0 g Sn^{2+} /100 mL.

1) Dissolve 10 g SnCl_2 in water containing 20 mL conc HCl and dilute to 100 mL.

2) Dissolve 11 g SnSO_4 in water containing 7 mL conc H_2SO_4 and dilute to 100 mL.

Both solutions decompose with aging. If a suspension forms, stir reagent continuously during use. Reagent volume is sufficient to process about 20 samples; adjust volumes prepared to

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accommodate number of samples processed.

i. *Sulfuric acid*, H_2SO_4 , conc.

4. Procedure

a. *Instrument operation*: See Section 3111B.4b. Set wavelength to 253.7 nm. Install absorption cell and align in light path to give maximum transmission. Connect associated equipment to absorption cell with glass or vinyl plastic tubing as indicated in Figure 3112:1. Turn on air and adjust flow rate to 2 L/min. Allow air to flow continuously. Alternatively, follow manufacturer's directions for operation. NOTE: Fluorescent lighting may increase baseline noise.

b. *Standardization*: Transfer 100 mL of each of the 1.0, 2.0, and 5.0 $\mu\text{g/L}$ Hg standard solutions and a blank of 100 mL water to 250-mL erlenmeyer reaction flasks. Add 5 mL conc H_2SO_4 and 2.5 mL conc HNO_3 to each flask. Add 15 mL KMnO_4 solution to each flask and let stand at least 15 min. Add 8 mL $\text{K}_2\text{S}_2\text{O}_8$ solution to each flask and heat for 2 h in a water bath at 95°C. Cool to room temperature.

Treating each flask individually, add enough NaCl-hydroxylamine solution to reduce excess KMnO_4 , then add 5 mL SnCl_2 or SnSO_4 solution and immediately attach flask to aeration apparatus. As Hg is volatilized and carried into the absorption cell, absorbance will increase to a maximum within a few seconds. As soon as recorder returns approximately to the base line, remove stopper holding the frit from reaction flask, and replace with a flask containing water. Flush system for a few seconds and run the next standard in the same manner. Construct a standard curve by plotting peak height versus micrograms Hg.

c. *Analysis of samples*: Transfer 100 mL sample or portion diluted to 100 mL containing not more than 5.0 $\mu\text{g Hg/L}$ to a reaction flask. Treat as in ¶ 4b. Seawaters, brines, and effluents high in chlorides require as much as an additional 25 mL KMnO_4 solution. During oxidation step, chlorides are converted to free chlorine, which absorbs at 253 nm. Remove all free chlorine before the Hg is reduced and swept into the cell by using an excess (25 mL) of hydroxylamine reagent.

Remove free chlorine by sparging sample gently with air or nitrogen after adding hydroxylamine reducing solution. Use a separate tube and frit to avoid carryover of residual stannous chloride, which could cause reduction and loss of mercury.

5. Calculation

Determine peak height of sample from recorder chart and read mercury value from standard curve prepared according to ¶ 4b.

6. Precision and Bias

Data on interlaboratory precision and bias for this method are given in Table 3112:I.

7. Reference

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3113 METALS BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY*#(81)

3113 A. Introduction

1. Applications

Electrothermal atomic absorption permits determination of most metallic elements with sensitivities and detection limits from 20 to 1000 times better than those of conventional flame techniques without extraction or sample concentration. This increase in sensitivity results from an increase in atom density within the furnace as compared to flame atomic absorption. Many

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elements can be determined at concentrations as low as 1.0 µg/L. An additional advantage of electrothermal atomic absorption is that only a very small volume of sample is required.

The electrothermal technique is used only at concentration levels below the optimum range of direct flame atomic absorption because it is subject to more interferences than the flame procedure and requires increased analysis time. The method of standard additions may be required to insure validity of data. Because of the high sensitivity of this technique, it is extremely susceptible to contamination; extra care in sample handling and analysis may be required.

2. Principle

Electrothermal atomic absorption spectroscopy is based on the same principle as direct flame atomization but an electrically heated atomizer or graphite furnace replaces the standard burner head. A discrete sample volume is dispensed into the graphite sample tube (or cup). Typically, determinations are made by heating the sample in three or more stages. First, a low current heats the tube to dry the sample. The second, or charring, stage destroys organic matter and volatilizes other matrix components at an intermediate temperature. Finally, a high current heats the tube to incandescence and, in an inert atmosphere, atomizes the element being determined. Additional stages frequently are added to aid in drying and charring, and to clean and cool the tube between samples. The resultant ground-state atomic vapor absorbs monochromatic radiation from the source. A photoelectric detector measures the intensity of transmitted radiation. The inverse of the transmittance is related logarithmically to the absorbance, which is directly proportional to the number density of vaporized ground-state atoms (the Beer-Lambert law) over a limited concentration range.

3. Interferences

Electrothermal atomization determinations may be subject to significant interferences from molecular absorption as well as chemical and matrix effects. Molecular absorption may occur when components of the sample matrix volatilize during atomization, resulting in broadband absorption. Several background correction techniques are available commercially to compensate for this interference. A continuum source such as a deuterium arc can correct for background up to absorbance levels of about 0.8. Continuum lamp intensity diminishes at long wavelengths and use of continuum background correction is limited to analytical wavelengths below 350 nm. Zeeman effect background correctors can handle background absorbance up to 1.5 to 2.0. The Smith-Hieftje correction technique can accommodate background absorbance levels as large as 2.5 to 3.0 (see Section 3111A.3). Both Zeeman and Smith-Hieftje background corrections are susceptible to rollover (development of a negative absorbance-concentration relationship) at high absorbances. The rollover absorbance for each element should be available in the manufacturer's literature. Curvature due to rollover should become apparent during calibration; dilution produces a more linear calibration plot. Use background correction when analyzing samples containing high concentrations of acid or dissolved solids and in determining elements for which an absorption line below 350 nm is used.

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Matrix modification can be useful in minimizing interference and increasing analytical sensitivity. Determine need for a modifier by evaluating recovery of a sample with a known addition. Recovery near 100% indicates that sample matrix does not affect analysis. Chemical modifiers generally modify relative volatilities of matrix and metal. Some modifiers enhance matrix removal, isolating the metal, while other modifiers inhibit metal volatilization, allowing use of higher ashing/charring temperatures and increasing efficiency of matrix removal. Chemical modifiers are added at high concentration (percent level) and can lead to sample contamination from impurities in the modifier solution. Heavy use of chemical modifiers may reduce the useful life (normally 50 to 100 firings) of the graphite tube. Some specific chemical modifiers and approximate concentrations are listed in Table 3113:I.

Addition of a chemical modifier directly to the sample before analysis is restricted to inexpensive additives (e.g. phosphoric acid). Use of palladium salts for matrix modification normally requires methods of co-addition, in which sample and modifier are added consecutively to the furnace either manually or, preferably, with an automatic sampler. Palladium salts (nitrate is preferred, chloride is acceptable) are listed in Table 3113:I as a modifier for many metals. The palladium solution (50 to 2000 mg/L) generally includes citric or ascorbic acid, which aids reduction of palladium in the furnace. Citric acid levels of 1 to 2% are typical. Use of hydrogen (5%) in the coolant gas (available commercially as a mixture) also reduces palladium, eliminating need for organic reducing acids. *CAUTION: Do not mix hydrogen and other gases in the laboratory; hydrogen gas is very flammable—handle with caution.* Use low levels of palladium (50 to 250 mg/L) for normal samples and higher levels for complex samples. Addition of excess palladium modifier may widen atomization peaks; in such cases peak area measurements may provide higher quality results. The recommended mode of modifier use is through co-addition to the furnace of about 10 μ L of the palladium (or other) modifier solution. Palladium may not be the best modifier in all cases and cannot be recommended unconditionally. Test samples requiring a modifier first with palladium; test other modifiers only if palladium is unsuccessful or to minimize modifier cost. See Section 3113B.3 for preparation of modifier solution.

Temperature ramping, i.e., gradual heating, can be used to decrease background interferences and permits analysis of samples with complex matrices. Ramping permits a controlled, continuous increase of furnace temperature in any of the various steps of the temperature sequence. Ramp drying is used for samples containing mixtures of solvents or for samples with a high salt content (to avoid spattering). If spattering is suspected, develop drying ramp by visual inspection of the drying stage, using a mirror. Samples that contain a complex mixture of matrix components sometimes require ramp charring to effect controlled, complete thermal decomposition. Ramp atomization may minimize background absorption by permitting volatilization of the element being determined before the matrix. This is especially applicable in the determination of such volatile elements as cadmium and lead. Use of time-resolved absorbance profiles (available on most modern instruments) greatly aids method development. Changes in atomization, notably the element peak appearance time and magnitude of background

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and metal absorbances, can be monitored directly.

Improve analysis by using a graphite platform, inserted into the graphite tube, as the atomization site. The platform is not heated as directly by the current flowing through the graphite tube; thus the metal atomizes later and under more uniform conditions.

Use standard additions to compensate for matrix interferences. When making standard additions, determine whether the added metal and that in the sample behave similarly under the specified conditions. [See Section 3113B.4d2)]. In the extreme, test every sample for recovery (85 to 115% recovery desired) to determine if standard addition is needed. Test every sample type for recovery. Recovery of only 40 to 85% generally indicates that standard addition is required. Often, as long as the samples are from sources of consistent properties, a representative recovery can be used to characterize the analysis and determine the necessity of standard addition. Test samples of unknown origin or of complex composition (digestates, for example) individually for metal recovery. Ideally, chemical modifiers and graphite platforms render the sample fit to be analyzed using a standard analytical calibration curve. Always verify this assumption; however, a properly developed method with judicious use of chemical modifiers should eliminate the necessity for standard addition in all but the most extreme samples.

Chemical interaction of the graphite tube with various elements to form refractory carbides occurs at high charring and atomization temperatures. Elements that form carbides are barium, molybdenum, nickel, titanium, vanadium, and silicon. Carbide formation is characterized by broad, tailing atomization peaks and reduced sensitivity. Using pyrolytically coated tubes for these metals minimizes the problem.

4. Sensitivity, Detection Limits, and Optimum Concentration Range

Estimated detection limits and optimum concentration ranges are listed in Table 3113:II. These values may vary with the chemical form of the element being determined, sample composition, or instrumental conditions.

For a given sample, increased sensitivity may be achieved by using a larger sample volume or by reducing flow rate of the purge gas or by using gas interrupt during atomization. Note, however, that these techniques also will increase the effects of any interferences present. Sensitivity can be decreased by diluting the sample, reducing sample volume, increasing purge-gas flow, or using a less sensitive wavelength. Use of argon, rather than nitrogen, as the purge gas generally improves sensitivity and reproducibility. Hydrogen mixed with the inert gas may suppress chemical interference and increase sensitivity by acting as a reducing agent, thereby aiding in producing more ground-state atoms. Pyrolytically coated graphite tubes can increase sensitivity for the more refractory elements and are recommended. The optical pyrometer/maximum power accessory available on some instruments also offers increased sensitivity with lower atomization temperatures for many elements.

Using the Stabilized Temperature Platform Furnace (STPF) technique, which is a combination of individual techniques, also offers significant interference reduction with improved sensitivity. Sensitivity changes with sample tube age. Discard graphite tubes when

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significant variations in sensitivity or poor reproducibility are observed. The use of high acid concentrations, brine samples, and matrix modifiers often drastically reduces tube life. Preferably use the graphite platform in such situations.

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3113 B. Electrothermal Atomic Absorption Spectrometric Method

1. General Discussion

This method is suitable for determination of micro quantities of aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, iron, lead, manganese, molybdenum, nickel, selenium, silver, and tin. It is also applicable to analysis of bismuth, gallium, germanium, gold, indium, mercury, tellurium, thallium, and vanadium, but precision and accuracy data are not yet available.

2. Apparatus

a. Atomic absorption spectrometer: See Section 3111A.6a. The instrument must have background correction capability.

b. Source lamps: See Section 3111A.6d.

c. Graphite furnace: Use an electrically heated device with electronic control circuitry designed to carry a graphite tube or cup through a heating program that provides sufficient thermal energy to atomize the elements of interest. Furnace heat controllers with only three heating steps are adequate only for fresh waters with low dissolved solids content. For salt waters, brines, and other complex matrices, use a furnace controller with up to seven individually programmed heating steps. Fit the furnace into the sample compartment of the spectrometer in place of the conventional burner assembly. Use argon as a purge gas to minimize oxidation of the furnace tube and to prevent the formation of metallic oxides. Use graphite tubes with platforms to minimize interferences and to improve sensitivity.

d. Readout: See Section 3111A.6c.

e. Sample dispensers: Use microliter pipets (5 to 100 μL) or an automatic sampling device designed for the specific instrument.

f. Vent: See Section 3111A.6f.

g. Cooling water supply: Cool with tap water flowing at 1 to 4 L/min or use a recirculating cooling device.

h. Membrane filter apparatus: Use an all-glass filtering device and 0.45- μm or smaller-pore-diameter membrane filters. For trace analysis of aluminum, use polypropylene or TFE devices.

3. Reagents

a. Metal-free water: See Section 3111B.3c.

b. Hydrochloric acid, HCl, 1 + 1 and conc.

c. Nitric acid, HNO₃, 1 + 1 and conc.

d. Matrix modifier stock solutions:

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1) *Magnesium nitrate*, 10 000 mg Mg/L: Dissolve 10.5 g $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ in water. Dilute to 100 mL.

2) *Nickel nitrate*, 10 000 mg Ni/L: Dissolve 4.96 g $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ in water. Dilute to 100 mL.

3) *Phosphoric acid*, 10% (v/v): Add 10 mL conc H_3PO_4 to water. Dilute to 100 mL.

4) *Palladium nitrate*, 4000 mg Pd/L: Dissolve 8.89 g $\text{Pd}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$ in water. Dilute to 1 L.

5) *Citric acid*, 4%: Dissolve 40 g citric acid in water. Dilute to 1 L.

NOTE: All of the modifier solutions recommended in Table 3113:I can be prepared with volumetric combination of the above solutions and water. For preparation of other matrix modifiers, see references or follow manufacturers' instructions.

e. *Stock metal solutions*: Refer to Section 3111B and Section 3114.

f. *Chelating resin*: 100 to 200 mesh#(82)* purified by heating at 60°C in 10N NaOH for 24 h. Cool resin and rinse 10 times each with alternating portions of 1N HCl, metal-free water, 1N NaOH, and metal-free water.

g. *Metal-free seawater (or brine)*: Fill a 1.4-cm-ID $\times$ 20-cm-long borosilicate glass column to within 2 cm of the top with purified chelating resin. Elute resin with successive 50-mL portions of 1N HCl, metal-free water, 1N NaOH, and metal-free water at the rate of 5 mL/min just before use. Pass salt water or brine through the column at a rate of 5 mL/min to extract trace metals present. Discard the first 10 bed volumes (300 mL) of eluate.

4. Procedures

a. *Sample pretreatment*: Before analysis, pretreat all samples as indicated below. Rinse all glassware with 1 + 1 HNO_3 and water. Carry out digestion procedures in a clean, dust-free laboratory area to avoid sample contamination. For digestion of trace aluminum, use polypropylene or TFE utensils to avoid leachable aluminum from glassware.

1) Dissolved metals—See Section 3030B. For samples requiring arsenic and/or selenium analysis add 3 mL 30% hydrogen peroxide/100 mL sample and an appropriate volume of nickel nitrate solution (see Table 3113:I) before analysis. For all other metals no further pretreatment is required except for adding an optional matrix modifier.

2) Total recoverable metals (Al, Sb, Ba, Be, Cd, Cr, Co, Cu, Fe, Pb, Mn, Mo, Ni, Ag, and Sn)—NOTE: Sb and Sn are not recovered unless HCl is used in the digestion. See Section 3030D. Quantitatively transfer digested sample to a 100-mL volumetric flask, add an appropriate amount of matrix modifier (see Table 3113:I), and dilute to volume with water.

3) Total recoverable metals (As, Se)—Transfer 100 mL of shaken sample, 1 mL conc HNO_3 , and 2 mL 30% H_2O_2 to a clean, acid-washed 250-mL beaker. Heat on a hot plate without allowing solution to boil until volume has been reduced to about 50 mL. Remove from hot plate and let cool to room temperature. Add an appropriate concentration of nickel (see Table 3113:I),

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and dilute to volume in a 100-mL volumetric flask with water. Substitution of palladium is uneconomical. Nickel may be deleted if palladium is co-added during analysis. Simultaneously prepare a digested blank by substituting water for sample and proceed with digestion as described above.

b. Instrument operation: Mount and align furnace device according to manufacturer's instructions. Turn on instrument and data collection system. Select appropriate light source and adjust to recommended electrical setting. Select proper wavelength and set all conditions according to manufacturer's instructions, including background correction. Background correction is important when elements are determined at short wavelengths or when sample has a high level of dissolved solids. Background correction normally is not necessary at wavelengths longer than 350 nm. If background correction above 350 nm is needed deuterium arc background correction is not useful and other types must be used.

Select proper inert- or sheath-gas flow. In some cases, it is desirable to interrupt the inert-gas flow during atomization. Such interruption results in increased sensitivity by increasing residence time of the atomic vapor in the optical path. Gas interruption also increases background absorption and intensifies interference effects, but modern background correction methods usually eliminate these problems. Consider advantages and disadvantages of this option for each matrix when optimizing analytical conditions.

To optimize graphite furnace conditions, carefully adjust furnace temperature settings to maximize sensitivity and precision and to minimize interferences. Follow manufacturer's instructions.

Use drying temperatures slightly above the solvent boiling point and provide enough time and temperature for complete evaporation without boiling or spattering.

Select atomization temperature by determining the lowest temperature providing maximum sensitivity without significantly eroding precision. Optimize by a series of successive determinations at various atomization temperatures using a standard solution giving an absorbance of 0.2 to 0.5.

The charring temperature must be high enough to maximize volatilization of interfering matrix components yet too low to volatilize the element of interest. With the drying and atomization temperatures set to their optimum values, analyze a standard solution at a series of charring temperatures in increasing increments of 50 to 100°C. When the optimum charring temperature is exceeded, there will be a significant drop in sensitivity. Plot charring temperature versus sample absorbance: the optimum charring temperature is the highest temperature without reduced sensitivity. Verify optimization with major changes in sample matrix.

c. Instrument calibration: Prepare standard solutions for instrument calibration by diluting metal stock solutions. Prepare standard solutions fresh daily.

Prepare a blank and at least three calibration standards in the appropriate concentration range (see Table 3113:II) for correlating element concentration and instrument response. Match the matrix of the standard solutions to those of the samples as closely as possible. In most cases, this simply requires matching the acid background of the samples. For seawaters or brines,

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however, use the metal-free matrix (¶ 3g) as the standard solution diluent. In addition, add the same concentration of matrix modifier (if required for sample analysis) to the standard solutions.

Inject a suitable portion of each standard solution, in order of increasing concentration. Analyze each standard solution in triplicate to verify method precision.

Construct an analytical curve by plotting the average peak absorbances or peak areas of the standard solution versus concentration on linear graph paper. Alternatively, use electronic instrument calibration if the instrument has this capability.

d. Sample analysis: Analyze all samples except those demonstrated to be free of matrix interferences (based on recoveries of 85% to 115% for known additions) using the method of standard additions. Analyze all samples at least in duplicate or until reproducible results are obtained. A variation of $\leq 10\%$ is considered acceptable reproducibility. Average replicate values.

1) Direct determination—Inject a measured portion of pretreated sample into the graphite furnace. Use the same volume as was used to prepare the calibration curve. Usually add modifier immediately after the sample, preferably using an automatic sampler or a micropipet. Some methods require modifier to be injected before the sample. Use the same volume and concentration of modifier for all standards and samples. Dry, char, and atomize according to the preset program. Repeat until reproducible results are obtained.

Compare the average absorbance value or peak area to the calibration curve to determine concentration of the element of interest. Alternatively, read results directly if the instrument is equipped with this capability. If absorbance (or concentration) or peak area of the sample is greater than absorbance (concentration) or peak area of the most concentrated standard solution, dilute sample and reanalyze. If very large dilutions are required, another technique (e.g., flame AA or ICP) may be more suitable for this sample. Large dilution factors magnify small errors on final calculation. Keep acid background and concentration of matrix modifier (if present in the solutions) constant. Dilute the sample in a blank solution of acid and matrix modifiers.

Proceed to ¶ 5a below.

2) Method of standard additions—Refer to ¶ 4c above. The method of standard additions is valid only when it falls in the linear portion of the calibration curve. Once instrument sensitivity has been optimized for the element of interest and the linear range for the element has been established, proceed with sample analyses.

Inject a measured volume of sample into furnace device. Dry, char or ash, and atomize samples according to preset program. Repeat until reproducible results are obtained. Record instrument response in absorbance or concentration as appropriate. Add a known concentration of the element of interest to a separate portion of sample so as not to change significantly the sample volume. Repeat the determination.

Add a known concentration (preferably twice that used in the first addition) to a separate sample portion. Mix well and repeat the determination.

Using linear graph paper, plot average absorbance or instrument response for the sample and

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the additions on the vertical axis against the concentrations of the added element on the horizontal axis, using zero as the concentration for the sample. Draw a straight line connecting the three points and extrapolate to zero absorbance. The intercept at the horizontal axis is the negative of the element concentration in the sample. The concentration axis to the left of the origin should be a mirror image of the axis to the right.

5. Calculations

a. Direct determination:

$$\mu\text{g metal/L} = C \times F$$

where:

C = metal concentration as read directly from the instrument or from the calibration curve, $\mu\text{g/L}$, and

F = dilution factor.

b. Method of additions:

$$\mu\text{g metal/L} = C \times F$$

where:

C = metal concentration as read from the method of additions plot, $\mu\text{g/L}$, and

F = dilution factor.

6. Precision and Bias

Data typical of the precision and bias obtainable are presented in Table 3113:III, Table 3113:IV, and Table 3113:V.

7. Quality Control

See Section 3020 for specific quality control procedures to be followed during analysis. Although previous indications were that very low optimum concentration ranges were attainable for most metals (see Table 3113:II), data in Table 3113:III using variations of these protocols show that this may not be so. Exercise extreme care when applying this method to the lower concentration ranges. Verify analyst precision at the beginning of each analytical run by making triplicate analyses. Verify autosampler precision by checking volumes (by weight) delivered by the autosampler at routinely used injection volume settings.

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3114 ARSENIC AND SELENIUM BY HYDRIDE GENERATION/ATOMIC ABSORPTION SPECTROMETRY*#(83)

3114 A. Introduction

For general introductory material on atomic absorption spectrometric methods, see Section 3111A.

Two methods are presented in this section: A manual method and a continuous-flow method

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especially recommended for selenium. Continuous-flow automated systems are preferable to manual hydride generators because the effect of sudden hydrogen generation on light-path transparency is removed and any blank response from contamination of the HCl reagent by the elements being determined is incorporated into the background base line.

3114 B. Manual Hydride Generation/Atomic Absorption Spectrometric Method

1. General Discussion

a. Principle: This method is applicable to the determination of arsenic and selenium by conversion to their hydrides by sodium borohydride reagent and transport into an atomic absorption atomizer.

Arsenous acid and selenous acid, the As(III) and Se(IV) oxidation states of arsenic and selenium, respectively, are instantaneously converted by sodium borohydride reagent in acid solution to their volatile hydrides. The hydrides are purged continuously by argon or nitrogen into a quartz cell heated electrically or by the flame of an atomic absorption spectrometer and converted to the gas-phase atoms. The sodium borohydride reducing agent, by rapid generation of the elemental hydrides in an appropriate reaction cell, minimizes dilution of the hydrides by the carrier gas and provides rapid, sensitive determinations of arsenic and selenium.

CAUTION: Arsenic and selenium and their hydrides are toxic. Handle with care.

At room temperature and solution pH values of 1 or less, arsenic acid, the As(V) oxidation state of arsenic, is reduced relatively slowly by sodium borohydride to As(III), which is then instantaneously converted to arsine. The arsine atomic absorption peaks commonly are decreased by one-fourth to one-third for As(V) when compared to As(III). Determination of total arsenic requires that all inorganic arsenic compounds be in the As(III) state. Organic and inorganic forms of arsenic are first oxidized to As(V) by acid digestion. The As(V) then is quantitatively reduced to As(III) with sodium or potassium iodide before reaction with sodium borohydride.

Selenic acid, the Se(VI) oxidation state of selenium, is not measurably reduced by sodium borohydride. To determine total selenium by atomic absorption and sodium borohydride, first reduce Se(VI) formed during the acid digestion procedure to Se(IV), being careful to prevent reoxidation by chlorine. Efficiency of reduction depends on temperature, reduction time, and HCl concentration. For 4N HCl, heat 1 h at 100°C. For 6N HCl, boiling for 10 min is sufficient.¹⁻³ Alternatively, autoclave samples in sealed containers at 121°C for 1 h. NOTE: Autoclaving in sealed containers may result in incomplete reduction, apparently due to the buildup of chlorine gas. To obtain equal instrument responses for reduced Se(VI) and Se(IV) solutions of equal concentrations, manipulate HCl concentration and heating time. For further details, see Section 3500-Se.

b. Equipment selection:

Certain atomic absorption atomizers and hydride reaction cells are available commercially

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for use with the sodium borohydride reagent. A functional manual system that can be constructed in the laboratory is presented in Figure 3114:1. Irrespective of the hydride reaction cell-atomizer system selected, it must meet the following quality-control considerations: (a) it must provide a precise and reproducible standard curve between 0 and 20 µg As or Se/L and an instrumental detection limit between 0.1 and 0.5 µg As or Se/L; (b) when carried through the entire procedure, oxidation state couples [As (III) - As (V) or Se (IV) - Se (VI)] must cause equal instrument response; and (c) sample digestion must yield 80% or greater recovery of added cacodylic acid (dimethyl arsinic acid) and 90% or greater recovery of added As(III), As(V), Se(VI), or Se(IV).

Quartz atomization cells provide for the most sensitive arsenic and selenium hydride determinations. The quartz cell can be heated electrically or by an air-acetylene flame in an atomic absorption unit.

c. Digestion techniques: Waters and wastewaters may contain varying amounts of organic arsenic compounds and inorganic compounds of As(III), As(V), Se(IV), and Se(VI). To measure total arsenic and selenium in these samples requires sample digestion to solubilize particulate forms and oxidize reduced forms of arsenic and selenium and to convert any organic compounds to inorganic ones. Organic selenium compounds rarely have been demonstrated in water. It is left to the experienced analyst's judgment whether sample digestion is required.

Various alternative digestion procedures are provided in ¶s 4c and 4d below. Consider sulfuric-nitric-perchloric acid digestion (¶ 4c) or sulfuric-nitric acid digestion (Section 3030F) as providing a measure of total recoverable arsenic rather than total arsenic because they do not completely convert certain organic arsenic compounds to As(V). The sulfuric-nitric-perchloric acid digestion effectively destroys organics and most particulates in untreated wastewaters or solid samples, but does not convert all organic arsenicals to As(V). The potassium persulfate digestion (¶ 4d) is effective for converting organic arsenic and selenium compounds to As(V) and Se(VI) in potable and surface waters and in most wastewaters.⁴

The HCl-autoclave reduction of Se(VI) described above and in ¶ 4 f is an effective digestion procedure for total inorganic selenium; however, it has not been found effective for converting benzene substituted selenium compounds to inorganic selenium. In all cases, verify the effectiveness of digestion methods by carrying samples with known additions of organic As or Se(IV) through the entire procedure.

d. Interferences: Interferences are minimized because the As and Se hydrides are removed from the solution containing most potential interfering substances. Slight response variations occur when acid matrices are varied. Control these variations by treating standards and samples in the same manner. Low concentrations of noble metals (approximately 100 µg/L of Ag, Au, Pt, Pd, etc.), concentrations of copper, lead, and nickel at or greater than 1 mg/L, and concentrations between 0.1 and 1 mg/L of hydride-forming elements (Bi, Sb, Sn, and Te) may suppress the response of As and Se hydrides. Interference by transition metals depends strongly on HCl concentration. Interferences are less pronounced at 4 to 6N HCl than at lower concentrations.⁵ The presence of As or Se in each other's matrices can cause similar suppression. Reduced

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nitrogen oxides resulting from HNO_3 digestion and nitrite also can suppress instrumental response for both elements. Large concentrations of iodide interfere with the Se determination by reducing Se to its elemental form. Do not use any glassware for determining Se that has been used for iodide reduction of As(V).

To prevent chlorine gas produced in the reduction of Se(VI) to Se(IV) from reoxidizing the Se(IV), generate the hydride within a few hours of the reduction steps or purge the chlorine from the samples by sparging.⁶

Interferences depend on system design and defy quantitative description because of their synergistic effects. Certain waters and wastewaters can contain interferences in sufficient concentration to suppress absorption responses of As and Se. For representative samples in a given laboratory and for initial analyses of unknown wastewaters, add appropriate inorganic forms of As or Se to digested sample portions and measure recovery. If average recoveries are less than 90%, consider using alternative analytical procedures.

e. Detection limit and optimum concentration range: For both arsenic and selenium, analyzed by aspiration into a nitrogen-hydrogen flame after reduction, the method detection limit is 2 $\mu\text{g/L}$ or lower and the optimum concentration range 2 to 20 $\mu\text{g/L}$.

2. Apparatus

a. Atomic absorption spectrometer equipped with air-acetylene flame and quartz cell with mounting bracket or an electrically heated quartz cell, As and Se electrodeless discharge lamps with power supply, background correction at measurement wavelengths, and appropriate strip-chart recorder. A good-quality 10-mV recorder with high sensitivity and a fast response time is needed.

b. Atomizer: Use one of the following:

- 1) *Cylindrical quartz cell*, 10 to 20 cm long, bracket-mountable above air-acetylene burner.
- 2) *Cylindrical quartz cell*, 10 to 20 cm long, electrically heated by external nichrome wire to 800 to 900°C.⁷
- 3) *Cylindrical quartz cell* with internal fuel rich hydrogen-oxygen (air) flame.⁸

The sensitivity of quartz cells deteriorates over several months of use. Sensitivity sometimes may be restored by treatment with 40% HF. CAUTION: *HF is extremely corrosive. Avoid all contact with exposed skin. Handle with care.*

c. Reaction cell for producing As or Se hydrides: See Figure 3114:1 for an example of a manual, laboratory-made system. A commercially available system is acceptable if it utilizes liquid sodium borohydride reagents; accepts samples digested in accordance with ¶s 4c, d, and e; accepts 4 to 6N HCl; and is efficiently and precisely stirred by the purging gas and/or a magnetic stirrer.

d. Eye dropper or syringe capable of delivering 0.5 to 3.0 mL sodium borohydride reagent. Exact and reproducible addition is required so that production of hydrogen gas does not vary

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significantly between determinations.

e. *Vent*: See Section 3111A.6 f.

3. Reagents

a. *Sodium borohydride reagent*: Dissolve 8 g NaBH_4 in 200 mL 0.1N NaOH. Prepare fresh daily.

b. *Sodium iodide prereductant solution*: Dissolve 50 g NaI in 500 mL water. Prepare fresh daily. Alternatively use an equivalent KI solution.

c. *Sulfuric acid*, 18N.

d. *Sulfuric acid*, 2.5N: Cautiously add 35 mL conc H_2SO_4 to about 400 mL water, let cool, and adjust volume to 500 mL.

e. *Potassium persulfate*, 5% solution: Dissolve 25 g $\text{K}_2\text{S}_2\text{O}_8$ in water and dilute to 500 mL. Store in glass and refrigerate. Prepare weekly.

f. *Nitric acid*, HNO_3 , conc.

g. *Perchloric acid*, HClO_4 , conc.

h. *Hydrochloric acid*, HCl, conc.

i. *Argon (or nitrogen)*, commercial grade.

j. *Arsenic(III) solutions*:

1) *Stock As(III) solution*: Dissolve 1.320 g arsenic trioxide, As_2O_3 , in water containing 4 g NaOH. Dilute to 1 L; 1.00 mL = 1.00 mg As(III).

2) *Intermediate As(III) solution*: Dilute 10 mL stock As solution to 1000 mL with water containing 5 mL conc HCl; 1.00 mL = 10.0 μg As(III).

3) *Standard As(III) solution*: Dilute 10 mL intermediate As(III) solution to 1000 mL with water containing the same concentration of acid used for sample preservation (2 to 5 mL conc HNO_3); 1.00 mL = 0.100 μg As(III). Prepare diluted solutions daily.

k. *Arsenic(V) solutions*:

1) *Stock As(V) solution*: Dissolve 1.534 g arsenic pentoxide, As_2O_5 , in distilled water containing 4 g NaOH. Dilute to 1 L; 1.00 mL = 1.00 mg As(V).

2) *Intermediate As(V) solution*: Prepare as for As(III) above; 1.00 mL = 10.0 μg As(V).

3) *Standard As(V) solution*: Prepare as for As(III) above; 1.00 mL = 0.100 μg As(V).

l. *Organic arsenic solutions*:

1) *Stock organic arsenic solution*: Dissolve 1.842 g dimethylarsinic acid (cacodylic acid), $(\text{CH}_3)_2\text{AsOOH}$, in water containing 4 g NaOH. Dilute to 1 L; 1.00 mL = 1.00 mg As. [NOTE: Check purity of cacodylic acid reagent against an intermediate arsenic standard (50 to 100 mg As/L) using flame atomic absorption.]

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2) *Intermediate organic arsenic solution*: Prepare as for As(III) above; 1.00 mL = 10.0 µg As.

3) *Standard organic arsenic solution*: Prepare as for As(III) above; 1.00 mL = 0.100 µg As.
m. Selenium(IV) solutions:

1) *Stock Se(IV) solution*: Dissolve 2.190 g sodium selenite, Na_2SeO_3 , in water containing 10 mL HCl and dilute to 1 L; 1.00 mL = 1.00 mg Se(IV).

2) *Intermediate Se(IV) solution*: Dilute 10 mL stock Se(IV) to 1000 mL with water containing 10 mL conc HCl; 1.00 mL = 10.0 µg Se(IV).

3) *Standard Se(IV) solution*: Dilute 10 mL intermediate Se(IV) solution to 1000 mL with water containing the same concentration of acid used for sample preservation (2 to 5 mL conc HNO_3). Prepare solution daily when checking the equivalency of instrument response for Se(IV) and Se(VI); 1.00 mL = 0.100 µg Se(IV).

n. Selenium(VI) solutions:

1) *Stock Se(VI) solution*: Dissolve 2.393 g sodium selenate, Na_2SeO_4 , in water containing 10 mL conc HNO_3 . Dilute to 1 L; 1.00 mL = 1.00 mg Se(VI).

2) *Intermediate Se(VI) solution*: Prepare as for Se(IV) above; 1.00 mL = 10.0 µg Se (VI).

3) *Standard Se(VI) solution*: Prepare as for Se(IV) above; 1.00 mL = 0.100 µg Se(VI).

4. Procedure

a. Apparatus setup: Either see Figure 3114:1 or follow manufacturer's instructions. Connect inlet of reaction cell with auxiliary purging gas controlled by flow meter. If a drying cell between the reaction cell and atomizer is necessary, use only anhydrous CaCl_2 but not CaSO_4 because it may retain SeH_2 . Before using the hydride generation/analysis system, optimize operating parameters. Align quartz atomizers for maximum absorbance. Aspirate a blank until memory effects are removed. Establish purging gas flow, concentration and rate of addition of sodium borohydride reagent, solution volume, and stirring rate for optimum instrument response for the chemical species to be analyzed. Optimize quartz cell temperature. If sodium borohydride reagent is added too quickly, rapid evolution of hydrogen will unbalance the system. If the volume of solution being purged is too large, the absorption signal will be decreased. Recommended wavelengths are 193.7 and 196.0 nm for As and Se, respectively.

b. Instrument calibration standards: Transfer 0.00, 1.00, 2.00, 5.00, 10.00, 15.00, and 20.00 mL standard solutions of As(III) or Se(IV) to 100-mL volumetric flasks and bring to volume with water containing the same acid concentration used for sample preservation (commonly 2 to 5 mL conc HNO_3 /L). This yields blank and standard solutions of 0, 1, 2, 5, 10, 15, and 20 µg As or Se/L. Prepare fresh daily. In all cases, standards must be carried through the same digestion protocol as the samples to monitor digestion effectiveness.

c. Preparation of samples and standards for total recoverable arsenic and selenium: Use

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digestion procedure described in 3030F for samples and standards. Alternatively, add 50 mL sample, As(III), or Se(IV) standard to 200-mL Berzelius beaker or 100-mL micro-kjeldahl flask. Add 7 mL 18N H_2SO_4 and 5 mL conc HNO_3 . Add a small boiling chip or glass beads if necessary. Evaporate to SO_3 fumes. Maintain oxidizing conditions at all times by adding small amounts of HNO_3 to prevent solution from darkening. Maintain an excess of HNO_3 until all organic matter is destroyed. Complete digestion usually is indicated by a light-colored solution. Cool slightly, add 25 mL water and 1 mL conc HClO_4 and again evaporate to SO_3 fumes to expel oxides of nitrogen. CAUTION: See Section 3030H for cautions on use of HClO_4 . Monitor effectiveness of either digestion procedure used by adding 5 mL of standard organic arsenic solution or 5 mL of a standard selenium solution to a 50-mL sample and measuring recovery, carrying standards and the sample with known addition through entire procedure. To report total recoverable arsenic as total arsenic, average recoveries of cacodylic acid must exceed 80%. After final evaporation of SO_3 fumes, dilute to 50 mL for arsenic measurements or to 30 mL for selenium measurements. For analysis of both elements in a single sample, increase sample volume to 100 mL and double the volumes of acids used in the digestion. Adjust final digestate volume to 100 mL. Use 50 mL for As and 30 mL for Se determinations, making appropriate volume corrections in calculating results.

d. Preparation of samples and standards for total arsenic and selenium: Add 50 mL undigested sample or standard to a 200-mL Berzelius beaker or 100-mL micro-kjeldahl flask. Add 1 mL 2.5N H_2SO_4 and 5 mL 5% $\text{K}_2\text{S}_2\text{O}_8$. Boil gently on a pre-heated hot plate for approximately 30 to 40 min or until a final volume of 10 mL is reached. Do not let sample go to dryness. Alternatively heat in an autoclave at 121°C for 1 h in capped containers. After manual digestion, dilute to 50 mL for subsequent arsenic measurements and to 30 mL for selenium measurements. Monitor effectiveness of digestion by measuring recovery of As or Se as above. If poor recovery of arsenic added as cacodylic acid is obtained, reanalyze using double the amount of $\text{K}_2\text{S}_2\text{O}_8$. For analysis of both elements in a single sample, increase sample volume to 100 mL and double the volumes of acids used in the digestion. Adjust final digestate volume to 100 mL. Use 50 mL for As and 30 mL for Se determinations, making appropriate volume corrections in calculating results.

e. Determination of arsenic with sodium borohydride: To 50 mL digested standard or sample in a 200-mL Berzelius beaker (see Figure 3114:1) add 5 mL conc HCl and mix. Add 5 mL NaI prereductant solution, mix, and wait at least 30 min. (NOTE: The NaI reagent has not been found necessary for certain hydride reaction cell designs if a 20 to 30% loss in instrument sensitivity is not important and variables of solution acid conditions, temperatures, and volumes for production of As(V) and arsine can be controlled strictly. Such control requires an automated delivery system; see Section 3114C.)

Attach one Berzelius beaker at a time to the rubber stopper containing the gas dispersion tube for the purging gas, the sodium borohydride reagent inlet, and the outlet to the quartz cell. Turn on strip-chart recorder and wait until the base line is established by the purging gas and all

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air is expelled from the reaction cell. Add 0.5 mL sodium borohydride reagent. After the instrument absorbance has reached a maximum and returned to the base line, remove beaker, rinse dispersion tube with water, and proceed to the next sample or standard. Periodically compare standard As(III) and As(V) curves for response consistency. Check for presence of chemical interferences that suppress instrument response for arsine by treating a digested sample with 10 µg/L As(III) or As(V) as appropriate. Average recoveries should be not less than 90%.

f. Determination of selenium with sodium borohydride: To 30 mL digested standard or sample in a 200-mL Berzelius beaker or 100-mL micro-kjeldahl flask, add 15 mL conc HCl and mix. Heat for a predetermined period at 90 to 100°C. Alternatively autoclave at 121°C in capped containers for 60 min, or heat for a predetermined time in open test tubes using a 90 to 100°C hot water bath or an aluminum block digester. Check effectiveness of the selected heating time by demonstrating equal instrument responses for calibration curves prepared either from standard Se(IV) or from Se(VI) solutions. Effective heat exposure for converting Se(VI) to Se(IV), with no loss of Se(IV), ranges between 5 and 60 min when open beakers or test tubes are used. Establish a heating time for effective conversion and apply this time to all samples and standards. Do not digest standard Se(IV) and Se(VI) solutions used for this check of equivalency. After prereduction of Se(VI) to Se(IV), attach Berzelius beakers, one at a time, to the purge apparatus. For each, turn on the strip-chart recorder and wait until the base line is established. Add 0.50 mL sodium borohydride reagent. After the instrument absorbance has reached a maximum and returned to the base line, remove beaker, rinse dispersion tube with water, and proceed to the next sample or standard. Check for presence of chemical interferences that suppress selenium hydride instrument response by treating a digested sample with 10 µg Se (IV)/L. Average recoveries should be not less than 90%.

5. Calculation

Construct a standard curve by plotting peak heights or areas of standards versus concentration of standards. Measure peak heights or areas of samples and read concentrations from curve. If sample was diluted (or concentrated) before sample digestion, apply an appropriate factor. On instruments so equipped, read concentrations directly after standard calibration.

6. Precision and Bias

Single-laboratory, single-operator data were collected for As(III) and organic arsenic by both manual and automated methods, and for the manual determination of selenium. Recovery values (%) from seven replicates are given below:

Method	As(III)	Org As	Se(IV)	Se(VI)
Manual with digestion	91.8	87.3	—	—
Manual without digestion	109.4	19.4	100.6	110.8
Automated with digestion	99.8	98.4	—	—

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Method	As(III)	Org As	Se(IV)	Se(VI)
Automated without digestion	92.5	10.4	—	—

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3114 C. Continuous Hydride Generation/Atomic Absorption Spectrometric Method

1. General Discussion

The continuous hydride generator offers the advantages of simplicity in operation, excellent reproducibility, low detection limits, and high sample volume throughput for selenium analysis following preparations as described in Section 3500-Se.B or Section 3114B.4c and d.

a. *Principle:* See Section 3114B.

b. *Interferences:* Free chlorine in hydrochloric acid is a common but difficult-to-diagnose interference. (The amount of chlorine varies with manufacturer and with each lot from the same manufacturer). Chlorine oxidizes the hydride and can contaminate the hydride generator to prevent recoveries under any conditions. When interference is encountered, or preferably before using each new bottle of HCl, eliminate chlorine from a 2.3-L bottle of conc HCl by bubbling with helium (commercial grade, 100 mL/min) for 3 h.

Excess oxidant (peroxide, persulfate, or permanganate) from the total selenium digestion can oxidize the hydride. Follow procedures in 3500-Se.B.2, 3, or 4 to ensure removal of all oxidizing agents before hydride generation.

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Nitrite is a common trace constituent in natural and waste waters, and at levels as low as 10 µg/L nitrite can reduce the recovery of hydrogen selenide from Se(IV) by over 50%. Moreover, during the reduction of Se(VI) to Se(IV) by digestion with HCl (3500-Se.B.5), some nitrate is converted to nitrite, which subsequently interferes. When this interference is suspected, add sulfanilamide after sample acidification (or HCl digestion). The diazotization reaction between nitrite and sulfanilamide completely removes the interferent effect (i.e., the standard addition slope is normal).

2. Apparatus

a. Continuous hydride generator: The basic unit is composed of two parts: a precision peristaltic pump, which is used to meter and mix reagents and sample solutions, and the gas-liquid separator. At the gas-liquid separator a constant flow of argon strips out the hydrogen and metal hydride gases formed in the reaction and carries them to the heated quartz absorption cell (Section 3114B.1b and Section 3114B.2b), which is supported by a metal bracket mounted on top of the regular air acetylene burner head. The spent liquid flows out of the separator via a constant level side drain to a waste bucket. Schematics and operating parameters are shown in Figure 3114:2.

Check flow rates frequently to ensure a steady flow; an uneven flow in any tubing will cause an erratic signal. Remove tubings from pump rollers when not in use. Typical flow rates are: sample, 7 mL/min; acid, 1 mL/min; borohydride reagent, 1 mL/min. Argon flow usually is pre-fixed, typically at 90 mL/min.

b. Atomic absorption spectrometric equipment: See Section 3111A.6.

3. Reagents

a. Hydrochloric acid, HCl, 5 + 1: Handle conc HCl under a fume hood. If necessary, remove free Cl₂ by stripping conc HCl with helium as described above.

b. Borohydride reagent: Dissolve 0.6 g NaBH₄ and 0.5 g NaOH in 100 mL water.

CAUTION: Sodium borohydride is toxic, flammable, and corrosive.

c. Selenium reference standard solution, 1000 mg/L: Use commercially available standard; verify that selenium is Se(IV).

d. Intermediate standard solution, 1 mg/L: Dilute 1 mL reference standard solution to 1 L in a volumetric flask with distilled water.

e. Working standard solutions, 5, 10, 20, 30, and 40 µg/L: Dilute 0.5, 1.0, 2.0, 3.0, and 4.0 mL intermediate standard solution to 100 mL in a volumetric flask.

f. Sulfanilamide solution: Prepare a 2.5% (w/v) solution daily; add several drops conc HCl per 50 mL solution to facilitate dissolution.

4. Procedure

a. Sample preparation: See Section 3500-Se or Section 3114B.4c and *d* for preparation

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steps for various Se fractions or total Se.

b. Preconditioning hydride generator: For newly installed tubing, turn on pump for at least 10 to 15 min before instrument calibration. Sample the highest standard for a few minutes to let volatile hydride react with the reactive sites in the transfer lines and on the quartz absorption cell surfaces.

c. Instrument calibration: Depending on total void volume in sample tubing, sampling time of 15 to 20 s generally is sufficient to obtain a steady signal. Between samples, submerge uptake tube in rinse water. Calibrate instrument daily after a 45-min lamp warmup time. Use either the hollow cathode or the electrodeless discharge lamp.

d. Antifoaming agents: Certain samples, particularly wastewater samples containing a high concentration of proteinaceous substances, can cause excessive foaming that could carry the liquid directly into the heated quartz absorption cell and cause splattering of salty deposits onto the windows of the spectrometer. Add a drop of antifoaming agent*#(84) to eliminate this problem.

e. Nitrite removal: After samples have been acidified, or after acid digestion, add 0.1 mL sulfanilamide solution per 10 mL sample and let react for 2 min.

f. Analysis: Follow manufacturer's instructions for operation of analytical equipment.

5. Calculation

Construct a calibration curve based on absorbance vs. standard concentration. Apply dilution factors on diluted samples.

6. Precision and Bias

Working standards were analyzed together with batches of water samples on a routine production basis. The standards were compounded using chemically pure sodium selenite and sodium selenate. The values of Se(IV) + Se(VI) were determined by converting Se(VI) to Se(IV) by digestion with HCl. Results are tabulated below.

No. Analyses	Mean Se(IV) μg/L	Rel. Dev. %	Se(IV) + Se(VI) μg/L	Rel. Del. %
21	4.3	12	10.3	7
26	8.5	12	19.7	6
22	17.2	7	39.2	8
20	52.8	5	106.0	6

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3120 METALS BY PLASMA EMISSION SPECTROSCOPY*(85)

3120 A. Introduction

1. General Discussion

Emission spectroscopy using inductively coupled plasma (ICP) was developed in the mid-1960's^{1,2} as a rapid, sensitive, and convenient method for the determination of metals in water and wastewater samples.³⁻⁶ Dissolved metals are determined in filtered and acidified samples. Total metals are determined after appropriate digestion. Care must be taken to ensure that potential interferences are dealt with, especially when dissolved solids exceed 1500 mg/L.

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3120 B. Inductively Coupled Plasma (ICP) Method

1. General Discussion

a. Principle: An ICP source consists of a flowing stream of argon gas ionized by an applied radio frequency field typically oscillating at 27.1 MHz. This field is inductively coupled to the ionized gas by a water-cooled coil surrounding a quartz “torch” that supports and confines the plasma. A sample aerosol is generated in an appropriate nebulizer and spray chamber and is carried into the plasma through an injector tube located within the torch. The sample aerosol is injected directly into the ICP, subjecting the constituent atoms to temperatures of about 6000 to 8000°K.¹ Because this results in almost complete dissociation of molecules, significant reduction in chemical interferences is achieved. The high temperature of the plasma excites atomic emission efficiently. Ionization of a high percentage of atoms produces ionic emission spectra. The ICP provides an optically “thin” source that is not subject to self-absorption except at very high concentrations. Thus linear dynamic ranges of four to six orders of magnitude are observed for many elements.²

The efficient excitation provided by the ICP results in low detection limits for many elements. This, coupled with the extended dynamic range, permits effective multielement determination of metals.³ The light emitted from the ICP is focused onto the entrance slit of either a monochromator or a polychromator that effects dispersion. A precisely aligned exit slit is used to isolate a portion of the emission spectrum for intensity measurement using a photomultiplier tube. The monochromator uses a single exit slit/photomultiplier and may use a computer-controlled scanning mechanism to examine emission wavelengths sequentially. The polychromator uses multiple fixed exit slits and corresponding photomultiplier tubes; it simultaneously monitors all configured wavelengths using a computer-controlled readout system. The sequential approach provides greater wavelength selection while the simultaneous

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approach can provide greater sample throughput.

b. Applicable metals and analytical limits: Table 3120:I lists elements for which this method applies, recommended analytical wavelengths, and typical estimated instrument detection limits using conventional pneumatic nebulization. Actual working detection limits are sample-dependent. Typical upper limits for linear calibration also are included in Table 3120:I.

c. Interferences: Interferences may be categorized as follows:

1) Spectral interferences—Light emission from spectral sources other than the element of interest may contribute to apparent net signal intensity. Sources of spectral interference include direct spectral line overlaps, broadened wings of intense spectral lines, ion-atom recombination continuum emission, molecular band emission, and stray (scattered) light from the emission of elements at high concentrations.⁴ Avoid line overlaps by selecting alternate analytical wavelengths. Avoid or minimize other spectral interference by judicious choice of background correction positions. A wavelength scan of the element line region is useful for detecting potential spectral interferences and for selecting positions for background correction. Make corrections for residual spectral interference using empirically determined correction factors in conjunction with the computer software supplied by the spectrometer manufacturer or with the calculation detailed below. The empirical correction method cannot be used with scanning spectrometer systems if the analytical and interfering lines cannot be precisely and reproducibly located. In addition, if using a polychromator, verify absence of spectral interference from an element that could occur in a sample but for which there is no channel in the detector array. Do this by analyzing single-element solutions of 100 mg/L concentration and noting for each element channel the apparent concentration from the interfering substance that is greater than the element's instrument detection limit.

2) Nonspectral interferences

a) Physical interferences are effects associated with sample nebulization and transport processes. Changes in the physical properties of samples, such as viscosity and surface tension, can cause significant error. This usually occurs when samples containing more than 10% (by volume) acid or more than 1500 mg dissolved solids/L are analyzed using calibration standards containing $\leq 5\%$ acid. Whenever a new or unusual sample matrix is encountered, use the test described in ¶ 4g. If physical interference is present, compensate for it by sample dilution, by using matrix-matched calibration standards, or by applying the method of standard addition (see ¶ 5d below).

High dissolved solids content also can contribute to instrumental drift by causing salt buildup at the tip of the nebulizer gas orifice. Using prehumidified argon for sample nebulization lessens this problem. Better control of the argon flow rate to the nebulizer using a mass flow controller improves instrument performance.

b) Chemical interferences are caused by molecular compound formation, ionization effects, and thermochemical effects associated with sample vaporization and atomization in the plasma. Normally these effects are not pronounced and can be minimized by careful selection of

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operating conditions (incident power, plasma observation position, etc.). Chemical interferences are highly dependent on sample matrix and element of interest. As with physical interferences, compensate for them by using matrix matched standards or by standard addition (§ 5d). To determine the presence of chemical interference, follow instructions in § 4g.

2. Apparatus

a. *ICP source:* The ICP source consists of a radio frequency (RF) generator capable of generating at least 1.1 KW of power, torch, tesla coil, load coil, impedance matching network, nebulizer, spray chamber, and drain. High-quality flow regulators are required for both the nebulizer argon and the plasma support gas flow. A peristaltic pump is recommended to regulate sample flow to the nebulizer. The type of nebulizer and spray chamber used may depend on the samples to be analyzed as well as on the equipment manufacturer. In general, pneumatic nebulizers of the concentric or cross-flow design are used. Viscous samples and samples containing particulates or high dissolved solids content (>5000 mg/L) may require nebulizers of the Babington type.⁵

b. *Spectrometer:* The spectrometer may be of the simultaneous (polychromator) or sequential (monochromator) type with air-path, inert gas purged, or vacuum optics. A spectral bandpass of 0.05 nm or less is required. The instrument should permit examination of the spectral background surrounding the emission lines used for metals determination. It is necessary to be able to measure and correct for spectral background at one or more positions on either side of the analytical lines.

3. Reagents and Standards

Use reagents that are of ultra-high-purity grade or equivalent. Redistilled acids are acceptable. Except as noted, dry all salts at 105°C for 1 h and store in a desiccator before weighing. Use deionized water prepared by passing water through at least two stages of deionization with mixed bed cation/anion exchange resins.⁶ Use deionized water for preparing all calibration standards, reagents, and for dilution.

a. *Hydrochloric acid*, HCl, conc and 1+1.

b. *Nitric acid*, HNO₃, conc.

c. *Nitric acid*, HNO₃, 1+1: Add 500 mL conc HNO₃ to 400 mL water and dilute to 1 L.

d. *Standard stock solutions:* See Section 3111B, Section 3111D, and Section 3114B.

CAUTION: *Many metal salts are extremely toxic and may be fatal if swallowed. Wash hands thoroughly after handling.*

1) *Aluminum:* See Section 3111D.3k1).

2) *Antimony:* See Section 3111B.3 j1).

3) *Arsenic:* See Section 3114B.3k1).

4) *Barium:* See Section 3111D.3k2).

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- 5) *Beryllium*: See Section 3111D.3k3).
- 6) *Boron*: *Do not dry* but keep bottle tightly stoppered and store in a desiccator. Dissolve 0.5716 g anhydrous H_3BO_3 in water and dilute to 1000 mL; 1 mL = 100 μg B.
- 7) *Cadmium*: See Section 3111B.3 j3).
- 8) *Calcium*: See Section 3111B.3 j4).
- 9) *Chromium*: See Section 3111B.3 j6).
- 10) *Cobalt*: See Section 3111B.3 j7).
- 11) *Copper*: See Section 3111B.3 j8).
- 12) *Iron*: See Section 3111B.3 j11).
- 13) *Lead*: See Section 3111B.3 j12).
- 14) *Lithium*: See Section 3111B.3 j13).
- 15) *Magnesium*: See Section 3111B.3 j14).
- 16) *Manganese*: See Section 3111B.3 j15).
- 17) *Molybdenum*: See Section 3111D.3k4).
- 18) *Nickel*: See Section 3111B.3 j16).
- 19) *Potassium*: See Section 3111B.3 j19).
- 20) *Selenium*: See Section 3114B.3n1).
- 21) *Silica*: See Section 3111D.3k7).
- 22) *Silver*: See Section 3111B.3 j22).
- 23) *Sodium*: See Section 3111B.3 j23).
- 24) *Strontium*: See Section 3111B.3 j24).
- 25) *Thallium*: See Section 3111B.3 j25).
- 26) *Vanadium*: See Section 3111D.3k10).
- 27) *Zinc*: See Section 3111B.3 j27).

e. *Calibration standards*: Prepare mixed calibration standards containing the concentrations shown in Table 3120:I by combining appropriate volumes of the stock solutions in 100-mL volumetric flasks. Add 2 mL 1+1 HNO_3 and 10 mL 1+1 HCl and dilute to 100 mL with water. Before preparing mixed standards, analyze each stock solution separately to determine possible spectral interference or the presence of impurities. When preparing mixed standards take care that the elements are compatible and stable. Store mixed standard solutions in an FEP fluorocarbon or unused polyethylene bottle. Verify calibration standards initially using the quality control standard; monitor weekly for stability. The following are recommended combinations using the suggested analytical lines in Table 3120:I. Alternative combinations are acceptable.

- 1) *Mixed standard solution I*: Manganese, beryllium, cadmium, lead, selenium, and zinc.
- 2) *Mixed standard solution II*: Barium, copper, iron, vanadium, and cobalt.

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3) *Mixed standard solution III*: Molybdenum, silica, arsenic, strontium, and lithium.

4) *Mixed standard solution IV*: Calcium, sodium, potassium, aluminum, chromium, and nickel.

5) *Mixed standard solution V*: Antimony, boron, magnesium, silver, and thallium. If addition of silver results in an initial precipitation, add 15 mL water and warm flask until solution clears. Cool and dilute to 100 mL with water. For this acid combination limit the silver concentration to 2 mg/L. Silver under these conditions is stable in a tap water matrix for 30 d. Higher concentrations of silver require additional HCl.

f. *Calibration blank*: Dilute 2 mL 1+1 HNO₃ and 10 mL 1+1 HCl to 100 mL with water. Prepare a sufficient quantity to be used to flush the system between standards and samples.

g. *Method blank*: Carry a reagent blank through entire sample preparation procedure. Prepare method blank to contain the same acid types and concentrations as the sample solutions.

h. *Instrument check standard*: Prepare instrument check standards by combining compatible elements at a concentration of 2 mg/L.

i. *Instrument quality control sample*: Obtain a certified aqueous reference standard from an outside source and prepare according to instructions provided by the supplier. Use the same acid matrix as the calibration standards.

j. *Method quality control sample*: Carry the instrument quality control sample (§ 3i) through the entire sample preparation procedure.

k. *Argon*: Use technical or welder's grade. If gas appears to be a source of problems, use prepurified grade.

4. Procedure

a. *Sample preparation*: See Section 3030F.

b. *Operating conditions*: Because of differences among makes and models of satisfactory instruments, no detailed operating instructions can be provided. Follow manufacturer's instructions. Establish instrumental detection limit, precision, optimum background correction positions, linear dynamic range, and interferences for each analytical line. Verify that the instrument configuration and operating conditions satisfy the analytical requirements and that they can be reproduced on a day-to-day basis. An atom-to-ion emission intensity ratio [Cu(I) 324.75 nm/ Mn(II) 257.61 nm] can be used to reproduce optimum conditions for multielement analysis precisely. The Cu/Mn intensity ratio may be incorporated into the calibration procedure, including specifications for sensitivity and for precision.⁷ Keep daily or weekly records of the Cu and Mn intensities and/or the intensities of critical element lines. Also record settings for optical alignment of the polychromator, sample uptake rate, power readings (incident, reflected), photomultiplier tube attenuation, mass flow controller settings, and system maintenance.

c. *Instrument calibration*: Set up instrument as directed (§ b). Warm up for 30 min. For polychromators, perform an optical alignment using the profile lamp or solution. Check

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alignment of plasma torch and spectrometer entrance slit, particularly if maintenance of the sample introduction system was performed. Make Cu/Mn or similar intensity ratio adjustment.

Calibrate instrument according to manufacturer's recommended procedure using calibration standards and blank. Aspirate each standard or blank for a minimum of 15 s after reaching the plasma before beginning signal integration. Rinse with calibration blank or similar solution for at least 60 s between each standard to eliminate any carryover from the previous standard. Use average intensity of multiple integrations of standards or samples to reduce random error.

Before analyzing samples, analyze instrument check standard. Concentration values obtained should not deviate from the actual values by more than $\pm 5\%$ (or the established control limits, whichever is lower).

d. Analysis of samples: Begin each sample run with an analysis of the calibration blank, then analyze the method blank. This permits a check of the sample preparation reagents and procedures for contamination. Analyze samples, alternating them with analyses of calibration blank. Rinse for at least 60 s with dilute acid between samples and blanks. After introducing each sample or blank let system equilibrate before starting signal integration. Examine each analysis of the calibration blank to verify that no carry-over memory effect has occurred. If carry-over is observed, repeat rinsing until proper blank values are obtained. Make appropriate dilutions and acidifications of the sample to determine concentrations beyond the linear calibration range.

e. Instrumental quality control: Analyze instrument check standard once per 10 samples to determine if significant instrument drift has occurred. If agreement is not within $\pm 5\%$ of the expected values (or within the established control limits, whichever is lower), terminate analysis of samples, correct problem, and recalibrate instrument. If the intensity ratio reference is used, resetting this ratio may restore calibration without the need for reanalyzing calibration standards. Analyze instrument check standard to confirm proper recalibration. Reanalyze one or more samples analyzed just before termination of the analytical run. Results should agree to within $\pm 5\%$, otherwise all samples analyzed after the last acceptable instrument check standard analysis must be reanalyzed.

Analyze instrument quality control sample within every run. Use this analysis to verify accuracy and stability of the calibration standards. If any result is not within $\pm 5\%$ of the certified value, prepare a new calibration standard and recalibrate the instrument. If this does not correct the problem, prepare a new stock solution and a new calibration standard and repeat calibration.

f. Method quality control: Analyze the method quality control sample within every run. Results should agree to within $\pm 5\%$ of the certified values. Greater discrepancies may reflect losses or contamination during sample preparation.

g. Test for matrix interference: When analyzing a new or unusual sample matrix verify that neither a positive nor negative nonlinear interference effect is operative. If the element is present at a concentration above 1 mg/L, use serial dilution with calibration blank. Results from the analyses of a dilution should be within $\pm 5\%$ of the original result. Alternately, or if the concentration is either below 1 mg/L or not detected, use a post-digestion addition equal to 1

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mg/L. Recovery of the addition should be either between 95% and 105% or within established control limits of ± 2 standard deviations around the mean. If a matrix effect causes test results to fall outside the critical limits, complete the analysis after either diluting the sample to eliminate the matrix effect while maintaining a detectable concentration of at least twice the detection limit or applying the method of standard additions.

5. Calculations and Corrections

a. *Blank correction:* Subtract result of an adjacent calibration blank from each sample result to make a baseline drift correction. (Concentrations printed out should include negative and positive values to compensate for positive and negative baseline drift. Make certain that the calibration blank used for blank correction has not been contaminated by carry-over.) Use the result of the method blank analysis to correct for reagent contamination. Alternatively, intersperse method blanks with appropriate samples. Reagent blank and baseline drift correction are accomplished in one subtraction.

b. *Dilution correction:* If the sample was diluted or concentrated in preparation, multiply results by a dilution factor (*DF*) calculated as follows:

$$DF = \frac{\text{Final weight or volume}}{\text{Initial weight or volume}}$$

c. *Correction for spectral interference:* Correct for spectral interference by using computer software supplied by the instrument manufacturer or by using the manual method based on interference correction factors. Determine interference correction factors by analyzing single-element stock solutions of appropriate concentrations under conditions matching as closely as possible those used for sample analysis. Unless analysis conditions can be reproduced accurately from day to day, or for longer periods, redetermine interference correction factors found to affect the results significantly each time samples are analyzed.^{7,8} Calculate interference correction factors (K_{ij}) from apparent concentrations observed in the analysis of the high-purity stock solutions:

$$K_{ij} = \frac{\text{Apparent concentration of element } i}{\text{Actual concentration of interfering element } j}$$

where the apparent concentration of element *i* is the difference between the observed concentration in the stock solution and the observed concentration in the blank. Correct sample concentrations observed for element *i* (already corrected for baseline drift), for spectral interferences from elements *j*, *k*, and *l*; for example:

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Concentration of element i corrected for spectral interference

$$\begin{aligned}
 & \text{Observed} \\
 = & \text{concentration of } i - (K_{ij}) \text{ concentration of interfering element } j - (K_{ik}) \text{ concentration of interfering element } k \\
 & - (K_{il}) \text{ concentration of interfering element } l
 \end{aligned}$$

Interference correction factors may be negative if background correction is used for element i . A negative K_{ij} can result where an interfering line is encountered at the background correction wavelength rather than at the peak wavelength. Determine concentrations of interfering elements j , k , and l within their respective linear ranges. Mutual interferences (i interferes with j and j interferes with i) require iterative or matrix methods for calculation.

d. Correction for nonspectral interference: If nonspectral interference correction is necessary, use the method of standard additions. It is applicable when the chemical and physical form of the element in the standard addition is the same as in the sample, or the ICP converts the metal in both sample and addition to the same form; the interference effect is independent of metal concentration over the concentration range of standard additions; and the analytical calibration curve is linear over the concentration range of standard additions.

Use an addition not less than 50% nor more than 100% of the element concentration in the sample so that measurement precision will not be degraded and interferences that depend on element/interferent ratios will not cause erroneous results. Apply the method to all elements in the sample set using background correction at carefully chosen off-line positions. Multielement standard addition can be used if it has been determined that added elements are not interferents.

e. Reporting data: Report analytical data in concentration units of milligrams per liter using up to three significant figures. Report results below the determined detection limit as not detected less than the stated detection limit corrected for sample dilution.

6. Precision and Bias

As a guide to the generally expected precision and bias, see the linear regression equations in Table 3120:II.⁹ Additional interlaboratory information is available.¹⁰

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3125 METALS BY INDUCTIVELY COUPLED PLASMA/MASS SPECTROMETRY*#(86)

3125 A. Introduction

1. General Discussion

This method is used for the determination of trace metals and metalloids in surface, ground, and drinking waters by inductively coupled plasma/mass spectrometry (ICP/MS). It may also be suitable for wastewater, soils, sediments, sludge, and biological samples after suitable digestion followed by dilution and/or cleanup.^{1,2} Additional sources of information on quality assurance and other aspects of ICP/MS analysis of metals are available.³⁻⁵

The method is intended to be performance-based, allowing extension of the elemental analyte

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list, implementation of “clean” preparation techniques as they become available, and other appropriate modifications of the base method as technology evolves. Preferably validate modifications to the base method by use of the quality control standards specified in the method.

Instrument detection limits for many analytes are between 1 and 100 ng/L. The method is best suited for the determination of metals in ambient or pristine fresh-water matrices. More complex matrices may require some type of cleanup to reduce matrix effects to a manageable level. Various cleanup techniques are available to reduce matrix interferences and/or concentrate analytes of interest.⁶⁻¹⁰

This method is ideally used by analysts experienced in the use of ICP/MS, the interpretation of spectral and matrix interference, and procedures for their correction. Preferably demonstrate analyst proficiency through analysis of a performance evaluation sample before the generation of data.

2. References

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3125 B. Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) Method

1. General Discussion

a. Principle: Sample material is introduced into an argon-based, high-temperature radio-frequency plasma, usually by pneumatic nebulization. Energy transfer from the plasma to the sample stream causes desolvation, atomization, and ionization of target elements. Ions generated by these energy-transfer processes are extracted from the plasma through a differential vacuum interface, and separated on the basis of their mass-to-charge ratio by a mass spectrometer. The mass spectrometer usually is of the quadrupole or magnetic sector type. The ions passing through the mass spectrometer are counted, usually by an electron multiplier detector, and the resulting information processed by a computer-based data-handling system.

b. Applicable elements and analytical limits: This method is suitable for aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium, silver, strontium, thallium, uranium, vanadium, and zinc. The method is also acceptable for other elemental analytes as long as the same quality assurance practices are followed. The basic element suite and recommended analytical masses are given in Table 3125:I.

Typical instrument detection limits (IDL)^{1,2} for method analytes are presented in Table 3125:I. Determine the IDL and method detection level (or limit) (MDL) for all analytes before method implementation. Section 1030 contains additional information and approaches for the evaluation of detection capabilities.

The MDL is defined in Section 1010C and elsewhere.² Determination of the MDL for each element is critical for complex matrices such as seawater, brines, and industrial effluents. The MDL will typically be higher than the IDL, because of background analyte in metals preparation and analysis laboratories and matrix-based interferences. Determine both IDL and MDL upon initial implementation of this method, and then yearly or whenever the instrument configuration changes or major maintenance occurs, whichever comes first.

Determine linear dynamic ranges (LDR) for all method analytes. LDR is defined as the maximum concentration of analyte above the highest calibration point where analyte response is within $\pm 10\%$ of the theoretical response. When determining linear dynamic ranges, avoid using unduly high concentrations of analyte that might damage the detector. Determine LDR on multielement mixtures, to account for possible interelement effects. Determine LDR on initial implementation of this method, and then yearly.

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c. Interferences: ICP/MS is subject to several types of interferences.

1) Isotopes of different elements that form ions of the same nominal mass-to-charge ratio are not resolved by the quadrupole mass spectrometer, and cause isobaric elemental interferences. Typically, ICP/MS instrument operating software will have all known isobaric interferences entered, and will perform necessary calculations automatically. Table 3125:II shows many of the commonly used corrections. Monitor the following additional masses: ^{83}Kr , ^{99}Ru , ^{118}Sn , and ^{125}Te . It is necessary to monitor these masses to correct for isobaric interference caused by ^{82}Kr on ^{82}Se , by ^{98}Ru on ^{98}Mo , by ^{114}Sn on ^{114}Cd , and by ^{123}Te on ^{123}Sb . Monitor ArCl at mass 77, to estimate chloride interferences. Verify that all elemental and molecular correction equations used in this method are correct and appropriate for the mass spectrometer used and sample matrix.

2) Abundance sensitivity is an analytical condition in which the tails of an abundant mass peak contribute to or obscure adjacent masses. Adjust spectrometer resolution to minimize these interferences.

3) Polyatomic (molecular) ion interferences are caused by ions consisting of more than one atom and having the same nominal mass-to-charge ratio as the isotope of interest. Most of the common molecular ion interferences have been identified and are listed in Table 3125:III. Because of the severity of chloride ion interference on important analytes, particularly arsenic and selenium, hydrochloric acid is not recommended for use in preparation of any samples to be analyzed by ICP/MS. The mathematical corrections for chloride interferences only correct chloride to a concentration of 0.4%. Because chloride ion is present in most environmental samples, it is critical to use chloride correction equations for affected masses. A high-resolution ICP/MS may be used to resolve interferences caused by polyatomic ions. Polyatomic interferences are strongly influenced by instrument design and plasma operating conditions, and can be reduced in some cases by careful adjustment of nebulizer gas flow and other instrument operating parameters.

4) Physical interferences include differences in viscosity, surface tension, and dissolved solids between samples and calibration standards. To minimize these effects, dissolved solid levels in analytical samples should not exceed 0.5%. Dilute water and wastewater samples containing dissolved solids at or above 0.5% before analysis. Use internal standards for correction of physical interferences. Any internal standards used should demonstrate comparable analytical behavior to the elements being determined.

5) Memory interferences occur when analytes from a previous sample or standard are measured in the current sample. Use a sufficiently long rinse or flush between samples to minimize this type of interference. If memory interferences persist, they may be indications of problems in the sample introduction system. Severe memory interferences may require disassembly and cleaning of the entire sample introduction system, including the plasma torch, and the sampler and skimmer cones.

6) Ionization interferences result when moderate (0.1 to 1%) amounts of a matrix ion change

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the analyte signal. This effect, which usually reduces the analyte signal, also is known as “suppression.” Correct for suppression by use of internal standardization techniques.

2. Apparatus

a. *Inductively coupled plasma/mass spectrometer*: Instrumentation, available from several manufacturers, includes a mass spectrometer detector, inductively coupled plasma source, mass flow controllers for regulation of ICP gas flows, peristaltic pump for sample introduction, and a computerized data acquisition and instrument control system. An x-y autosampler also may be used with appropriate control software.

b. *Laboratory ware*: Use precleaned plastic laboratory ware for standard and sample preparation. Teflon, [®] either tetrafluoroethylene hexafluoropropylene-copolymer (FEP), polytetrafluoroethylene (PTFE), or perfluoroalkoxy PTFE (PFA) is preferred for standard preparation and sample digestion, while high-density polyethylene (HDPE) and other dense, metal-free plastics may be acceptable for internal standards, known-addition solutions, etc. Check each new lot of autosampler tubes for suitability, and preclean autosampler tubes and pipettor tips (see Section 3010C.2).

c. *Air displacement pipets*, 10 to 100 μL , 100 to 1000 μL , and 1 to 10 mL size.

d. *Analytical balance*, accurate to 0.1 mg.

e. *Sample preparation apparatus*, such as hot plates, microwave digestors, and heated sand baths. Any sample preparation device has the potential to introduce trace levels of target analytes to the sample.

f. *Clean hood* (optional), Class 100 (certified to contain less than 100 particles/ m^3), for sample preparation and manipulation. Preferably perform all sample manipulations, digestions, dilutions, etc. in a certified Class 100 environment. Alternatively, handle samples in glove boxes, plastic fume hoods, or other environments where random contamination by trace metals can be minimized.

3. Reagents

a. *Acids*: Use ultra-high-purity grade (or equivalent) acids to prepare standards and to process sample. Redistilled acids are acceptable if each batch is demonstrated to be free from contamination by target analytes. Use extreme care in the handling of acids in the laboratory to avoid contamination of the acids with trace levels of metals.

1) *Nitric acid*, HNO_3 , conc (specific gravity 1.41).

2) *Nitric acid*, 1 + 1: Add 500 mL conc HNO_3 to 500 mL reagent water.

3) *Nitric acid*, 2%: Add 20 mL conc HNO_3 to 100 mL reagent water; dilute to 1000 mL.

4) *Nitric acid*, 1%: Add 10 mL conc HNO_3 to 100 mL reagent water; dilute to 1000 mL.

b. *Reagent water*: Use water of the highest possible purity for blank, standard, and sample preparation (see Section 1080). Alternatively, use the procedure described below to produce

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water of acceptable quality. Other water preparation regimes may be used, provided that the water produced is metal-free. Reagent water containing trace amounts of analyte elements will cause erroneous results.

Produce reagent water using a softener/reverse osmosis unit with subsequent UV sterilization. After the general deionization system use a dual-column strong acid/strong base ion exchange system to polish laboratory reagent water before production of metal-free water. Use a multi-stage reagent water system, with two strong acid/strong base ion exchange columns and an activated carbon filter for organics removal for final polishing of laboratory reagent water. Use only high-purity water for preparation of samples and standards.

c. Stock, standard, and other required solutions: See Section 3120B.3d for preparation of standard stock solutions from elemental materials (pure metals, salts). Preferably, purchase high-purity commercially prepared stock solutions and dilute to required concentrations. Single- or multi-element stock solutions (1000 mg/L) of the following elements are required: aluminum, antimony, arsenic, barium, beryllium, cerium, cadmium, chromium, cobalt, copper, germanium, indium, lead, magnesium, manganese, molybdenum, nickel, rhodium, scandium, selenium, silver, strontium, terbium, thallium, thorium, uranium, vanadium, and zinc. Prepare internal standard stock separately from target element stock solution. The potential for incompatibility between target elements and/or internal standards exists, and could cause precipitation or other solution instability.

1) *Internal standard stock solution:* Lithium, scandium, germanium, indium, and thorium are suggested as internal standards. The following masses are monitored: ^6Li , ^{45}Sc , ^{72}Ge , ^{115}In , and ^{232}Th . Add to all samples, standards, and quality control (QC) samples a level of internal standard that will give a suitable counts/second (cps) signal (for most internal standards, 200 000 to 500 000 cps; for lithium, 20 000 to 70 000 cps). Minimize error introduced by dilution during this addition by using an appropriately high concentration of internal standard mix solution. Maintain volume ratio for all internal standard additions.

Prepare internal standard mix as follows: Prepare a nominal 50-mg/L solution of ^6Li by dissolving 0.15 g $^6\text{Li}_2\text{CO}_3$ (isotopically pure, i.e., 95% or greater purity†#(88)) in a minimal amount of 1:1 HNO_3 . Pipet 5.0 mL 1000-mg/L scandium, germanium, indium, and thorium standards into the lithium solution, dilute resulting solution to 500.0 mL, and mix thoroughly. The resultant concentration of Sc, Ge, In, and Th will be 10 mg/L. Older instruments may require higher levels of internal standard to achieve acceptable levels of precision.

Other internal standards, such as rhodium, yttrium, terbium, holmium, and bismuth may also be used in this method. Ensure that internal standard mix used is stable and that there are no undesired interactions between elements.

Screen all samples for internal standard elements before analysis. The analysis of a few representative samples for internal standards should be sufficient. Analyze samples “as received” or “as digested” (before addition of internal standard), then add internal standard mix and reanalyze. Monitor counts at the internal standard masses. If the “as received” or “as

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digested'' samples show appreciable detector counts (10% or higher of samples with added internal standard), dilute sample or use an alternate internal standard. If the internal standard response of the sample with the addition is not within 70 to 125% of the response for a calibration blank with the internal standard added, either dilute the sample before analysis, or use an alternate internal standard. During actual analysis, monitor internal standard masses and note all internal standard recoveries over 125% of internal standard response in calibration blank. Interpret results for these samples with caution.

The internal standard mix may be added to blanks, standards, and samples by pumping the solution so it is mixed with the sample stream in the sample introduction process.

2) *Instrument optimization/tuning solution*, containing the following elements: barium, beryllium, cadmium, cerium, cobalt, copper, germanium, indium, magnesium, rhodium, scandium, terbium, thallium, and lead. Prepare this solution in 2% HNO₃. This mix includes all common elements used in optimization and tuning of the various ICP/MS operational parameters. It may be possible to use fewer elements in this solution, depending on the instrument manufacturer's recommendations.

3) *Calibration standards*, 0, 5, 10, 20, 50, and 100 µg/L. (89) Other calibration regimes are acceptable, provided the full suite of quality assurance samples and standards is run to validate these method changes. Fewer standards may be used, and a two-point blank/mid-range calibration technique commonly used in ICP optical methods should also produce acceptable results. Calibrate all analytes using the selected concentrations. Prepare all calibration standards and blanks in a matrix of 2% nitric acid. Add internal standard mix to all calibration standards to provide appropriate count rates for interference correction. NOTE: All standards and blanks used in this method have the internal standard mix added at the same ratio.

4) *Method blank*, consisting of reagent water (¶ 3b) taken through entire sample preparation process. For dissolved samples, take reagent water through same filtration and preservation processes used for samples. For samples requiring digestion, process reagent water with the same digestion techniques as samples. Add internal standard mix to method blank.

5) *Calibration verification standard*: Prepare a mid-range standard, from a source different from the source of the calibration standards, in 2% HNO₃, with equivalent addition of internal standard.

6) *Calibration verification blank*: Use 2% HNO₃.

7) *Laboratory fortified blank* (optional): Prepare solution with 2% nitric acid and method analytes added at about 50 µg/L. This standard, sometimes called a laboratory control sample (LCS), is used to validate digestion techniques and known-addition levels.

8) *Reference materials*: Externally prepared reference material, preferably from National Institute of Standards and Technology (NIST) 1643 series or equivalent.

9) *Known-addition solution for samples*: Add stock standard to sample in such a way that volume change is less than 5%. In the absence of information on analyte levels in the sample,

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prepare known additions at around 50 µg/L. If analyte concentration levels are known, add at 50 to 200% of the sample levels. For samples undergoing digestion, make additions before digestion. For the determination of dissolved metals, make additions after filtration, preferably immediately before analysis.

10) *Low-level standards:* Use both a 0.3- and a 1.0-µg/L standard when expected analyte concentration is below 5 µg/L. Prepare both these standards in 2% nitric acid.

Prepare volumetrically a mixed standard containing the method analytes at desired concentration(s) (0.30 µg/L, 1.0 µg/L, or both). Prepare weekly in 100-mL quantities.

d. Argon: Use a prepurified grade of argon unless it can be demonstrated that other grades can be used successfully. The use of prepurified argon is usually necessary because of the presence of krypton as an impurity in technical argon. ^{82}Kr interferes with the determination of ^{82}Se . Monitor ^{83}Kr at all times.

4. Procedures

a. Sample preparation: See Section 3010 and Section 3020 for general guidance regarding sampling and quality control. See Section 3030E for recommended sample digestion technique for all analytes except silver and antimony. If silver and antimony are target analytes, use method given in 3030F, paying special attention to interferences caused by chloride ion, and using all applicable elemental corrections. Alternative digestion techniques and additional guidance on sample preparation are available.^{3,4}

Ideally use a “clean” environment for any sample handling, manipulation, or preparation. Preferably perform all sample manipulations in a Class 100 clean hood or room to minimize potential contamination artifacts in digested or filtered samples.

b. Instrument operating conditions: Follow manufacturer’s standard operating procedures for initialization, mass calibration, gas flow optimization, and other instrument operating conditions. Maintain complete and detailed information on the operational status of the instrument whenever it is used.

c. Analytical run sequence: A suggested analytical run sequence, including instrument tuning/optimization, checking of reagent blanks, instrument calibration and calibration verification, analysis of samples, and analysis of quality control samples and blanks, is given in Table 3125:IV.

d. Instrument tuning and optimization: Follow manufacturer’s instructions for optimizing instrument performance. The most important optimization criteria include nebulizer gas flows, detector and lens voltages, radio-frequency forward power, and mass calibration. Periodically check mass calibration and instrument resolution. Ideally, optimize the instrument to minimize oxide formation and doubly-charged species formation. Measure the CeO/Ce ratio to monitor oxide formation, and measure doubly-charged species by determination of the $\text{Ba}^{2+}/\text{Ba}^{+}$ ratio. Both these ratios should meet the manufacturer’s criteria before instrument calibration. Monitor background counts at mass 220 after optimization and compare with manufacturer’s criteria. A

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summary of performance criteria related to optimization and tuning, calibration, and analytical performance for this method is given in Table 3125:V.

e. Instrument calibration: After optimization and tuning, calibrate instrument using an appropriate range of calibration standards. Use appropriate regression techniques to determine calibration lines or curves for each analyte. For acceptable calibrations, correlation coefficients for regression curves are ideally 0.995 or greater.

Immediately after calibration, run initial calibration verification standard, ¶ 3c5); acceptance criteria are $\pm 10\%$ of known analyte concentration. Next run initial calibration verification blank, ¶ 3c6); acceptance criteria are ideally $\pm$ the absolute value of the instrument detection limit for each analyte, but in practice, $\pm$ the absolute value of the laboratory reporting limit or the laboratory method detection limit for each analyte is acceptable. Verify low-level calibration by running 0.3- and/or 1.0- $\mu\text{g/L}$ standards, if analyte concentrations are less than 5 $\mu\text{g/L}$.

f. Sample analysis: Ensure that all vessels and reagents are free from contamination. During analytical run (see Table 3125:IV), include quality control analyses according to schedule of Table 3125:VI, or follow project-specific QA/QC protocols.

Internal standard recoveries must be between 70% and 125% of internal standard response in the laboratory-fortified blank; otherwise, dilute sample, add internal standard mix, and reanalyze.

Make known-addition analyses for each separate matrix in a digestion or filtration batch.

5. Calculations and Corrections

Configure instrument software to report internal standard corrected results. For water samples, preferably report results in micrograms per liter. Report appropriate number of significant figures.

a. Correction for dilutions and solids: Correct all results for dilutions, and raise reporting limit for all analytes reported from the diluted sample by a corresponding amount. Similarly, if results for solid samples are to be determined, use Method 2540B to determine total solids. Report results for solid samples as micrograms per kilogram, dry weight. Correct all results for solids content of solid samples. Use the following equation to correct solid or sediment sample results for dilution during digestion and moisture content:

$$R_{corr} = \frac{R_{uncorr} \times V}{W \times \% TS/100}$$

where:

R_{corr} = corrected result, $\mu\text{g/kg}$,

R_{uncorr} = uncorrected elemental result, $\mu\text{g/L}$,

V = volume of digestate (after digestion), L,

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W = mass of the wet sample, kg, and

% TS = percent total solids determined in the solid sample.

b. Compensation for interferences: Use instrument software to correct for interferences listed previously for this method. See Table 3125:III for a listing of the most common molecular ion interferences.

c. Data reporting: Establish appropriate reporting limits for method analytes based on instrument detection limits and the laboratory blank. For regulatory programs, ensure that reporting limits for method analytes are a factor of three below relevant regulatory criteria.

If method blank contamination is typically random, sporadic, or otherwise not in statistical control, do not correct results for the method blank. Consider the correction of results for laboratory method blanks only if it can be demonstrated that the concentration of analytes in the method blank is within statistical control over a period of months. Report all method blank data explicitly in a manner identical to sample reporting procedures.

d. Documentation: Maintain documentation for the following (where applicable): instrument tuning, mass calibration, calibration verification, analyses of blanks (method, field, calibration, and equipment blanks), IDL and MDL studies, analyses of samples and duplicates with known additions, laboratory and field duplicate information, serial dilutions, internal standard recoveries, and any relevant quality control charts.

Also maintain, and keep available for review, all raw data generated in support of the method.⁵

6. Method Performance

Table 3125:I presents instrument detection limit (IDL) data generated by this method; this represents optimal state-of-the-art instrument detection capabilities, not recommended method detection or reporting limits. Table 3125:VII through IX contain single-laboratory, single-operator, single-instrument performance data generated by this method for calibration verification standards, low-level standards, and known-addition recoveries for fresh-water matrices. Performance data for this method for some analytes are not currently available. However, performance data for similar ICP/MS methods are available in the literature.^{1,4}

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3130 METALS BY ANODIC STRIPPING VOLTAMMETRY*(90)

3130 A. Introduction

Anodic stripping voltammetry (ASV) is one of the most sensitive metal analysis techniques; it is as much as 10 to 100 times more sensitive than electrothermal atomic absorption spectroscopy for some metals. This corresponds to detection limits in the nanogram-per-liter range. The technique requires no sample extraction or preconcentration, it is nondestructive, and it allows simultaneous determination of four to six trace metals, utilizing inexpensive instrumentation. The disadvantages of ASV are that it is restricted to amalgam-forming metals, analysis time is longer than for spectroscopic methods, and interferences and high sensitivity can present severe limitations. The analysis should be performed only by analysts skilled in ASV methodology because of the interferences and potential for trace background contamination.

3130 B. Determination of Lead, Cadmium, and Zinc

1. General Discussion

a. Principle: Anodic stripping voltammetry is a two-step electroanalytical technique. In the preconcentration step, metal ions in the sample solution are reduced at negative potential and concentrated into a mercury electrode. The concentration of the metal in the mercury is 100 to 1000 times greater than that of the metal ion in the sample solution. The preconcentration step is followed by a stripping step applying a positive potential scan. The amalgamated metal is oxidized rapidly and the accompanying current is proportional to metal concentration.

b. Detection limits and working range: The limit of detection for metal determination using ASV depends on the metal determined, deposition time, stirring rate, solution pH, sample matrix, working electrode (hanging mercury drop electrode, HMDE, or thin mercury film electrode, TMFE), and mode of the stripping potential scan (square wave or differential pulse). Cadmium, lead, and zinc are concentrated efficiently during pre-electrolysis because of their high solubility

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in mercury and thus have low detection limits ($<1 \mu\text{g/L}$). Long deposition times and high stirring rates increase the concentration of metal preconcentrated in the mercury phase and reduce detection limits. The effects of solution pH and matrix are more complicated. In general, add a high concentration of inert electrolyte to samples to maintain a high, constant ionic strength. Acidify sample to a low pH or add a pH buffer. If the pH buffer or other component of the sample matrix complexes the metal (3130B.1c), detection limits often are increased.

The choice of working electrode is determined largely by the working range of concentration required. The HMDE is best suited for analysis from approximately $1 \mu\text{g/L}$ to 10 mg/L , while the TMFE is superior for detection below $1 \mu\text{g/L}$.

c. Interferences: Major interferences include intermetallic compound formation, overlapping stripping peaks, adsorption of organics, and complexation. Intermetallic compounds can form in the mercury phase when high concentrations of certain metals are present simultaneously. Zinc forms intermetallic compounds with cobalt and nickel, and both zinc and cadmium form intermetallic compounds with copper, silver, and gold. As a result, the stripping peak for the constituent metals may be severely depressed or shifted and additional peaks due to intermetallic compound stripping may be observed. Minimize or avoid intermetallic compound formation by use of a hanging mercury drop electrode instead of a thin film mercury electrode when metal concentrations are above $1 \mu\text{g/L}$, application of a preconcentration potential sufficiently negative to reduce the desired but not the interfering metal, and use of a relatively short preconcentration period followed by a relatively large pulse modulation (50 mV) during the stripping stage. In general, suspect formation of intermetallic compounds if metals are present in concentrations above 1 mg/L . If metals are present at concentrations above 10 mg/L , do not use anodic stripping voltammetry. Concentrations above 10 mg/L usually can be quantitated by methods such as those given in Section 3111 and Section 3120.

Separate overlapping stripping peaks by various methods, including appropriate choice of buffer and electrolyte.¹⁻³ If only one of the metal peaks is of interest, eliminate interfering peaks by selective complexation with a suitable ligand, such as EDTA. Judicious choice of preconcentration potential can result in the deposition of the selected metal but not the interfering metal in the mercury electrode. Selection of buffer/ligand also may help to distinguish metals during the preconcentration step. Alternatively, use “medium exchange,” in which preconcentration is performed with the electrodes in the sample and stripping is performed in a different electrolyte solution. In this procedure, metals are deposited from the sample into the amalgam as usual, but they may be stripped into a medium that provides different stripping peak potentials for the overlapping metals.

Minimize interferences from adsorption of organic compounds and complexation by removal of the organic matter. Digest samples with high-purity acids as described in Section 3030. Make standard additions to determine if complexation or adsorption remains a problem. Analyze a metal-free solution with a matrix similar to that of the sample both before and after addition of known quantities of standard. Repeat procedure for sample. If the slope of the stripping current versus added metal is significantly different in the sample relative to the metal-free solution,

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digest sample further. The choice of stripping waveform also is important. While both square-wave and differential-pulse stripping attempt to minimize the contribution of adsorption currents to the total measured stripping current, square-wave stripping does this more effectively. Thus, use square-wave stripping instead of differential-pulse stripping when adsorption occurs.

2. Apparatus

a. Electrochemical analyzer: The basic electrochemical analyzer for ASV applications contains a three-electrode potentiostat, which very precisely controls potential applied to the working electrode relative to the reference electrode, and a sensitive current measuring device. It is capable of delivering potential pulses of various amplitudes and frequencies, and provides several scan rates and current ranges. More advanced ASV instruments offer automated timing, gas purge and stirring, and data processing routines including curve smoothing, baseline correction, and background subtraction.

Two variations of stripping waveforms are commonly used: differential pulse (DPASV) and square wave (SWASV) waveforms. The differential-pulse waveform consists of a series of pulses superimposed on a linear voltage ramp, while the square-wave waveform consists of a series of pulses superimposed on a staircase potential waveform. Square-wave stripping is significantly faster than differential-pulse stripping and is typically ten times more sensitive. Most commercially available ASV instruments perform both differential-pulse and square-wave stripping.

b. Electrodes and cell: Provide working, reference, and auxiliary electrodes. Working electrodes are either hanging mercury drop or thin mercury film electrodes. Hanging mercury drop electrodes must be capable of dispensing mercury in very precisely controlled drop sizes. Three types of electrodes meet this requirement: static mercury drop electrodes, controlled growth mercury drop electrodes, or Kemula-type electrodes. In any case, use a drop knocker to remove an old drop before dispensing a fresh mercury drop.

When the lowest detection limits are required, a thin mercury film electrode is preferred. This electrode consists of a rotating glassy carbon disk plated with mercury in situ during preconcentration of the analyte. A high-precision, constant-speed rotator controls the rotation rate of the electrode and provides reproducible mass transport.

Reference electrodes may be either saturated calomel or silver/ silver chloride electrodes. Use a platinum wire for the auxiliary electrode.

Use cells constructed of glass, or preferably fused silica or TFE, because they are more resistant to solution adsorption or leaching. Cover cell with a lid that provides reproducible placement of the electrodes and gas purging tubes. Provide an additional hole in the cell lid for addition of standards. Most commercially available mercury drop electrodes include electrolytic cells, reference and auxiliary electrodes, and gas purging tubes.

Use a constant-speed stirring mechanism to provide reproducible mass transport in samples and standards.

Locate the cell in an area where temperature is relatively constant. Alternatively, use a

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constant-temperature water bath and cell jacket.

c. *Oxygen-removal apparatus*: Oxygen interferes in electrochemical analyses; remove it from solution before preconcentration by purging with nitrogen or argon. Provide two gas inlet tubes through the cell lid: one extends into the solution and the second purges the space above the solution. A gas outlet hole in the lid provides for removal of oxygen and excess purging gas.

d. *Recording device*: If the electrochemical analyzer is not equipped with a digital data acquisition system, use an XY plotter to record stripping voltammograms.

e. *Timer*: If preconcentration and equilibration periods are not controlled by the instrument, use an accurate timing device.

f. *Polishing wheel*: To obtain the high polish required for a glassy carbon disk electrode, use a motorized polishing wheel.

3. Reagents

CAUTION: *Follow proper practices for disposal of any solutions containing mercury.*

a. *Metal-free water*: Use deionized water to prepare buffers, electrolytes, standards, etc. Use water with at least 18 megohm-cm resistivity (see Section 1080).

b. *Nitric acid*, HNO_3 , conc, high-purity.*#(91)

c. *Nitric acid*, HNO_3 , 6N, 1.6N (10%), and 0.01N.

d. *Purging gas (nitrogen or argon)*, high-purity. Remove traces of oxygen in nitrogen or argon gas before purging the solution. Pass gas through sequential scrubbing columns containing vanadous chloride in the first, deionized water in the second, and buffer (or electrolyte) solution in the third column.

e. *Metal standards*: Prepare stock solutions containing 1 mg metal/mL in polyethylene bottles. Purchase these solutions commercially or prepare as in Section 3111. Daily prepare dilutions of stock standards in a matrix similar to that of the samples to cover the concentration range desired.

f. *Electrolyte/buffer*: Use one of the following:

1) *Acetate buffer*, pH 4.5: Dissolve 16.4 g anhydrous sodium acetate, $\text{NaC}_2\text{H}_3\text{O}_2$, in 800 mL water. Adjust to pH 4.5 with high-purity glacial acetic acid.*#(92) Dilute to 1 L with water.

2) *Citrate buffer*, pH 3: Dissolve 42.5 g citric acid (monohydrate) in 700 mL water. Adjust to pH 3 with high-purity NH_4OH .*(93) Dilute to 1 L with water.

3) *Phosphate buffer*, pH 6.8: Dissolve 24 g NaH_2PO_4 in 500 mL water. Adjust to pH 6.8 with 1N NaOH. Dilute to 1 L with water.

g. *Mercury*: Use commercially available triply distilled metallic mercury for hanging mercury drop electrodes. CAUTION: *Mercury vapors are highly toxic. Use only in well-ventilated area.*

h. *Mercuric nitrate solution*, $\text{Hg}(\text{NO}_3)_2$: For thin mercury film electrodes, dissolve 0.325 g

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$\text{Hg}(\text{NO}_3)_2$ in 100 mL 0.01N HNO_3 .

i. *Reference electrode filling solution:* Available from electrode manufacturer.

j. *Amalgamated zinc:* Dissolve 2 g $\text{Hg}(\text{NO}_3)_2$ in 25 mL conc HNO_3 ; dilute to 250 mL with water. In a separate beaker, clean approximately 50 g mossy zinc by gently oxidizing the surface with 10% HNO_3 and rinse with water. Add $\text{Hg}(\text{NO}_3)_2$ solution to cleaned zinc and stir with a glass rod. If barely visible bubbles do not appear, add a small amount of 6N HNO_3 . Zinc should rapidly acquire a shiny, metallic appearance. Decant solution and store for amalgamating future batches of zinc. Rinse amalgamated zinc copiously with water and transfer to a gas scrubbing column.

k. *Hydrochloric acid, HCl, conc.*

l. *Vanadous chloride:* Add 2 g ammonium metavanadate, NH_4VO_3 , to 25 mL conc HCl and heat to boiling. Solution should turn blue-green. Dilute to 250 mL with water. Pour solution into gas scrubbing column packed with amalgamated zinc and bubble purging gas through it until the solution turns a clear violet color. When the violet color is replaced by a blue, green, or brown color, regenerate vanadous chloride by adding HCl.

m. *Siliconizing solution:* Preferably use commercially available solutions in sealed ampules for siliconizing capillaries used for hanging mercury drops. CAUTION: *Most commercial siliconizing reagents contain CCl_4 , a toxic and cancer-suspect agent. Handle with gloves and avoid breathing vapors.*

n. *Alumina suspensions, 1, 0.3, and 0.05 μm .* Use commercially available alumina suspensions in water, or make a suspension by adding a small amount of water to the alumina.

o. *Hydrofluoric acid, HF, 5%:* Dilute 5 mL conc HF to 100 mL with water.

p. *Methanol.*

q. *Sodium hydroxide, NaOH, 1N.*

4. Procedure

a. *Sample preparation and storage:* Collect samples in pre-cleaned, acid-soaked polyethylene or TFE bottles. Add 2 mL conc HNO_3 /L sample and mix well. Cap tightly and store in refrigerator or freezer until ready for analysis.

b. *Cell preparation:* Soak clean cell in 6N HNO_3 overnight and rinse well with water before use.

c. *Electrode preparation:*

1) HMDE—Follow manufacturer's guidance for capillary cleaning. If not available, use the following procedure. Remove all mercury from the capillary. Aspirate the following through the capillary in the order listed: 6N HNO_3 , water, 5% HF, water, methanol, and air. Dry capillary at 100°C for 1 h. Siliconize cooled capillary using a siliconizing solution. Between uses, fill

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capillary with clean mercury and immerse tip in clean mercury. If the capillary fails to suspend a drop of mercury, repeat cleaning.

2) TMFE—Polish glassy carbon disks used for thin mercury film electrodes to a high metallic sheen with alumina suspensions, progressively decreasing particle size from 1 μm to 0.05 μm . Use a motorized polishing wheel for best results. Completely rinse off all traces of alumina with water. Check disk frequently for etching or pitting; repolish as necessary to maintain reproducible mercury film deposition.

d. *Instrumental conditions:* Use the following conditions:

Variable	Value
Initial potential	−1.00 V (Pb, Cd); −1.20 V (Zn)
Final potential	0.00 V
Equilibration potential	−1.00 V (Pb, Cd); −1.20 V (Zn)
HMDE drop size	medium
TMFE rotation rate	2000 rpm
DPASV:	
Pulse amplitude	25 mV
Pulse period	0.5 s
Pulse width	50 ms
Sample width	17 ms
Scan rate	5 mV/s
SWASV:	
SW amplitude	25 mV
Step potential	4 mV
Frequency	100 Hz

e. *Deoxygenation:* Pipet 2 mL sample and 3 mL electrolyte/ buffer into cell. If using a TMFE, add 10 μL $\text{Hg}(\text{NO}_3)_2$ solution. Place electrodes in cell and secure cell lid. Deoxygenate solution with purified purging gas for 10 min while stirring. When solution purge is completed, purge the space above the solution with purified purging gas. Continue head-space purge throughout analysis.

f. *Preconcentration:* If using a HMDE, dispense a new mercury drop. Start preconcentration, stirring and timing simultaneously. Precisely control and keep constant preconcentration times and stirring rates for solutions and standards. Generally use 120 s and a rotation rate of 2000 rpm for the TMFE.

After the metal is sufficiently concentrated in the amalgam, stop stirring or TMFE rotation

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for an equilibration period of precisely 30 s.

g. Anodic stripping: After equilibration period, begin anodic stripping without stirring and make potential at the working electrode progressively more positive as a function of time. Monitor stripping current and plot as a function of applied potential in stripping voltammograms. Use peak current to quantify metal concentration and peak potential to identify the metal.

h. Add 5 to 50 μL standard solution and repeat analysis, beginning with deoxygenation of sample. Adjust volume of added standard solution to obtain 30 to 70% increase of the stripping peak. If 50 μL addition is not sufficient, use standard solution with a higher concentration of metal. Shorten deoxygenation step to 1 min after initial gas purge.

5. Calculations

Calculate the concentration of metal in the original sample using the following equation:

$$C_o = \frac{C_s \times V_s}{V_o} \times \frac{i_o}{(i_s - i_o)}$$

where:

C_o = concentration of metal in sample, mg/ L,

C_s = concentration of metal in standard solution, mg/L,

i_o = stripping peak height in original sample,

i_s = stripping peak height in sample with standard addition,

V_o = volume of sample, mL, and

V_s = volume of standard solution added, mL.

6. Quality Control

Follow quality control guidelines outlined in Section 3020 with respect to use of additions, duplicates, and blanks for best results. Blanks are critical because of the high sensitivity of the method.

7. Precision and Bias

Table 3130:I gives precision data for analyses of samples with various matrices.

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3500-AI ALUMINUM*#(94)

3500-AI A. Introduction

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1. Occurrence and Significance

Aluminum (Al) is the second element in Group IIIA of the periodic table; it has an atomic number of 13, an atomic weight of 26.98, and a valence of 3. The average abundance in the earth's crust is 8.1%; in soils it is 0.9 to 6.5%; in streams it is 400 µg/L; in U.S. drinking waters it is 54 µg/L, and in groundwater it is <0.1 µg/L. Aluminum occurs in the earth's crust in combination with silicon and oxygen to form feldspars, micas, and clay minerals. The most important minerals are bauxite and corundum, which is used as an abrasive. Aluminum and its alloys are used for heat exchangers, aircraft parts, building materials, containers, etc. Aluminum potassium sulfate (alum) is used in water-treatment processes to flocculate suspended particles, but it may leave a residue of aluminum in the finished water.

Aluminum's occurrence in natural waters is controlled by pH and by very finely suspended mineral particles. The cation Al^{3+} predominates at pH less than 4. Above neutral pH, the predominant dissolved form is $\text{Al}(\text{OH})_4^-$. Aluminum is nonessential for plants and animals. Concentrations exceeding 1.5 mg/L constitute a toxicity hazard in the marine environment, and levels below 200 µg/L present a minimal risk. The United Nations Food and Agriculture Organization's recommended maximum level for irrigation waters is 5 mg/L. The possibility of a link between elevated aluminum levels in brain tissues and Alzheimer's disease has been raised. The proposed U.S. EPA secondary drinking water standard MCL is 0.05 mg/L.

2. Selection of Method

The atomic absorption spectrometric methods (Section 3111D and Section 3111E, and Section 3113B) and the inductively coupled plasma methods (Section 3120 and Section 3125) are free from such common interferences as fluoride and phosphate, and are preferred. The Eriochrome cyanine R colorimetric method (B) provides a means for estimating aluminum with simpler instrumentation.

3500-AL B. Eriochrome Cyanine R Method

1. General Discussion

a. Principle: With Eriochrome cyanine R dye, dilute aluminum solutions buffered to a pH of 6.0 produce a red to pink complex that exhibits maximum absorption at 535 nm. The intensity of the developed color is influenced by the aluminum concentration, reaction time, temperature, pH, alkalinity, and concentration of other ions in the sample. To compensate for color and turbidity, the aluminum in one portion of sample is complexed with EDTA to provide a blank. The interference of iron and manganese, two elements commonly found in water when aluminum is present, is eliminated by adding ascorbic acid. The optimum aluminum range lies between 20 and 300 µg/L but can be extended upward by sample dilution.

b. Interference: Negative errors are caused by both fluoride and polyphosphates. When the fluoride concentration is constant, the percentage error decreases with increasing amounts of

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aluminum. Because the fluoride concentration often is known or can be determined readily, fairly accurate results can be obtained by adding the known amount of fluoride to a set of standards. A simpler correction can be determined from the family of curves in Figure 3500-Al:1. A procedure is given for the removal of complex phosphate interference. Orthophosphate in concentrations under 10 mg/L does not interfere. The interference caused by even small amounts of alkalinity is removed by acidifying the sample just beyond the neutralization point of methyl orange. Sulfate does not interfere up to a concentration of 2000 mg/L.

c. Minimum detectable concentration: The minimum aluminum concentration detectable by this method in the absence of fluorides and complex phosphates is approximately 6 µg/L.

d. Sample handling: Collect samples in clean, acid-rinsed bottles, preferably plastic, and examine them as soon as possible after collection. If only soluble aluminum is to be determined, filter a portion of sample through a 0.45-µm membrane filter; discard first 50 mL of filtrate and use succeeding filtrate for the determination. Do not use filter paper, absorbent cotton, or glass wool for filtering any solution that is to be tested for aluminum, because they will remove most of the soluble aluminum.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

- 1) *Spectrophotometer*, for use at 535 nm, with a light path of 1 cm or longer.
- 2) *Filter photometer*, providing a light path of 1 cm or longer and equipped with a green filter with maximum transmittance between 525 and 535 nm.
- 3) *Nessler tubes*, 50-mL, tall form, matched.

b. Glassware: Treat all glassware with warm 1 + 1 HCl and rinse with aluminum-free distilled water to avoid errors due to materials absorbed on the glass. Rinse sufficiently to remove all acid.

3. Reagents

Use reagents low in aluminum, and aluminum-free distilled water.

a. Stock aluminum solution: Use either the metal (1) or the salt (2) for preparing stock solution; 1.00 mL = 500 µg Al:

- 1) Dissolve 500.0 mg aluminum metal in 10 mL conc HCl by heating gently. Dilute to 1000 mL with water, or
- 2) Dissolve 8.791 g aluminum potassium sulfate (also called potassium alum), $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, in water and dilute to 1000 mL. Correct this weight by dividing by the decimal fraction of assayed $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ in the reagent used.

b. Standard aluminum solution: Dilute 10.00 mL stock aluminum solution to 1000 mL with water; 1.00 mL = 5.00 µg Al. Prepare daily.

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c. *Sulfuric acid*, H_2SO_4 , 0.02N and 6N.

d. *Ascorbic acid solution*: Dissolve 0.1 g ascorbic acid in water and make up to 100 mL in a volumetric flask. Prepare fresh daily.

e. *Buffer reagent*: Dissolve 136 g sodium acetate, $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$, in water, add 40 mL 1N acetic acid, and dilute to 1 L.

f. *Stock dye solution*: Use any of the following products:

1) *Solochrome cyanine R-200**#(95) or *Eriochrome cyanine*:†#(96) Dissolve 100 mg in water and dilute to 100 mL in a volumetric flask. This solution should have a pH of about 2.9.

2) *Eriochrome cyanine R*:‡#(97) Dissolve 300 mg dye in about 50 mL water. Adjust pH from about 9 to about 2.9 with 1 + 1 acetic acid (approximately 3 mL will be required). Dilute with water to 100 mL.

3) *Eriochrome cyanine R*:§#(98) Dissolve 150 mg in about 50 mL water. Adjust pH from about 9 to about 2.9 with 1 + 1 acetic acid (approximately 2 mL will be required). Dilute with water to 100 mL.

Stock solutions have excellent stability and can be kept for at least a year.

g. *Working dye solution*: Dilute 10.0 mL of selected stock dye solution to 100 mL in a volumetric flask with water. Working solutions are stable for at least 6 months.

h. *Methyl orange indicator solution*, or bromcresol green indicator solution specified in the total alkalinity determination (Section 2320B.3d).

i. *EDTA (sodium salt of ethylenediamine-tetraacetic acid dihydrate)*, 0.01M: Dissolve 3.7 g in water, and dilute to 1 L.

j. *Sodium hydroxide*, NaOH, 1N and 0.1N.

4. Procedure

a. *Preparation of calibration curve*:

1) Prepare a series of aluminum standards from 0 to 7 μg (0 to 280 $\mu\text{g/L}$ based on a 25-mL sample) by accurately measuring the calculated volumes of standard aluminum solution into 50-mL volumetric flasks or nessler tubes. Add water to a total volume of approximately 25 mL.

2) Add 1 mL 0.02N H_2SO_4 to each standard and mix. Add 1 mL ascorbic acid solution and mix. Add 10 mL buffer solution and mix. With a volumetric pipet, add 5.00 mL working dye reagent and mix. Immediately make up to 50 mL with distilled water. Mix and let stand for 5 to 10 min. The color begins to fade after 15 min.

3) Read transmittance or absorbance on a spectrophotometer, using a wavelength of 535 nm or a green filter providing maximum transmittance between 525 and 535 nm. Adjust instrument to zero absorbance with the standard containing no aluminum.

Plot concentration of Al (micrograms Al in 50 mL final volume) against absorbance.

b. *Sample treatment in absence of fluoride and complex phosphates*: Place 25.0 mL sample,

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or a portion diluted to 25 mL, in a porcelain dish or flask, add a few drops of methyl orange indicator, and titrate with 0.02N H₂SO₄ to a faint pink color. Record reading and discard sample. To two similar samples at room temperature add the same amount of 0.02N H₂SO₄ used in the titration and 1 mL in excess.

To one sample add 1 mL EDTA solution. This will serve as a blank by complexing any aluminum present and compensating for color and turbidity. To both samples add 1 mL ascorbic acid, 10 mL buffer reagent, and 5.00 mL working dye reagent as prescribed in ¶ a2) above.

Set instrument to zero absorbance or 100% transmittance using the EDTA blank. After 5 to 10 min contact time, read transmittance or absorbance and determine aluminum concentration from the calibration curve previously prepared.

c. *Visual comparison:* If photometric equipment is not available, prepare and treat standards and a sample, as described above, in 50-mL nessler tubes. Make up to mark with water and compare sample color with the standards after 5 to 10 min contact time. A sample treated with EDTA is not needed when nessler tubes are used. If the sample contains turbidity or color, the use of nessler tubes may result in considerable error.

d. *Removal of phosphate interference:* Add 1.7 mL 6N H₂SO₄ to 100 mL sample in a 200-mL erlenmeyer flask. Heat on a hot plate for at least 90 min, keeping solution temperature just below the boiling point. At the end of the heating period solution volume should be about 25 mL. Add water if necessary to keep it at or above that volume.

After cooling, neutralize to a pH of 4.3 to 4.5 with NaOH, using 1N NaOH at the start and 0.1N for the final fine adjustment. Monitor with a pH meter. Make up to 100 mL with water, mix, and use a 25-mL portion for the aluminum test.

Run a blank in the same manner, using 100 mL distilled water and 1.7 mL 6N H₂SO₄. Subtract blank reading from sample reading or use it to set instrument to zero absorbance before reading the sample.

e. *Correction for samples containing fluoride:* Measure sample fluoride concentration by the SPADNS or electrode method. Either:

- 1) Add the same amount of fluoride as in the sample to each aluminum standard, or
- 2) Determine fluoride correction from the set of curves in Figure 3500-Al:1.

5. Calculation

$$\text{mg Al/L} = \frac{\mu\text{g Al (in 50 mL final volume)}}{\text{mL sample}}$$

6. Precision and Bias

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A synthetic sample containing 520 µg Al/L and no interference in distilled water was analyzed by the Eriochrome cyanine R method in 27 laboratories. Relative standard deviation was 34.4% and relative error 1.7%.

A second synthetic sample containing 50 µg Al/L, 500 µg Ba/L, and 5 µg Be/L in distilled water was analyzed in 35 laboratories. Relative standard deviation was 38.5% and relative error 22.0%.

A third synthetic sample containing 500 µg Al/L, 50 µg Cd/L, 110 µg Cr/L, 1000 µg Cu/L, 300 µg Fe/L, 70 µg Pb/L, 50 µg Mn/L, 150 µg Ag/L, and 650 µg Zn/L in distilled water was analyzed in 26 laboratories. Relative standard deviation was 28.8% and relative error 6.2%.

A fourth synthetic sample containing 540 µg Al/L and 2.5 mg polyphosphate/L in distilled water was analyzed in 16 laboratories that hydrolyzed the sample in the prescribed manner. Relative standard deviation was 44.3% and relative error 1.3%. In 12 laboratories that applied no corrective measures, the relative standard deviation was 49.2% and the relative error 8.9%.

A fifth synthetic sample containing 480 µg Al/L and 750 µg F/L in distilled water was analyzed in 16 laboratories that relied on the curve to correct for the fluoride content. Relative standard deviation was 25.5% and relative error 2.3%. The 17 laboratories that added fluoride to the aluminum standards showed a relative standard deviation of 22.5% and a relative error of 7.1%.

7. Bibliography

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3500-Sb ANTIMONY

Antimony (Sb) is the fourth element in Group VA in the periodic table; it has an atomic number of 51, an atomic weight of 121.75, and valences of 3 and 5. The average abundance of Sb in the earth's crust is 0.2 ppm; in soils it is 1 ppm; in streams it is 1 µg/L, and in groundwaters it is <0.1 mg/L. Antimony is sometimes found native, but more commonly in stibnite (Sb₂S₃). It is used in alloys of lead and in batteries, bullets, solder, pyrotechnics, and semiconductors.

The common aqueous species are SbO₂⁻, HSbO₂, and complexes with carbonate and sulfate. Soluble salts of antimony are toxic. The U.S. EPA primary drinking water standard MCL is 6 µg/L.

The electrothermal atomic absorption spectrometric method (Section 3113B) or the inductively coupled plasma/mass spectrometric method (Section 3125) are the methods of choice because of their sensitivity. Alternatively use the flame atomic absorption spectrometric method (Section 3111B) or the inductively coupled plasma method (Section 3120) when high sensitivity is not required.

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3500-As ARSENIC*#(99)

3500-As A. Introduction

1. Occurrence and Significance

Arsenic (As) is the third element in Group VA of the periodic table; it has an atomic number of 33, an atomic weight of 74.92, and valences of 3 and 5. The average abundance of As in the earth's crust is 1.8 ppm; in soils it is 5.5 to 13 ppm; in streams it is less than 2 µg/L, and in groundwater it is generally less than 100 µg/L. It occurs naturally in sulfide minerals such as pyrite. Arsenic is used in alloys with lead, in storage batteries, and in ammunition. Arsenic compounds are widely used in pesticides and in wood preservatives.

Arsenic is nonessential for plants but is an essential trace element in several animal species. The predominant form between pH 3 and pH 7 is H_2AsO_4^- , between pH 7 and pH 11 it is HAsO_4^{2-} , and under reducing conditions it is $\text{HAsO}_2(\text{aq})$ (or H_3AsO_3). Aqueous arsenic in the form of arsenite, arsenate, and organic arsenicals may result from mineral dissolution, industrial discharges, or the application of pesticides. The chemical form of arsenic depends on its source (inorganic arsenic from minerals, industrial discharges, and pesticides; organic arsenic from industrial discharges, pesticides, and biological action on inorganic arsenic).

Severe poisoning can arise from the ingestion of as little as 100 mg arsenic trioxide; chronic effects may result from the accumulation of arsenic compounds in the body at low intake levels. Carcinogenic properties also have been imputed to arsenic compounds. The toxicity of arsenic depends on its chemical form. Arsenite is many times more toxic than arsenate. For the protection of aquatic life, the average concentration of As^{3+} in water should not exceed 72 µg/L and the maximum should not exceed 140 µg/L. The United Nations Food and Agriculture Organization's recommended maximum level for irrigation waters is 100 µg/L. The U.S. EPA primary drinking water standard MCL is 0.05 mg/L.

2. Selection of Method

Methods are available to identify and determine total arsenic, arsenite, and arsenate. Unpolluted fresh water normally does not contain organic arsenic compounds, but may contain inorganic arsenic compounds in the form of arsenate and arsenite. The electrothermal atomic absorption spectrometric method (Section 3113B) is the method of choice in the absence of overwhelming interferences. The hydride generation-atomic absorption method (Section 3114B) is preferred when interferences are present that cannot be overcome by standard electrothermal techniques (e.g., matrix modifiers, background correction). The silver diethyldithiocarbamate method (B), in which arsine is generated by reaction with sodium borohydride in acidic solution, is applicable to the determination of total inorganic arsenic when interferences are absent and

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when the sample contains no methylarsenic compounds. This method also provides the advantage of being able to identify and quantify arsenate and arsenite separately by generating arsine at different pHs. The inductively coupled plasma (ICP) emission spectroscopy method (Section 3120) is useful at higher concentrations (greater than 50 µg/L) while the ICP-mass spectrometric method (Section 3125) is applicable at lower concentrations if chloride does not interfere. When measuring arsenic species, document that speciation does not change over time. No universal preservative for speciation measurements has been identified.

3500-As B. Silver Diethyldithiocarbamate Method

1. General Discussion

a. Principle: Arsenite, containing trivalent arsenic, is reduced selectively by aqueous sodium borohydride solution to arsine, AsH_3 , in an aqueous medium of pH 6. Arsenate, methylarsonic acid, and dimethylarsenic acid are not reduced under these conditions. The generated arsine is swept by a stream of oxygen-free nitrogen from the reduction vessel through a scrubber containing glass wool or cotton impregnated with lead acetate solution into an absorber tube containing silver diethyldithiocarbamate and morpholine dissolved in chloroform. The intensity of the red color that develops is measured at 520 nm. To determine total inorganic arsenic in the absence of methylarsenic compounds, a sample portion is reduced at a pH of about 1. Alternatively, arsenate is measured in a sample from which arsenite has been removed by reduction to arsine gas at pH 6 as above. The sample is then acidified with hydrochloric acid and another portion of sodium borohydride solution is added. The arsine formed from arsenate is collected in fresh absorber solution.

b. Interferences: Although certain metals—chromium, cobalt, copper, mercury, molybdenum, nickel, platinum, silver, and selenium—influence the generation of arsine, their concentrations in water are seldom high enough to interfere, except in the instance of acid rock drainage. H_2S interferes, but the interference is removed with lead acetate. Antimony is reduced to stibine, which forms a colored complex with an absorption maximum at 510 nm and interferes with the arsenic determination. Methylarsenic compounds are reduced at pH 1 to methylarsines, which form colored complexes with the absorber solution. If methylarsenic compounds are present, measurements of total arsenic and arsenate are unreliable. The results for arsenite are not influenced by methylarsenic compounds.

c. Minimum detectable quantity: 1 µg arsenic.

2. Apparatus

a. Arsine generator, scrubber, and absorption tube: See Figure 3500-As:1. Use a 200-mL three-necked flask with a sidearm (19/ 22 or similar size female ground-glass joint) through which the inert gas delivery tube reaching almost to the bottom of the flask is inserted; a 24/40 female ground-glass joint to carry the scrubber; and a second side arm closed with a rubber

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septum, or preferably by a screw cap with a hole in its top for insertion of a TFE-faced silicone septum. Place a small magnetic stirring bar in the flask. Fit absorber tube (20 mL capacity) to the scrubber and fill with silver diethyldithiocarbamate solution. Do not use rubber or cork stoppers because they may absorb arsine. Clean glass equipment with concentrated nitric acid.

b. *Fume hood*: Use apparatus in a well-ventilated hood with flask secured on top of a magnetic stirrer.

c. *Photometric equipment*:

1) *Spectrophotometer*, for use at 520 nm.

2) *Filter photometer*, with green filter having a maximum transmittance in the 500- to 540-nm range.

3) *Cells*, for spectrophotometer or filter photometer, 1-cm, clean, dry, and each equipped with a tightly fitting cover (TFE stopper) to prevent chloroform evaporation.

3. Reagents

a. *Reagent water*: See Section 1080A.

b. *Acetate buffer*, pH 5.5: Mix 428 mL 0.2M sodium acetate, $\text{NaC}_2\text{H}_3\text{O}_2$, and 72 mL 0.2M acetic acid, CH_3COOH .

c. *Sodium acetate*, 0.2M: Dissolve 16.46 g anhydrous sodium acetate or 27.36 g sodium acetate trihydrate, $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$, in water. Dilute to 1000 mL with water.

d. *Acetic acid*, 0.2M: Dissolve 11.5 mL glacial acetic acid in water. Dilute to 1000 mL.

e. *Sodium borohydride solution*, 1%: Dissolve 0.4 g sodium hydroxide, NaOH (4 pellets), in 400 mL water. Add 4.0 g sodium borohydride, NaBH_4 (check for absence of arsenic). Shake to dissolve and to mix. Prepare fresh every few days.

f. *Hydrochloric acid*, HCl, 2M: Dilute 165 mL conc HCl to 1000 mL with water.

g. *Lead acetate solution*: Dissolve 10.0 g $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$ in 100 mL water.

h. *Silver diethyldithiocarbamate solution*: Dissolve 1.0 mL morpholine (CAUTION: Corrosive—avoid contact with skin) in 70 mL chloroform, CHCl_3 . Add 0.30 g silver diethyldithiocarbamate, $\text{AgSCSN}(\text{C}_2\text{H}_5)_2$; shake in a stoppered flask until most is dissolved. Dilute to 100 mL with chloroform. Filter and store in a tightly closed brown bottle in a refrigerator.

i. *Standard arsenite solution*: Dissolve 0.1734 g NaAsO_2 in water and dilute to 1000 mL with water. CAUTION: Toxic—avoid contact with skin and do not ingest. Dilute 10.0 mL to 100 mL with water; dilute 10.0 mL of this intermediate solution to 100 mL with water; 1.00 mL = 1.00 μg As.

j. *Standard arsenate solution*: Dissolve 0.416 g $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ in water and dilute to 1000 mL. Dilute 10.0 mL to 100 mL with water; dilute 10 mL of this intermediate solution to

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100 mL; 1.00 mL = 1.00 µg As.

4. Procedure

a. Arsenite:

1) Preparation of scrubber and absorber—Dip glass wool into lead acetate solution; remove excess by squeezing glass wool. Press glass wool between pieces of filter paper, then fluff it. Alternatively, if cotton is used treat it similarly but dry in a desiccator and fluff thoroughly when dry. Place a plug of loose glass wool or cotton in scrubber tube. Add 4.00 mL silver diethyldithiocarbamate solution to absorber tube (5.00 mL may be used to provide enough volume to rinse spectrophotometer cell).

2) Loading of arsine generator—Pipet not more than 70 mL sample containing not more than 20.0 µg As (arsenite) into the generator flask. Add 10 mL acetate buffer. If necessary, adjust total volume of liquid to 80 mL. Flush flask with nitrogen at the rate of 60 mL/min.

3) Arsine generation and measurement—While nitrogen is passing through the system, use a 30-mL syringe to inject through the septum 15 mL 1% sodium borohydride solution within 2 min. Stir vigorously with magnetic stirrer. Pass nitrogen through system for an additional 15 min to flush arsine into absorber solution. Pour absorber solution into a clean and dry spectrophotometric cell and measure absorbance at 520 nm against chloroform. Determine concentration from a calibration curve obtained with arsenite standards. If arsenate also is to be determined for this sample by using the same sample portion, save the liquid in the generator flask.

4) Preparation of standard curves—Treat standard arsenite solution containing 0.0, 1.0, 2.0, 5.0, 10.0, and 20.0 µg As described in ¶s 1) through 3) above. Plot absorbance versus micrograms arsenic in the standard.

b. Arsenate: After removal of arsenite as arsine, treat sample to convert arsenate to arsine:

If the lead acetate-impregnated glass wool has become ineffective in removing hydrogen sulfide (if it has become gray to black) replace glass wool [see ¶ a1)]. Pass nitrogen through system at the rate of 60 mL/min. Cautiously add 10 mL 2.0N HCl. Generate arsine as directed in ¶ 4a3) and prepare standard curves with standard solutions of arsenate according to procedure of ¶ 4a4).

c. Total inorganic arsenic: Prepare scrubber and absorber as directed in ¶ 4a1) and load arsine generator as directed in ¶ 4a2) using 10 mL 2.0N HCl instead of acetate buffer. Generate arsine and measure as directed in ¶ 4a3). Prepare standard curves according to ¶ 4a4). Curves obtained with standard arsenite solution are almost identical to those obtained with arsenate standard solutions. Therefore, use either arsenite or arsenate standards.

5. Calculation

Calculate arsenite, arsenate, and total inorganic arsenic from readings and calibration curves obtained in 4a, b, and c, respectively, as follows:

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$$\text{mg As/L} = \frac{\mu\text{g As (from calibration curve)}}{\text{mL sample in generator flask}}$$

6. Precision and Bias

Interlaboratory comparisons are not available. The relative standard deviation of results obtained with arsenite/arsenate mixtures containing approximately 10 µg arsenic was less than 10%.

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3500-Ba BARIUM

Barium (Ba) is the fifth element in Group IIA in the periodic table; it has an atomic number of 56, an atomic weight of 137.33, and a valence of 2. The average abundance of Ba in the earth's crust is 390 ppm; in soils it is 63 to 810 ppm; in streams it is 10 mg/L; in U.S. drinking waters it is 49 µg/L; and in groundwaters it is 0.05 to 1 mg/L. It is found chiefly in barite (BaSO₄) or in witherite (BaCO₃). Barium's main use is in mud slurries used in drilling oil and exploration wells, but it is also used in pigments, rat poisons, pyrotechnics, and in medicine.

The solubility of barium in natural waters is controlled by the solubility of BaSO₄, and somewhat by its adsorption on hydroxides. High concentrations of barium occur in some brines. Concentrations exceeding 1 mg/L constitute a toxicity hazard in the marine environment. The U.S. EPA primary drinking water standard MCL is 1 mg/L.

Perform analyses by the atomic absorption spectrometric methods (Section 3111D or Section 3111E), the electrothermal atomic absorption method (Section 3113B), or the inductively

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coupled plasma methods (Section 3120 or Section 3125).

3500-Be BERYLLIUM

Beryllium (Be) is the first element in Group IIA of the periodic table; it has an atomic number of 4, an atomic weight of 9.01, and a valence of 2. The average abundance of Be in the earth's crust is 2 ppm; in soils it is 0.8 to 1.3 ppm; in streams it is 0.2 µg/L, in U.S. drinking waters and in groundwaters it is typically <0.1 µg/L. Beryllium occurs in nature in deposits of beryls in granitic rocks. Beryllium is used in high-strength alloys of copper and nickel, windows in X-ray tubes, and as a moderator in nuclear reactors.

Beryllium solubility is controlled in natural waters by the solubility of beryllium hydroxides. The solubility at pH 6.0 is approximately 0.1 µg/L. It is nonessential for plants and animals. Acute toxicity occurs at 130 µg/L, and chronic toxicity at 5 µg/L in freshwater species. The United Nations Food and Agriculture Organization recommended maximum level for irrigation waters is 100 µg/L. The U.S. EPA primary drinking water standard MCL for beryllium is 4 µg/L.

The atomic absorption spectrometric methods (Section 3111D and Section 3111E, and Section 3113B) and the inductively coupled plasma (ICP) methods (Section 3120 and Section 3125) are the methods of choice. If atomic absorption or ICP instrumentation is not available, the aluminon colorimetric method detailed in the 19th Edition of *Standard Methods* may be used. This method has poorer precision and bias than the methods of choice.

3500-Bi BISMUTH

Bismuth (Bi) is the fifth element in Group VA in the periodic table; it has an atomic number of 83, an atomic weight of 208.98, and valences of 3 and 5. The average abundance of Bi in the earth's crust is 0.08 ppm; in streams it is <0.02 mg/L, and in groundwaters it is <0.1 mg/L. Bismuth occurs in association with lead and silver ores, and occasionally as the native element. ²¹⁰Bi, ²¹²Bi, and ²¹⁴Bi are naturally occurring radioisotopes produced in the decay of uranium and thorium. The metal is used in alloys of lead, tin, and cadmium, and in some pharmaceuticals.

In natural water, Bi³⁺ ion will occur, and complex ions with nitrate and chloride also might be expected. The iodide and telluride compounds are toxic by ingestion or inhalation.

Perform analyses by the atomic absorption spectrometric method (Section 3111B) or by the electrothermal atomic absorption method (Section 3113B). The inductively coupled plasma mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though bismuth is not specifically listed as an analyte in the method.

3500-B BORON

See Section 4500-B.

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3500-Cd CADMIUM

Cadmium (Cd) is the second element in Group IIB of the periodic table; it has an atomic number of 48, an atomic weight of 112.41, and a valence of 2. The average abundance of Cd in the earth's crust is 0.16 ppm; in soils it is 0.1 to 0.5 ppm; in streams it is 1 µg/L, and in groundwaters it is from 1 to 10 µg/L. Cadmium occurs in sulfide minerals that also contain zinc, lead, or copper. The metal is used in electroplating, batteries, paint pigments, and in alloys with various other metals. Cadmium is usually associated with zinc at a ratio of about 1 part cadmium to 500 parts zinc in most rocks and soils.

The solubility of cadmium is controlled in natural waters by carbonate equilibria. Guidelines for maximum cadmium concentrations in natural water are linked to the hardness or alkalinity of the water (i.e., the softer the water, the lower the permitted level of cadmium). It is nonessential for plants and animals. Cadmium is extremely toxic and accumulates in the kidneys and liver, with prolonged intake at low levels sometimes leading to dysfunction of the kidneys. The United Nations Food and Agriculture Organization recommended maximum level for cadmium in irrigation waters is 10 µg/L. The U.S. EPA primary drinking water standard MCL is 10 µg/L.

The electrothermal atomic absorption spectrometric method (Section 3113B) is preferred. The flame atomic absorption methods (Section 3111B and Section 3111C) and inductively coupled plasma methods (Section 3120 and Section 3125) provide acceptable precision and bias, with higher detection limits. Anodic stripping voltammetry (Section 3130B) can achieve superior detection limits, but is susceptible to interferences from copper, silver, gold, and organic compounds. When atomic absorption spectrometric or inductively coupled plasma apparatus is unavailable and the desired precision is not as great, the dithizone method detailed in the 19th Edition of *Standard Methods* is suitable.

3500-Ca CALCIUM*#(100)

3500-Ca A. Introduction

1. Occurrence and Significance

Calcium (Ca) is the third element in Group IIA of the periodic table; it has an atomic number of 20, an atomic weight of 40.08, and a valence of 2. The average abundance of Ca in the earth's crust is 4.9%; in soils it is 0.07 to 1.7%; in streams it is about 15 mg/L; and in groundwaters it is from 1 to >500 mg/L. The most common forms of calcium are calcium carbonate (calcite) and calcium-magnesium carbonate (dolomite). Calcium compounds are widely used in pharmaceuticals, photography, lime, de-icing salts, pigments, fertilizers, and plasters. Calcium carbonate solubility is controlled by pH and dissolved CO₂. The CO₂, HCO₃⁻,

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and CO_3^{2-} equilibrium is the major buffering mechanism in fresh waters. Hardness is based on the concentration of calcium and magnesium salts, and often is used as a measure of potable water quality.

NOTE: Calcium is necessary in plant and animal nutrition and is an essential component of bones, shells, and plant structures. The presence of calcium in water supplies results from passage over deposits of limestone, dolomite, gypsum, and gypsiferous shale. Small concentrations of calcium carbonate combat corrosion of metal pipes by laying down a protective coating. Because precipitation of calcite in pipes and in heat-exchangers can cause damage, the amount of calcium in domestic and industrial waters is often controlled by water softening (e.g., ion exchange, reverse osmosis). Calcium carbonate saturation and water hardness are discussed in Section 2330 and Section 2340, respectively.

Calcium contributes to the total hardness of water. Chemical softening treatment, reverse osmosis, electrodialysis, or ion exchange is used to reduce calcium and the associated hardness.

2. Selection of Method

The atomic absorption methods (Section 3111B, Section 3111D, and Section 3111E) and inductively coupled plasma method (Section 3120) are accurate means of determining calcium. The EDTA titration method gives good results for control and routine applications, but for samples containing high P levels (>50 mg/L) only the atomic absorption or atomic emission methods are recommended because of interferences often encountered with EDTA indicators.

3. Storage of Samples

The customary precautions are sufficient if care is taken to redissolve any calcium carbonate that may precipitate on standing.

3500-Ca B. EDTA Titrimetric Method

1. General Discussion

a. Principle: When EDTA (ethylenediaminetetraacetic acid or its salts) is added to water containing both calcium and magnesium, it combines first with the calcium. Calcium can be determined directly, with EDTA, when the pH is made sufficiently high that the magnesium is largely precipitated as the hydroxide and an indicator is used that combines with calcium only. Several indicators give a color change when all of the calcium has been complexed by the EDTA at a pH of 12 to 13.

b. Interference: Under conditions of this test, the following concentrations of ions cause no interference with the calcium hardness determination: Cu^{2+} , 2 mg/L; Fe^{2+} , 20 mg/L; Fe^{3+} , 20 mg/L; Mn^{2+} , 10 mg/L; Zn^{2+} , 5 mg/L; Pb^{2+} , 5 mg/L; Al^{3+} , 5 mg/L; and Sn^{4+} , 5 mg/L. Orthophosphate precipitates calcium at the pH of the test. Strontium and barium give a positive interference and alkalinity in excess of 300 mg/L may cause an indistinct end point in hard

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waters.

2. Reagents

a. *Sodium hydroxide*, NaOH, 1N.

b. *Indicators*: Many indicators are available for the calcium titration. Some are described in the literature (see Bibliography ¶ 6); others are commercial preparations and also may be used. Murexide (ammonium purpurate) was the first indicator available for detecting the calcium end point; directions for its use are presented in this procedure. Individuals who have difficulty recognizing the murexide end point may find the indicator Eriochrome Blue Black R (color index number 202) or Solochrome Dark Blue an improvement because of the color change from red to pure blue. Eriochrome Blue Black R is sodium-1-(2-hydroxy-1-naphthylazo)-2-naphthol-4-sulfonic acid. Other indicators specifically designed for use as end-point detectors in EDTA titration of calcium may be used.

1) *Murexide (ammonium purpurate) indicator*: This indicator changes from pink to purple at the end point. Prepare by dissolving 150 mg dye in 100 g absolute ethylene glycol. Water solutions of the dye are not stable for longer than 1 d. A ground mixture of dye powder and sodium chloride (NaCl) provides a stable form of the indicator. Prepare by mixing 200 mg murexide with 100 g solid NaCl and grinding the mixture to 40 to 50 mesh. Titrate immediately after adding indicator because it is unstable under alkaline conditions. Facilitate end-point recognition by preparing a color comparison blank containing 2.0 mL NaOH solution, 0.2 g solid indicator mixture (or 1 to 2 drops if a solution is used), and sufficient standard EDTA titrant (0.05 to 0.10 mL) to produce an unchanging color.

2) *Eriochrome Blue Black R indicator*: Prepare a stable form of the indicator by grinding together in a mortar 200 mg powdered dye and 100 g solid NaCl to 40 to 50 mesh. Store in a tightly stoppered bottle. Use 0.2 g of ground mixture for the titration in the same manner as murexide indicator. During titration the color changes from red through purple to bluish purple to a pure blue with no trace of reddish or purple tint. The pH of some (not all) waters must be raised to 14 (rather than 12 to 13) by the use of 8N NaOH to get a good color change.

c. *Standard EDTA titrant*, 0.01M: Prepare standard EDTA titrant and standardize against standard calcium solution as described in Section 2340C to obtain EDTA/CaCO₃ equivalence. Standard EDTA titrant, 0.0100M, is equivalent to 1000 mg CaCO₃/1.00 mL; use titrated equivalent for *B* in the calculations in 4.

3. Procedure

a. *Pretreatment of water and wastewater samples*: Follow the procedure described in Section 3030E or I if samples require preliminary digestion.

b. *Sample preparation*: Because of the high pH used in this procedure, titrate immediately after adding alkali and indicator. Use 50.0 mL sample, or a smaller portion diluted to 50 mL so that the calcium content is about 5 to 10 mg. Analyze hard waters with alkalinity higher than 300

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mg CaCO₃/L by taking a smaller portion and diluting to 50 mL. Alternatively, adjust sample pH into the acid range (pH <6), boil for 1 min to dispel CO₂, and cool before beginning titration.

c. *Titration:* Add 2.0 mL NaOH solution or a volume sufficient to produce a pH of 12 to 13. Stir. Add 0.1 to 0.2 g indicator mixture selected (or 1 to 2 drops if a solution is used). Add EDTA titrant slowly, with continuous stirring to the proper end point. When using murexide, check end point by adding 1 to 2 drops of titrant in excess to make certain that no further color change occurs.

4. Calculation

$$\text{mg Ca/L} = \frac{A \times B \times 400.8}{\text{mL sample}}$$

$$\text{Calcium hardness as mg CaCO}_3\text{/L} = \frac{A \times B \times 1000}{\text{mL sample}}$$

where:

A = mL titrant for sample and

B = mg CaCO₃ equivalent to 1.00 mL EDTA titrant at the calcium indicator end point.

5. Precision and Bias

A synthetic sample containing 108 mg Ca/L, 82 mg Mg/L, 3.1 mg K/L, 19.9 mg Na/L, 241 mg Cl⁻/L, 1.1 mg NO₃⁻-N/L, 0.25 mg NO₂⁻-N/L, 259 mg SO₄²⁻/L, and 42.5 mg total alkalinity/L (contributed by NaHCO₃) in distilled water was analyzed in 44 laboratories by the EDTA titrimetric method, with a relative standard deviation of 9.2% and a relative error of 1.9%.

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magnesium. *J. Amer. Water Works Assoc.* 56:121.

3500-Cs CESIUM

Cesium (Cs) is the sixth element in Group IA of the periodic table; it has an atomic number of 55, an atomic weight of 132.90, and a valence of 1. The average abundance of Cs in the earth's crust is 2.6 ppm; in soils it is 1 to 5 ppm; in streams it is 0.02 mg/L; and in groundwaters it is generally <0.1 mg/L. Cesium is found in lepidolite and in the water of certain mineral springs. ^{137}Cs , with a 33-year half-life, is widely dispersed on the earth's surface as a result of the radioactive fallout from the atmospheric testing of nuclear weapons. Cesium compounds are used in photoelectric cells, as a catalyst, and in brewing. Some cesium compounds are fire hazards.

Perform analyses by the flame atomic absorption spectrometric method (Section 3111B). The inductively coupled plasma/mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though cesium is not specifically listed as an analyte in the method.

3500-Cr CHROMIUM*#(101)

3500-Cr A. Introduction

1. Occurrence and Significance

Chromium (Cr) is the first element in Group VIB in the periodic table; it has an atomic number of 24, an atomic weight of 51.99, and valences of 1 through 6. The average abundance of Cr in the earth's crust is 122 ppm; in soils Cr ranges from 11 to 22 ppm; in streams it averages about 1 $\mu\text{g/L}$, and in groundwaters it is generally 100 $\mu\text{g/L}$. Chromium is found chiefly in chrome-iron ore ($\text{FeO}\cdot\text{Cr}_2\text{O}_3$). Chromium is used in alloys, in electroplating, and in pigments. Chromate compounds frequently are added to cooling water for corrosion control.

In natural waters trivalent chromium exists as Cr^{3+} , $\text{Cr}(\text{OH})^{2+}$, $\text{Cr}(\text{OH})_2^+$, and $\text{Cr}(\text{OH})_4^-$; in the hexavalent form chromium exists as CrO_4^{2-} and as $\text{Cr}_2\text{O}_7^{2-}$. Cr^{3+} would be expected to form strong complexes with amines, and would be adsorbed by clay minerals. Chromium may exist in water supplies in both the hexavalent and the trivalent state although the trivalent form rarely occurs in potable water.

Chromium is considered nonessential for plants, but an essential trace element for animals. Hexavalent compounds have been shown to be carcinogenic by inhalation and are corrosive to tissue. The chromium guidelines for natural water are linked to the hardness or alkalinity of the water (i.e., the softer the water, the lower the permitted level for chromium). The United Nations

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Food and Agriculture Organization recommended maximum level for irrigation waters is 100 µg/L. The U.S. EPA primary drinking water standard MCL is 0.1 mg/L for total chromium.

2. Selection of Method

The colorimetric method (B) is useful for the determination of hexavalent chromium in a natural or treated water in the range from 100 to 1000 µg/L. This range can be extended by appropriate sample dilution or concentration and/or use of longer cell paths. The ion chromatographic method with photometric detection (C) is suitable for determining dissolved hexavalent chromium in drinking water, groundwater, and industrial wastewater effluents. The electrothermal atomic absorption spectrometric method (Section 3113B) is suitable for determining low levels of total chromium (< 50 µg/L) in water and wastewater, and the flame atomic absorption spectrometric methods (Section 3111B and Section 3111C) and the inductively coupled plasma methods (Section 3120 and Section 3125) are appropriate for measuring concentrations up to milligram-per-liter levels.

3. Sample Handling

If only the dissolved metal content is desired, filter sample through a 0.45-µm membrane filter at time of collection, and after filtration acidify filtrate with conc nitric acid (HNO₃) to pH <2. If only dissolved hexavalent chromium is desired, adjust pH of filtrate to 8 or above with 1N sodium hydroxide solution and refrigerate. If the total chromium content is desired, acidify unfiltered sample at time of collection with conc HNO₃ to pH <2. If total hexavalent chromium is desired, adjust the pH of unfiltered sample to 8 or above with 1N sodium hydroxide and refrigerate.

3500-Cr B. Colorimetric Method

1. General Discussion

a. Principle: This procedure measures only hexavalent chromium (Cr⁶⁺). Therefore, to determine total chromium convert all the chromium to the hexavalent state by oxidation with potassium permanganate. NOTE: The oxidation process may not provide total conversion of all chromium species to Cr⁶⁺.¹⁻³ For total chromium determination, acid-digest the sample (see Section 3030) and follow with a suitable instrumental analysis technique. The hexavalent chromium is determined colorimetrically by reaction with diphenylcarbazide in acid solution. A red-violet colored complex of unknown composition is produced. The reaction is very sensitive, the molar absorptivity based on chromium being about 40 000 L g⁻¹ cm⁻¹ at 540 nm. To determine total chromium, digest the sample with a sulfuric-nitric acid mixture and then oxidize with potassium permanganate before reacting with the diphenylcarbazide.

b. Interferences: The reaction with diphenylcarbazide is nearly specific for chromium. Hexavalent molybdenum and mercury salts will react to form color with the reagent but the

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intensities are much lower than that for chromium at the specified pH. Concentrations as high as 200 mg Mo or Hg/L can be tolerated. Vanadium interferes strongly but concentrations up to 10 times that of chromium will not cause trouble. Potential interference from permanganate is eliminated by prior reduction with azide. Iron in concentrations greater than 1 mg/L may produce a yellow color but the ferric ion (Fe^{3+}) color is not strong and no difficulty is encountered normally if the absorbance is measured photometrically at the appropriate wavelength. Interfering amounts of molybdenum, vanadium, iron, and copper can be removed by extraction of the cupferrates of these metals into chloroform (CHCl_3). A procedure for this extraction is provided but do not use it unless necessary, because residual cupferron and CHCl_3 in the aqueous solution complicate the later oxidation. Therefore, follow the extraction by additional treatment with acid fuming to decompose these compounds.

2. Apparatus

a. *Colorimetric equipment:* One of the following is required:

- 1) *Spectrophotometer*, for use at 540 nm, with a light path of 1 cm or longer.
- 2) *Filter photometer*, providing a light path of 1 cm or longer and equipped with a greenish yellow filter having maximum transmittance near 540 nm.

b. *Separatory funnels*, 125-mL, Squibb form, with glass or TFE stopcock and stopper.

c. *Acid-washed glassware:* New and unscratched glassware will minimize chromium adsorption on glass surfaces during the oxidation procedure. Do not use glassware previously treated with chromic acid. Thoroughly clean other used glassware and new glassware with nitric or hydrochloric acid to remove chromium traces.

3. Reagents

Use reagent water (see Section 1080) for reagent preparation and analytical procedure.

a. *Stock chromium solution:* Dissolve 141.4 mg $\text{K}_2\text{Cr}_2\text{O}_7$ in water and dilute to 100 mL; 1.00 mL = 500 μg Cr.

b. *Standard chromium solution:* Dilute 1.00 mL stock chromium solution to 100 mL; 1.00 mL = 5.00 μg Cr.

c. *Nitric acid*, HNO_3 , conc.

d. *Sulfuric acid*, H_2SO_4 , conc, 18N, and 6N.

e. *Sulfuric acid*, H_2SO_4 , 0.2N: Dilute 17 mL 6N H_2SO_4 to 500 mL with water.

f. *Phosphoric acid*, H_3PO_4 , conc.

g. *Methyl orange indicator solution.*

h. *Hydrogen peroxide*, H_2O_2 , 30%.

i. *Ammonium hydroxide*, NH_4OH , conc.

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- j. *Potassium permanganate solution*: Dissolve 4 g KMnO_4 in 100 mL water.
- k. *Sodium azide solution*: Dissolve 0.5 g NaN_3 in 100 mL water.
- l. *Diphenylcarbazide solution*: Dissolve 250 mg 1,5-diphenylcarbazide (1,5-diphenylcarbohydrazide) in 50 mL acetone. Store in a brown bottle. Discard when solution becomes discolored.
- m. *Chloroform*, CHCl_3 : Avoid or redistill material that comes in containers with metal or metal-lined caps.
- n. *Cupferron solution*: Dissolve 5 g cupferron, $\text{C}_6\text{H}_5\text{N}(\text{NO})\text{ONH}_4$, in 95 mL water.
- o. *Sodium hydroxide, 1N*: Dissolve 40 g NaOH in 1 L water. Store in plastic bottle.

4. Procedure

a. *Preparation of calibration curve*: To compensate for possible slight losses of chromium during digestion or other analytical operations, treat standards by the same procedure as the sample. Accordingly, pipet measured volumes of standard chromium solution ($5\text{ }\mu\text{g/mL}$) ranging from 2.00 to 20.0 mL, to give standards for 10 to 100 $\mu\text{g Cr}$, into 250-mL beakers or conical flasks. Depending on pretreatment used in ¶ b below, proceed with subsequent treatment of standards as if they were samples, also carrying out cupferron treatment of standards if this is required for samples.

Develop color as for samples, transfer a suitable portion of each colored solution to a 1-cm absorption cell, and measure absorbance at 540 nm, using reagent water as reference. Correct absorbance readings of standards by subtracting absorbance of a reagent blank carried through the method.

Construct a calibration curve by plotting corrected absorbance values against micrograms chromium in 102 mL final volume.

b. *Treatment of sample*: If sample has been filtered and/or only hexavalent chromium is desired, start analysis within 24 h of collection and proceed to ¶ 4e. NOTE: Recent evidence⁴ suggests that preserved samples can be held for 30 d without substantial changes to Cr^{6+} concentrations. If total dissolved chromium is desired and there are interfering amounts of molybdenum, vanadium, copper, or iron present, proceed to ¶ 4c. If interferences are not present, proceed to ¶ 4d.

If sample is unfiltered and total chromium is desired, digest with HNO_3 and H_2SO_4 as in Section 3030G. If interferences are present, proceed to ¶ 4c, ¶ 4d, and ¶ 4e. If there are no interferences, proceed to ¶ 4d and ¶ 4e.

c. *Removal of molybdenum, vanadium, iron, and copper with cupferron*: Pipet a portion of sample containing 10 to 100 $\mu\text{g Cr}$ into a 125-mL separatory funnel. Dilute to about 40 mL with water and chill in an ice bath. Add 5 mL ice-cold cupferron solution, shake well, and let stand in ice bath for 1 min. Extract in separatory funnel with three successive 5-mL portions of CHCl_3 ;

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shake each portion thoroughly with aqueous solution, let layers separate, and withdraw and discard CHCl_3 extract. Transfer extracted aqueous solution to a 125-mL conical flask. Wash separatory funnel with a small amount of water and add wash water to flask. Boil for about 5 min to volatilize CHCl_3 and cool. Add 5 mL HNO_3 and 3 mL H_2SO_4 . Boil samples to the appearance of SO_3 fumes. Cool slightly, carefully add 5 mL HNO_3 , and again boil to fumes to complete decomposition of organic matter. Cool, wash sides of flask, and boil once more to SO_3 fumes, assuming elimination of all HNO_3 . Cool and add 25 mL water.

d. Oxidation of trivalent chromium: Pipet a portion of digested sample with or without interferences removed, and containing 10 to 100 μg Cr, into a 125-mL conical flask. Add several drops of methyl orange indicator, then add conc NH_4OH until solution just begins to turn yellow. Add 1 + 1 H_2SO_4 dropwise until it is acidic, plus 1 mL (20 drops) in excess. Adjust volume to about 40 mL, add two or more acid-washed glass beads, and heat to boiling. Add 2 drops KMnO_4 solution to give a dark red color. If fading occurs, add KMnO_4 dropwise to maintain an excess of about 2 drops. Boil for 2 min longer. Add 1 mL NaN_3 solution and continue boiling gently. If red color does not fade completely after boiling for approximately 30 s, add another 1 mL NaN_3 solution. Continue boiling for 1 min after color has faded completely, then cool.

e. Color development and measurement: Add 0.25 mL (5 drops) H_3PO_4 . Use 0.2N H_2SO_4 and a pH meter to adjust solution to $\text{pH } 1.0 \pm 0.3$. NOTE: Recent work⁵ identifies the optimum pH range for color development to be 1.6 to 2.2; the matter of optimum pH range is currently being considered by *Standard Methods*. Transfer solution to a 100-mL volumetric flask, dilute to 100 mL, and mix. Add 2.0 mL diphenylcarbazide solution, mix, and let stand 5 to 10 min for full color development. Transfer an appropriate portion to a 1-cm absorption cell and measure its absorbance at 540 nm, using reagent water as reference. Correct absorbance reading of sample by subtracting absorbance of a blank carried through the method (see also note below). From the corrected absorbance, determine micrograms chromium present by reference to the calibration curve.

NOTE: If the solution is turbid after dilution to 100 mL in ¶ e above, take an absorbance reading before adding carbazide reagent and correct absorbance reading of final colored solution by subtracting the absorbance measured previously.

5. Calculation

For digested samples:

$$\text{mg Cr/L} = \frac{\mu\text{g Cr (in 102 mL final volume)}}{A \times B} \times 100$$

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where:

A = mL original sample, and

B = mL portion from 100 mL digested sample.

For undigested samples:

$$\text{mg Cr/L} = \frac{\mu\text{g Cr (in 102 mL final volume)}}{A}$$

6. Precision and Bias

Collaborative test data from 16 laboratories were obtained on reagent water, tap water, 10% NaCl solution, treated water from synthetic organic industrial waste, EPA extraction leachate, process water, lake water, and effluent from a steel pickle liquor treatment plant.⁶ The test data yielded the following relationships:

Reagent water:

$$S_t = 0.037x + 0.006$$

$$S_o = 0.022x + 0.004$$

Drinking or wastewater:

$$S_t = 0.067x + 0.004$$

$$S_o = 0.037x + 0.002$$

Leachate:

$$S_t = 0.032x + 0.007$$

$$S_o = 0.017x + 0.004$$

where:

S_t = overall precision,

S_o = single-operator precision, and

x = chromium concentration, mg/L.

7. References

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3500-Cr C. Ion Chromatographic Method

1. General Discussion

a. Principle: This method is applicable to determination of dissolved hexavalent chromium in drinking water, groundwater, and industrial wastewater effluents. An aqueous sample is filtered and its pH adjusted to 9 to 9.5 with a concentrated buffer. This pH adjustment reduces the solubility of trivalent chromium and preserves the hexavalent chromium oxidation state. The sample is introduced into the instrument's eluent stream of ammonium sulfate and ammonium

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hydroxide. Trivalent chromium in solution is separated from the hexavalent chromium by the column. After separation, hexavalent chromium reacts with an azide dye to produce a chromogen that is measured at 530 nm. Hexavalent chromium is identified on the basis of retention time.

Although this method was developed using specific commercial equipment, use of another manufacturer's equipment should be acceptable if appropriate adjustments are made.

b. Interferences: Interferences may come from several sources. Use a good grade of salts for the buffer because trace amounts of chromium may be included.

Several soluble species of trivalent chromium in the sample may be oxidized to the hexavalent form in an alkaline medium in the presence of such oxidants as hydrogen peroxide, ozone, and manganese dioxide. The hexavalent form can be reduced to the trivalent in the presence of reducing species in an acid medium.

High ionic concentration may cause column overload. Samples high in chloride and/or sulfate might show this phenomenon, which is characterized by a change in peak geometry.

Interfering organic compounds are removed by the guard column.

c. Minimum detectable concentrations: The method detection limits obtained in a single laboratory with a 250- μ L loop were as follows:

Reagent water	0.4 μ g/L
Drinking water	0.3 μ g/L
Groundwater	0.3 μ g/L
Primary wastewater effluent	0.3 μ g/L
Electroplating waste	0.3 μ g/L

d. Sample preservation and holding time: Filter sample through a 0.45- μ m filter. Use a portion of sample to rinse syringe filter unit and filter, then collect the required volume of filtrate. Adjust pH to 9 to 9.5 by adding buffer solution dropwise while checking pH with a pH meter.

Ship and store sample at 4°C. Bring to room temperature before analysis. Analyze samples within 24 h of collection.

2. Apparatus

a. Ion chromatograph equipped with a pump capable of precisely delivering a flow of 1 to 5 mL/min. The metallic parts of the pump must not contact sample, eluent, or reagent. Sample loops should be available or the instrument should be capable of delivering from 50 to 250- μ L injections of sample. The visible absorption cell should not contain metallic parts that contact the eluent-sample flow. The cell must be usable at 530 nm. Use plastic pressurized containers to deliver eluent and post-column reagent. Use high-purity helium (99.995%) to pressurize the eluent and post-column reagent vessel.

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b. *Guard column*, to be placed before the separator column, containing an adsorbent capable of adsorbing organic compounds and particulates that would damage or interfere with the analysis or equipment.*#(102)

c. *Separator column*, packed with a high-capacity anion-exchange resin capable of resolving chromate from other sample constituents.†#(103)

d. *Recorder, integrator, or computer* for receiving signals from the detector as a function of time.

e. *Labware*: Soak all reusable labware (glass, plastic, etc.) including sample containers, overnight in laboratory-grade detergent, rinse, and soak for 4 h in a mixture of nitric acid (1 part), hydrochloric acid (2 parts), and reagent water (9 parts). Rinse with tap water and reagent water. NOTE: Never use chromic acid cleaning solution.

f. *Syringe*, equipped with male luer-type fitting and a capacity of at least 3 mL.

3. Reagents

a. *Reagent water*: Deionized or distilled water free from interferences at the minimum detection limit of each constituent, filtered through a 0.2- μ m membrane filter and having a conductance of less than 0.1 μ S/cm. Use for preparing all reagents.

b. *Cr(VI) stock solution*, 100 mg Cr⁶⁺/L: Prepare from primary standard grade potassium dichromate. Dissolve 0.1414 g K₂Cr₂O₇ in water and dilute to 500 mL in a volumetric flask. pH adjustment is not required. Store in plastic. CAUTION: *Hexavalent chromium is toxic and a suspected carcinogen; handle with care.*

c. *Eluent*: Dissolve 33 g ammonium sulfate (NH₄)₂SO₄ in 500 mL water and add 6.5 mL conc ammonium hydroxide, NH₄OH. Dilute to 1 L with water.

d. *Post-column reagent*: Dissolve 0.5 g 1,5-diphenylcarbazide in 100 mL HPLC-grade methanol. Add with stirring to 500 mL water containing 28 mL conc H₂SO₄. Dilute to 1 L with water. Reagent is stable for 4 or 5 d; prepare only as needed.

e. *Buffer solution*: Dissolve 33 g ammonium sulfate, (NH₄)₂SO₄ in 75 mL water and add 6.5 mL conc ammonium hydroxide, NH₄OH. Dilute to 100 mL with water.

4. Procedure

a. *Instrument setup*: Establish ion chromatograph operating conditions as indicated in Table 3500-Cr:I. Set flow rate of the eluent pump at 1.5 mL/min and adjust pressure of reagent delivery module so that the system flow rate, measured after the detector, is 2.0 mL/min. Measure system flow rate using a graduated cylinder and stopwatch. Allow approximately 30 min after adjustment before measuring flow.

Use an injection loop size based on required sensitivity. A 50- μ L loop is sufficient, although a 250- μ L loop was used to determine the method detection limit.

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b. Calibration: Before sample analysis, construct a calibration curve using a minimum of a blank and three standards that bracket the expected sample concentration range. Prepare calibration standards from the stock standard (3b) by appropriate dilution with water in volumetric flasks. Adjust to pH 9 to 9.5 with buffer solution (3e) before final dilution. Injection volumes of standards should be about 10 times the injection loop volume to insure complete loop flushing.

c. Sample analysis: Bring chilled, pH-adjusted sample to ambient temperature. Fill a clean syringe with sample, attach a 0.45- μ m syringe filter, and inject 10 times the sample loop volume into the instrument. Dilute any sample that has a concentration greater than the highest calibration standard.

5. Calculation

Determine area or height of the Cr(VI) peak in the calibration standard chromatograms. Calculate a calibration line by regressing peak area (or height) against standard concentration in mg/L. A correlation coefficient less than 0.995 may indicate a problem with the analysis.

For samples, measure area (or height) of Cr(VI) peak in sample chromatogram, as determined by retention time. Calculate Cr(VI) concentration by interpolating from the calibration line. Correct data for any dilutions made.

Currently available instrumentation automates the entire measurement process (peak measurement, calibration, and sample measurements and calculations). Ensure that enough quality control samples are analyzed to monitor the instrumental processes.

6. Quality Control

a. Initial demonstration of performance: Before sample analysis, set up instrument and analyze enough known samples to determine estimates for the method detection limit and linear calibration range. Use the initial demonstration of performance to characterize instrument performance, i.e., method detection levels (MDLs) and linear calibration range.

b. Initial and continuing calibration performance: Initially, after every 10 samples, and after the final sample, analyze an independent check sample and a calibration blank. The concentration of the calibration check sample should be near the mid-calibration range; prepare from a source independent of the calibration standards. Use acceptance criteria for check standard recovery and calibration standard concentration based on project goals for precision and accuracy. Typical values for recovery of the check standard range from 90 to 110%. The acceptance criteria for the calibration blank are typically set at $\pm$ the nominal MDL.

c. Reagent blank analysis: Analyze one laboratory reagent blank with each batch of samples. Significant Cr(VI) detected in the reagent blank is a sign of contamination. Identify source and eliminate contamination.

d. Laboratory-fortified matrix (also known as matrix spike) analysis: To a portion of a sample, add a known quantity of Cr(VI). After analysis, calculate percent recovery of the known addition. If the recovery falls outside the control limits (typically 75 to 125%), the matrix may be

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interfering with the results. Perform additional testing to determine whether analysis by the method of standard additions will overcome the interference. Analyze fortified matrix samples as frequently as dictated by project goals and anticipated similarity of matrices in the sample set.

e. Laboratory control sample: Analyze a laboratory control sample (LCS) from an external source with every sample batch. Process LCS and samples identically, including filtering and pH adjustment. Base acceptance criteria for LCS recovery on project goals for precision and bias. Typical values for acceptable recovery range from 90 to 110%.

7. Precision and Bias

The instrument operating conditions and data from a single- laboratory test of the method are shown in Table 3500-Cr:I and Table 3500-Cr:II, respectively.

Multilaboratory test data are shown in Table 3500-Cr:III.†‡(104) Fifteen laboratories analyzed samples ranging from 1.2 to 960 µg Cr/L.

For reagent water matrix:

$$S_o = 0.033x + 0.106$$

$$S_t = 0.050x + 0.559$$

$$\text{Mean recovery} = 1.04x + 0.183$$

where:

S_o = single-operator precision,

S_t = overall precision, and

x = added amount.

For wastewater matrix:

$$S_o = 0.041x + 0.039$$

$$S_t = 0.059x + 1.05$$

$$\text{Mean recovery} = 0.989x - 0.41$$

The eleven water samples consisted of a reagent water blank and five Youden pairs. The nine wastewater samples consisted of a wastewater blank and four Youden pairs.

8. Bibliography

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3500-Co COBALT

Cobalt (Co) is the second element in Group VIII in the periodic table; it has an atomic number of 27, an atomic weight of 58.93, and valences of 1, 2, and 3. The average abundance of Co in the earth's crust is 29 ppm; in soils it is 1.0 to 14 ppm; in streams it is 0.2 µg/L; and in groundwaters it is 1 to 10 µg/L. Cobalt occurs only sparingly in ores, usually as the sulfide or the arsenide. It is widely used in alloys of various steels, in electroplating, in fertilizers, and in porcelain and glass.

The solubility of cobalt is controlled by coprecipitation or adsorption by oxides or manganese and iron, by carbonate precipitation, and by the formation of complex ions. Cobalt dust is flammable and is toxic by inhalation. Cobalt is considered essential for algae and some bacteria, nonessential for higher plants, and an essential trace element for animals. The United Nations Food and Agriculture Organization recommended maximum level for irrigation waters is 100 µg/L.

Perform analyses by the flame atomic absorption spectrometric methods (Section 3111B and Section 3111C), by the electrothermal atomic absorption method (Section 3113B), or by the inductively coupled plasma methods (Section 3120 and Section 3125).

3500-Cu COPPER*(105)

3500-Cu A. Introduction

1. Occurrence and Significance

Copper (Cu) is the first element in Group IB in the periodic table; it has an atomic number of 29, an atomic weight of 63.54, and valences of 1 and 2. The average abundance of Cu in the earth's crust is 68 ppm; in soils it is 9 to 33 ppm; in streams it is 4 to 12 µg/L; and in groundwater it is <0.1 mg/L. Copper occurs in its native state, but is also found in many minerals, the most important of which are those containing sulfide compounds (e.g., chalcopyrite), but also those with oxides and carbonates. Copper is widely used in electrical wiring, roofing, various alloys, pigments, cooking utensils, piping, and in the chemical industry.

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Copper salts are used in water supply systems to control biological growths in reservoirs and distribution pipes and to catalyze the oxidation of manganese. Copper forms a number of complexes in natural waters with inorganic and organic ligands. Among the common aqueous species are Cu^{2+} , $\text{Cu}(\text{OH})_2$, and CuHCO_3^+ . Corrosion of copper-containing alloys in pipe fittings may introduce measurable amounts of copper into the water in a pipe system.

Copper is considered an essential trace element for plants and animals. Some compounds are toxic by ingestion or inhalation. The United Nations Food and Agriculture Organization recommended maximum level for irrigation waters is 200 $\mu\text{g/L}$. Under the lead-copper rule, the U.S. EPA drinking water 90th percentile action level is 1.3 mg/L .

2. Selection of Method

The atomic absorption spectrometric methods (Section 3111B and Section 3111C), the inductively coupled plasma methods (Section 3120 and Section 3125), and the neocuproine method (B) are recommended because of their freedom from interferences. The electrothermal atomic absorption method (Section 3113B) also may be used with success with an appropriate matrix modifier. The bathocuproine method (C) may be used for potable waters.

3. Sampling and Storage

Copper ion tends to be adsorbed on the surface of sample containers. Therefore, analyze samples as soon as possible after collection. If storage is necessary, use 0.5 mL 1 + 1 HCl/100 mL sample, or acidify to pH <2 with HNO_3 , to prevent this adsorption.

3500-Cu B. Neocuproine Method

1. General Discussion

a. Principle: Cuprous ion (Cu^+) in neutral or slightly acidic solution reacts with 2,9-dimethyl-1,10-phenanthroline (neocuproine) to form a complex in which 2 moles of neocuproine are bound by 1 mole of Cu^+ ion. The complex can be extracted by a number of organic solvents, including a chloroform-methanol ($\text{CHCl}_3\text{-CH}_3\text{OH}$) mixture, to give a yellow solution with a molar absorptivity of about 8000 at 457 nm. The reaction is virtually specific for copper; the color follows Beer's law up to a concentration of 0.2 mg Cu/25 mL solvent; full color development is obtained when the pH of the aqueous solution is between 3 and 9; the color is stable in $\text{CHCl}_3\text{-CH}_3\text{OH}$ for several days.

The sample is treated with hydroxylamine-hydrochloride to reduce cupric ions to cuprous ions. Sodium citrate is used to complex metallic ions that might precipitate when the pH is raised. The pH is adjusted to 4 to 6 with NH_4OH , a solution of neocuproine in methanol is added, and the resultant complex is extracted into CHCl_3 . After dilution of the CHCl_3 to an exact volume with CH_3OH , the absorbance of the solution is measured at 4571 nm.

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b. Interference: Large amounts of chromium and tin may interfere. Avoid interference from chromium by adding sulfurous acid to reduce chromate and complex chromic ion. In the presence of much tin or excessive amounts of other oxidizing ions, use up to 20 mL additional hydroxylamine-hydrochloride solution.

Cyanide, sulfide, and organic matter interfere but can be removed by a digestion procedure.

c. Minimum detectable concentration: The minimum detectable concentration, corresponding to 0.01 absorbance or 98% transmittance, is 3 µg Cu when a 1-cm cell is used and 0.6 µg Cu when a 5-cm cell is used.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

- 1) *Spectrophotometer*, for use at 4571 nm, providing a light path of 1 cm or longer.
- 2) *Filter photometer*, providing a light path of 1 cm or longer and equipped with a narrow-band violet filter having maximum transmittance in the range 450 to 460 nm.

b. Separatory funnels, 125-mL, Squibb form, with glass or TFE stopcock and stopper.

3. Reagents

a. Redistilled water, copper-free: Because most ordinary distilled water contains detectable amounts of copper, use redistilled water, prepared by distilling singly distilled water in a resistant-glass still, or distilled water passed through an ion-exchange unit, to prepare all reagents and dilutions.

b. Stock copper solution: To 200.0 mg polished electrolytic copper wire or foil in a 250-mL conical flask, add 10 mL water and 5 mL conc HNO₃. After the reaction has slowed, warm gently to complete dissolution of the copper and boil to expel oxides of nitrogen, using precautions to avoid loss of copper. Cool, add about 50 mL water, transfer quantitatively to a 1-L volumetric flask, and dilute to the mark with water; 1 mL = 200 µg Cu.

c. Standard copper solution: Dilute 50.00 mL stock copper solution to 500 mL with water; 1.00 mL = 20.0 µg Cu.

d. Sulfuric acid, H₂SO₄, conc.

e. Hydroxylamine-hydrochloride solution: Dissolve 50 g NH₂OH·HCl in 450 mL water.

f. Sodium citrate solution: Dissolve 150 g Na₃C₆H₅O₇·2H₂O in 400 mL water. Add 5 mL NH₂OH·HCl solution and 10 mL neocuproine reagent. Extract with 50 mL CHCl₃ to remove copper impurities and discard CHCl₃ layer.

g. Ammonium hydroxide, NH₄OH, 5N: Dilute 330 mL conc NH₄OH (28-29%) to 1000 mL with water. Store in a polyethylene bottle.

h. Congo red paper, or other pH test paper showing a color change in the pH range of 4 to 6.

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i. *Neocuproine reagent*: Dissolve 100 mg 2,9-dimethyl-1,10-phenanthroline hemihydrate*#(106) in 100 mL methanol. This solution is stable under ordinary storage conditions for a month or more.

j. *Chloroform*, CHCl_3 : Avoid or redistill material that comes in containers with metal-lined caps.

k. *Methanol*, CH_3OH , reagent grade.

l. *Nitric acid*, HNO_3 , conc.

m. *Hydrochloric acid*, HCl , conc.

4. Procedure

a. *Preparation of calibration curve*: Pipet 50 mL water into a 125-mL separatory funnel for use as a reagent blank. Prepare standards by pipetting 1.00 to 10.00 mL (20.0 to 200 μg Cu) standard copper solution into a series of 125-mL separatory funnels, and dilute to 50 mL with water. Add 1 mL conc H_2SO_4 and use the extraction procedure given in ¶ 4b below.

Construct a calibration curve by plotting absorbance versus micrograms of copper.

To prepare a calibration curve for smaller amounts of copper, dilute 10.0 mL standard copper solution to 100 mL. Carry 1.00-to 10.00-mL volumes of this diluted standard through the previously described procedure, but use 5-cm cells to measure absorbance.

b. *Treatment of sample*: Transfer 100 mL sample to a 250-mL beaker, add 1 mL conc H_2SO_4 and 5 mL conc HNO_3 . Add a few boiling chips and cautiously evaporate to dense white SO_3 fumes on a hot plate. If solution remains colored, cool, add another 5 mL conc HNO_3 , and again evaporate to dense white fumes. Repeat, if necessary, until solution becomes colorless.

Cool, add about 80 mL water, and bring to a boil. Cool and filter into a 100-mL volumetric flask. Make up to 100 mL with water using mostly beaker and filter washings.

Pipet 50.0 mL or other suitable portion containing 4 to 200 μg Cu, from the solution obtained from preliminary treatment, into a 125-mL separatory funnel. Dilute, if necessary, to 50 mL with water. Add 5 mL $\text{NH}_2\text{OH}\cdot\text{HCl}$ solution and 10 mL sodium citrate solution, and mix thoroughly. Adjust pH to approximately 4 by adding 1-mL increments of NH_4OH until Congo red paper is just definitely red (or other suitable pH test paper indicates a value between 4 and 6).

Add 10 mL neocuproine reagent and 10 mL CHCl_3 . Stopper and shake vigorously for 30 s or more to extract the copper-neocuproine complex into the CHCl_3 . Let mixture separate into two layers and withdraw lower CHCl_3 layer into a 25-mL volumetric flask, taking care not to transfer any of the aqueous layer. Repeat extraction of the water layer with an additional 10 mL CHCl_3 and combine extracts. Dilute combined extracts to 25 mL with CH_3OH , stopper, and mix thoroughly.

Transfer an appropriate portion of extract to a suitable absorption cell (1 cm for 40 to 200 μg

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Cu; 5 cm for lesser amounts) and measure absorbance at 4571 nm or with a 450- to 460-nm filter. Use a sample blank prepared by carrying 50 mL water through the complete digestion and analytical procedure.

Determine micrograms copper in final solution by reference to the appropriate calibration curve.

5. Calculation

$$\text{mg Cu/L} = \frac{\mu\text{g Cu (in 25 mL final volume)}}{\text{mL portion taken for extraction}}$$

6. Bibliography

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3500-Cu C. Bathocuproine Method

1. General Discussion

a. Principle: Cuprous ion forms a water-soluble orange-colored chelate with bathocuproine disulfonate (2,9-dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonic acid, disodium salt). While the color forms over the pH range 3.5 to 11.0, the recommended pH range is between 4 and 5. The sample is buffered at a pH of about 4.3 and reduced with hydroxylamine hydrochloride. The absorbance is measured at 4841 nm. The method can be applied to copper concentrations up to at least 5 mg/L with a sensitivity of 20 µg/L.

b. Interference: The following substances can be tolerated with an error of less than ±2%:

Substance	Concentration mg/L
Cations	

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Substance	Concentration mg/L
Aluminum	100
Beryllium	10
Cadmium	100
Calcium	1000
Chromium (III)	10
Cobalt (II)	5
Iron (II)	100
Iron (III)	100
Lithium	500
Magnesium	100
Manganese (II)	500
Nickel (II)	500
Sodium	1000
Strontium	200
Thorium (IV)	100
Zinc	200
Anions	
Chlorate	1000
Chloride	1000
Fluoride	500
Nitrate	200
Nitrite	200
Orthophosphate	1000
Perchlorate	1000
Sulfate	1000
Compounds	
Residual chlorine	1
Linear alkylate sulfonate (LAS)	40

Cyanide, thiocyanate, persulfate, and EDTA also can interfere.

c. Minimum detectable concentration: 20 µg/L with a 5-cm cell.

2. Apparatus

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a. *Colorimetric equipment*: One of the following, with a light path of 1 to 5 cm (unless nessler tubes are used):

- 1) *Spectrophotometer*, for use at 4841 nm.
- 2) *Filter photometer*, equipped with a blue-green filter exhibiting maximum light transmission near 4841 nm.
- 3) *Nessler tubes*, matched, 100-mL, tall form.

b. *Acid-washed glassware*: Rinse all glassware with conc HCl and then with copper-free water.

3. Reagents

a. *Copper-free water*: See Method B, ¶ 3a.

b. *Stock copper solution*: Prepare as directed in Method B, ¶ 3b, but use 20.00 mg copper wire or foil; 1.00 mL = 20.00 µg Cu.

c. *Standard copper solution*: Dilute 250 mL stock copper solution to 1000 mL with water; 1.00 mL = 5.00 µg Cu. Prepare daily.

d. *Hydrochloric acid*, HCl, 1 + 1.

e. *Hydroxylamine hydrochloride solution*: See Method B, ¶ 3e.

f. *Sodium citrate solution*: Dissolve 300 g $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ in water and make up to 1000 mL.

g. *Disodium bathocuproine disulfonate solution*: Dissolve 1.000 g $\text{C}_{12}\text{H}_4\text{N}_2(\text{CH}_3)_2(\text{C}_6\text{H}_4)_2(\text{SO}_3\text{Na})_2$ in water and make up to 1000 mL.

4. Procedure

Pipet 50.0 mL sample, or a suitable portion diluted to 50.0 mL, into a 250-mL erlenmeyer flask. In separate 250-mL erlenmeyer flasks, prepare a 50.0-mL water blank and a series of 50.0-mL copper standards containing 5.0, 10.0, 15.0, 20.0, and 25.0 µg Cu. To sample, blank, and standards add, mixing after each addition, 1.00 mL 1 + 1 HCl, 5.00 mL $\text{NH}_2\text{OH} \cdot \text{HCl}$ solution, 5.00 mL sodium citrate solution, and 5.00 mL disodium bathocuproine disulfonate solution. Transfer to cells and read sample absorbance against the blank at 4841 nm. Plot absorbance against micrograms Cu in standards for the calibration curve. Estimate concentration from the calibration curve.

5. Calculation

$$\text{mg Cu/L} = \frac{\mu\text{g Cu (in 66 mL final volume)}}{\text{mL sample}}$$

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6. Precision and Bias

A synthetic sample containing 1000 µg Cu/L, 500 µg Al/L, 50 µg Cd/L, 110 µg Cr/L, 300 µg Fe/L, 70 µg Pb/L, 50 µg Mn/L, 150 µg Ag/L, and 650 µg Zn/L was analyzed in 33 laboratories by the bathocuproine method, with a relative standard deviation of 4.1% and a relative error of 0.3%.

7. Bibliography

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3500-Ga GALLIUM

Gallium (Ga) is the third element in Group IIIA in the periodic table; it has an atomic number of 31, an atomic weight of 67.72, and valences of 1, 2, and 3. The average abundance of Ga in the earth's crust is 19 ppm; in soils it is 1.9 to 29 ppm; in streams it is 0.09 µg/L; and in groundwaters it is <0.1 mg/L. Gallium occurs in many zinc ores, and nearly always in bauxite. Gallium compounds are used in semiconducting devices.

The element exists as Ga^{3+} in natural water, and its solubility is controlled by formation of the hydroxide. It is considered nonessential for plants and animals.

Perform analyses by the electrothermal atomic absorption method (Section 3113B). The inductively coupled plasma/mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though gallium is not specifically listed as an analyte in the method.

3500-Ge GERMANIUM

Germanium (Ge) is the third element in Group IVA in the periodic table; it has an atomic number of 32, an atomic weight of 72.59, and valences of 2 and 4. The average abundance of Ge in the earth's crust is 1.5 ppm; in streams it is 0.03 to 0.1 µg/L; and in groundwaters it is <0.1 mg/L. Germanium is found in germanite, in certain zinc ores, and in elevated levels in certain

hot spring waters. Germanium alloys are used in transistors, gold alloys, phosphors, and semiconducting devices.

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Germanium is present in natural waters in the tetravalent state, and its distribution in natural waters probably is controlled by adsorption on clay mineral surfaces. It is nonessential for plants and animals.

Perform analyses by the electrothermal atomic absorption method (Section 3113B). The inductively coupled plasma/mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though germanium is not specifically listed as an analyte in the method.

3500-Au GOLD

Gold (Au) is the third element in Group IB in the periodic table; it has an atomic number of 79, an atomic weight of 196.97, and valences of 1 and 3. The average abundance of Au in the earth's crust is 0.004 ppm; in streams it is 2 µg/L; and in groundwater it is <0.1 mg/L. Gold occurs in the native form, and is associated with quartz or pyrite. The main uses of gold are in jewelry, dentistry, electronics, and the aerospace industry.

Gold solubility is restricted to acidic waters in the presence of oxidizing agents and chloride, or in alkaline solutions in the presence of hydrogen sulfide. Its solubility may be influenced by natural organic acids. Compounds of gold containing thiosulfate and cyanide have some human toxicity.

Perform analyses by the atomic absorption spectrometric method (Section 3111B) or by the electrothermal atomic absorption method (Section 3113B). The inductively coupled plasma mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though gold is not specifically listed as an analyte in the method.

3500-In INDIUM

Indium (In) is the fourth element in Group IIIA in the periodic table; it has an atomic number of 49, an atomic weight of 114.82, and valences of 1, 2, and 3. The average abundance of indium in the earth's crust is 0.19 ppm; in streams it is <0.01 µg/L; and in groundwaters it is <0.1 mg/L. Indium often occurs in combination with zinc ores, and sometimes with pyrites and siderite. Indium is used in alloys for bearings, brazing, solder, and in electrical devices.

Indium exists as In^{3+} and as a number of complex ions. Its solubility is controlled by the formation of the insoluble hydroxide. The metal and its compounds are toxic by inhalation.

Perform analyses by the electrothermal atomic absorption method (Section 3113B). The inductively coupled plasma mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though indium is not specifically listed as an analyte in the method.

3500-Ir IRIDIUM

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Iridium (Ir) is the eighth element in Group VIII of the periodic table; it has an atomic number of 77, an atomic weight of 192.2, and valences of 1, 3, and 4. The average abundance of Ir in the earth's crust is probably <0.001 ppm, and in groundwaters it is <0.1 mg/L. Iridium occurs uncombined with platinum and other metals. It is used in alloys with platinum in catalysts, thermocouples, electrodes, and wires.

The aqueous chemistry is controlled by complex compounds, although the solubility in natural waters is relatively unknown.

Perform analyses by the flame atomic absorption spectrometric method (Section 3111B). The inductively coupled plasma/mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though iridium is not specifically listed as an analyte in the method.

3500-Fe IRON*#(107)

3500-Fe A. Introduction

1. Occurrence and Significance

Iron (Fe) is the first element in Group VIII of the periodic table; it has an atomic number of 26, an atomic weight of 55.85, and common valences of 2 and 3 (and occasionally valences of 1, 4, and 6). The average abundance of Fe in the earth's crust is 6.22%; in soils Fe ranges from 0.5 to 4.3%; in streams it averages about 0.7 mg/L; and in groundwater it is 0.1 to 10 mg/L. Iron occurs in the minerals hematite, magnetite, taconite, and pyrite. It is widely used in steel and in other alloys.

The solubility of ferrous ion (Fe^{2+}) is controlled by the carbonate concentration. Because groundwater is often anoxic, any soluble iron in groundwater is usually in the ferrous state. On exposure to air or addition of oxidants, ferrous iron is oxidized to the ferric state (Fe^{3+}) and may hydrolyze to form red, insoluble hydrated ferric oxide. In the absence of complex-forming ions, ferric iron is not significantly soluble unless the pH is very low.

Elevated iron levels in water can cause stains in plumbing, laundry, and cooking utensils, and can impart objectionable tastes and colors to foods. The United Nations Food and Agriculture Organization recommended level for irrigation waters is 5 mg/L. The U.S. EPA secondary drinking water standard MCL is 0.3 mg/L.

2. Selection of Method

Sensitivity and detection limits for the atomic absorption spectrometric methods (Section 3111B and Section 3111C), the inductively coupled plasma method (Section 3120), and the phenanthroline colorimetric procedure described here (B) are similar and generally adequate for analysis of natural or treated waters. Lower detection limits can be achieved with electrothermal

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atomic absorption spectrometry (Section 3113B) when an appropriate matrix modifier is used. The complexing reagents used in the colorimetric procedures are specific for ferrous iron but the atomic absorption procedures are not. However, because of the instability of ferrous iron, which is changed easily to the ferric form in solutions in contact with air, determination of ferrous iron requires special precautions and may need to be done in the field at the time of sample collection.

The procedure for determining ferrous iron using 1,10-phenanthroline (Section 3500-Fe.B.4c) has a somewhat limited applicability; avoid long storage time or exposure of samples to light. A rigorous quantitative distinction between ferrous and ferric iron can be obtained with a special procedure using bathophenanthroline. Spectrophotometric methods using bathophenanthroline¹⁻⁶ and other organic complexing reagents such as ferrozine⁷ or TPTZ⁸ are capable of determining iron concentrations as low as 1 µg/L. A chemiluminescence procedure⁹ is stated to have a detection limit of 5 ng/L. Additional procedures are described elsewhere.¹⁰⁻¹³

3. Sampling and Storage

Plan in advance the methods of collecting, storing, and pretreating samples. Clean sample container with acid and rinse with reagent water. Equipment for membrane filtration of samples in the field may be required to determine iron in solution (dissolved iron). Dissolved iron, considered to be that passing through a 0.45-µm membrane filter, may include colloidal iron. The value of the determination depends greatly on the care taken to obtain a representative sample. Iron in well or tap water samples may vary in concentration and form with duration and degree of flushing before and during sampling. When taking a sample portion for determining iron in suspension, shake the sample bottle often and vigorously to obtain a uniform suspension of precipitated iron. Use particular care when colloidal iron adheres to the sample bottle. This problem can be acute with plastic bottles.

For a precise determination of total iron, use a separate container for sample collection. Treat with acid at the time of collection to place the iron in solution and prevent adsorption or deposition on the walls of the sample container. Take account of the added acid in measuring portions for analysis. The addition of acid to the sample may eliminate the need for adding acid before digestion (Section 3500-Fe.B.4a).

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3500-Fe B. Phenanthroline Method

1. General Discussion

a. Principle: Iron is brought into solution, reduced to the ferrous state by boiling with acid and hydroxylamine, and treated with 1,10-phenanthroline at pH 3.2 to 3.3. Three molecules of phenanthroline chelate each atom of ferrous iron to form an orange-red complex. The colored solution obeys Beer's law; its intensity is independent of pH from 3 to 9. A pH between 2.9 and 3.5 insures rapid color development in the presence of an excess of phenanthroline. Color standards are stable for at least 6 months.

b. Interference: Among the interfering substances are strong oxidizing agents, cyanide, nitrite, and phosphates (polyphosphates more so than orthophosphate), chromium, zinc in concentrations exceeding 10 times that of iron, cobalt and copper in excess of 5 mg/L, and nickel in excess of 2 mg/L. Bismuth, cadmium, mercury, molybdate, and silver precipitate phenanthroline. The initial boiling with acid converts polyphosphates to orthophosphate and removes cyanide and nitrite that otherwise would interfere. Adding excess hydroxylamine eliminates errors caused by excessive concentrations of strong oxidizing reagents. In the presence of interfering metal ions, use a larger excess of phenanthroline to replace that complexed by the interfering metals. Where excessive concentrations of interfering metal ions

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are present, the extraction method may be used.

If noticeable amounts of color or organic matter are present, it may be necessary to evaporate the sample, gently ash the residue, and redissolve in acid. The ashing may be carried out in silica, porcelain, or platinum crucibles that have been boiled for several hours in 6N HCl. The presence of excessive amounts of organic matter may necessitate digestion before use of the extraction procedure.

c. Minimum detectable concentration: Dissolved or total concentrations of iron as low as 10 µg/L can be determined with a spectrophotometer using cells with a 5 cm or longer light path.

Carry a blank through the entire procedure to allow for correction.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

- 1) *Spectrophotometer*, for use at 510 nm, providing a light path of 1 cm or longer.
- 2) *Filter photometer*, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 510 nm.
- 3) *Nessler tubes*, matched, 100-mL, tall form.

b. Acid-washed glassware: Wash all glassware with conc hydrochloric acid (HCl) and rinse with reagent water before use to remove deposits of iron oxide.

c. Separatory funnels: 125-mL, Squibb form, with ground-glass or TFE stopcocks and stoppers.

3. Reagents

Use reagents low in iron. Use reagent water (see Section 1080 and Section 3111B.3c) in preparing standards and reagent solutions and in procedure. Store reagents in glass-stoppered bottles. The HCl and ammonium acetate solutions are stable indefinitely if tightly stoppered. The hydroxylamine, phenanthroline, and stock iron solutions are stable for several months. The standard iron solutions are not stable; prepare daily as needed by diluting the stock solution. Visual standards in nessler tubes are stable for several months if sealed and protected from light.

a. Hydrochloric acid, HCl, conc, containing less than 0.5 ppm iron.

b. Hydroxylamine solution: Dissolve 10 g $\text{NH}_2\text{OH}\cdot\text{HCl}$ in 100 mL water.

c. Ammonium acetate buffer solution: Dissolve 250 g $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ in 150 mL water. Add 700 mL conc (glacial) acetic acid. Because even a good grade of $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ contains a significant amount of iron, prepare new reference standards with each buffer preparation.

d. Sodium acetate solution: Dissolve 200 g $\text{NaC}_2\text{H}_3\text{O}_2\cdot 3\text{H}_2\text{O}$ in 800 mL water.

e. Phenanthroline solution: Dissolve 100 mg 1,10-phenanthroline monohydrate, $\text{C}_{12}\text{H}_8\text{N}_2\cdot\text{H}_2\text{O}$, in 100 mL water by stirring and heating to 80°C. Do not boil. Discard the solution if it darkens. Heating is unnecessary if 2 drops conc HCl are added to the water. (NOTE:

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One milliliter of this reagent is sufficient for no more than 100 µg Fe.)

f. Potassium permanganate, 0.1M: Dissolve 0.316 KMnO_4 in reagent water and dilute to 100 mL.

g. Stock iron solution: Use metal (1) or salt (2) for preparing the stock solution.

1) Use electrolytic iron wire, or “iron wire for standardizing,” to prepare the solution. If necessary, clean wire with fine sandpaper to remove any oxide coating and to produce a bright surface. Weigh 200.0 mg wire and place in a 1000-mL volumetric flask. Dissolve in 20 mL 6N sulfuric acid (H_2SO_4) and dilute to mark with water; 1.00 mL = 200 µg Fe.

2) If ferrous ammonium sulfate is preferred, slowly add 20 mL conc H_2SO_4 to 50 mL water and dissolve 1.404 g $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$. Add 0.1M potassium permanganate (KMnO_4) dropwise until a faint pink color persists. Dilute to 1000 mL with water and mix; 1.00 mL = 200 µg Fe.

h. Standard iron solutions: Prepare daily for use.

1) Pipet 50.00 mL stock solution into a 1000-mL volumetric flask and dilute to mark with water; 1.00 mL = 10.0 µg Fe.

2) Pipet 5.00 mL stock solution into a 1000-mL volumetric flask and dilute to mark with water; 1.00 mL = 1.00 µg Fe.

i. Diisopropyl or isopropyl ether. CAUTION: *Ethers may form explosive peroxides; test before using.*

4. Procedure

a. Total iron: Mix sample thoroughly and measure 50.0 mL into a 125-mL erlenmeyer flask. If this sample volume contains more than 200 µg iron use a smaller accurately measured portion and dilute to 50.0 mL. Add 2 mL conc HCl and 1 mL $\text{NH}_2\text{OH} \cdot \text{HCl}$ solution. Add a few glass beads and heat to boiling. To insure dissolution of all the iron, continue boiling until volume is reduced to 15 to 20 mL. (If the sample is ashed, take up residue in 2 mL conc HCl and 5 mL water.) Cool to room temperature and transfer to a 50- or 100-mL volumetric flask or nessler tube. Add 10 mL $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ buffer solution and 4 mL phenanthroline solution, and dilute to mark with water. Mix thoroughly and allow a minimum of 10 min for maximum color development.

b. Dissolved iron: Immediately after collection filter sample through a 0.45-µm membrane filter into a vacuum flask containing 1 mL conc HCl/100 mL sample. Analyze filtrate for total dissolved iron (§ 4a) and/or dissolved ferrous iron (§ 4c). (This procedure also can be used in the laboratory if it is understood that normal sample exposure to air during shipment may result in precipitation of iron.)

Calculate suspended iron by subtracting dissolved from total iron.

c. Ferrous iron: Determine ferrous iron at sampling site because of the possibility of change

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in the ferrous-ferric ratio with time in acid solutions. To determine ferrous iron only, acidify a separate sample with 2 mL conc HCl/100 mL sample at time of collection. Fill bottle directly from sampling source and stopper. Immediately withdraw a 50-mL portion of acidified sample and add 20 mL phenanthroline solution and 10 mL $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ solution with vigorous stirring. Dilute to 100 mL and measure color intensity within 5 to 10 min. Do not expose to sunlight. (Color development is rapid in the presence of excess phenanthroline. The phenanthroline volume given is suitable for less than 50 μg total iron; if larger amounts are present, use a correspondingly larger volume of phenanthroline or a more concentrated reagent.)

Calculate ferric iron by subtracting ferrous from total iron.

d. Color measurement: Prepare a series of standards by accurately pipetting calculated volumes of standard iron solutions [use solution described in ¶ 3h2) to measure 1- to 10- μg portions] into 125-mL erlenmeyer flasks, diluting to 50 mL by adding measured volumes of water, and carrying out the steps in ¶ 4a beginning with transfer to a 100-mL volumetric flask or nessler tube.

For visual comparison, prepare a set of at least 10 standards, ranging from 1 to 100 μg Fe in the final 100-mL volume. Compare colors in 100-mL tall-form nessler tubes.

For photometric measurement, use Table 3500-Fe:I as a rough guide for selecting proper light path at 510 nm. Read standards against water set at zero absorbance and plot a calibration curve, including a blank (see ¶ 3c and General Introduction).

If samples are colored or turbid, carry a second set of samples through all steps of the procedure without adding phenanthroline. Instead of water, use the prepared blanks to set photometer to zero absorbance and read each sample developed with phenanthroline against the corresponding blank without phenanthroline. Translate observed photometer readings into iron values by means of the calibration curve. This procedure does *not* compensate for interfering ions.

e. Samples containing organic interferences: Digest samples containing substantial amounts of organic substances according to the directions given in Section 3030G or Section 3030H.

1) If a digested sample has been prepared according to the directions given in Section 3030G or Section 3030H, pipet 10.0 mL or other suitable portion containing 20 to 500 μg Fe into a 125-mL separatory funnel. If the volume taken is less than 10 mL, add water to make up to 10 mL. To the separatory funnel add 15 mL conc HCl for a 10-mL aqueous volume; or, if the portion taken was greater than 10.0 mL, add 1.5 mL conc HCl/mL sample. Mix, cool, and proceed with ¶ 4e3) below.

2) To prepare a sample solely for determining iron, measure a suitable volume containing 20 to 500 μg Fe and carry it through the digestion procedure described in either Section 3030G or Section 3030H. However, use only 5 mL H_2SO_4 or HClO_4 and omit H_2O_2 . When digestion is complete, cool, dilute with 10 mL water, heat almost to boiling to dissolve slowly soluble salts, and, if the sample is still cloudy, filter through a glass-fiber, sintered-glass, or porcelain filter, washing with 2 to 3 mL water. Quantitatively transfer filtrate or clear solution to a 25-mL

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volumetric flask and make up to 25 mL with water. Empty flask into a 125-mL separatory funnel, rinse flask with 5 mL conc HCl and add to the funnel. Add 25 mL conc HCl measured with the same flask. Mix and cool to room temperature.

3) Extract the iron from the HCl solution in the separatory funnel by shaking for 30 s with 25 mL isopropyl ether (CAUTION). Draw off lower acid layer into a second separatory funnel. Extract acid solution again with 25 mL isopropyl ether, drain acid layer into a suitable clean vessel, and add ether layer to the ether in the first funnel. Pour acid layer back into second separatory funnel and re-extract with 25 mL isopropyl ether. Withdraw and discard acid layer and add ether layer to first funnel. Persistence of a yellow color in the HCl solution after three extractions does not signify incomplete separation of iron because copper, which is not extracted, gives a similar yellow color.

Shake combined ether extracts with 25 mL water to return iron to aqueous phase and transfer lower aqueous layer to a 100-mL volumetric flask. Repeat extraction with a second 25-mL portion of water, adding this to the first aqueous extract. Discard ether layer.

4) Add 1 mL $\text{NH}_2\text{OH}\cdot\text{HCl}$ solution, 10 mL phenanthroline solution, and 10 mL $\text{NaC}_2\text{H}_3\text{O}_2$ solution. Dilute to 100 mL with water, mix thoroughly, and let stand for a minimum of 10 min. Measure absorbance at 510 nm using a 5-cm absorption cell for amounts of iron less than 100 μg or 1-cm cell for quantities from 100 to 500 μg . As reference, use either water or a sample blank prepared by carrying the specified quantities of acids through the entire analytical procedure. If water is used as reference, correct sample absorbance by subtracting absorbance of a sample blank.

Determine micrograms of iron in the sample from the absorbance (corrected, if necessary) by reference to the calibration curve prepared by using a suitable range of iron standards containing the same amounts of phenanthroline, hydroxylamine, and sodium acetate as the sample.

5. Calculation

When the sample has been treated according to ¶ 4a, ¶ 4b, ¶ 4c, or ¶ 4e2):

$$\text{mg Fe/L} = \frac{\mu\text{g Fe (in 100 mL final volume)}}{\text{mL sample}}$$

When the sample has been treated according to ¶ 4e1):

$$\text{mg Fe/L} = \frac{\mu\text{g Fe (in 100 mL final volume)}}{\text{mL sample}} \times \frac{100}{\text{mL portion}}$$

Report details of sample collection, storage, and pretreatment if they are pertinent to interpretation of results.

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6. Precision and Bias

Precision and bias depend on the method of sample collection and storage, the method of color measurement, the iron concentration, and the presence of interfering color, turbidity, and foreign ions. In general, optimum reliability of visual comparison in nessler tubes is not better than 5% and often only 10%, whereas, under optimum conditions, photometric measurement may be reliable to 3% or 3 µg, whichever is greater. The sensitivity limit for visual observation in nessler tubes is approximately 1 µg Fe. Sample variability and instability may affect precision and bias of this determination more than will the errors of analysis. Serious divergences have been found in reports of different laboratories because of variations in methods of collecting and treating samples.

A synthetic sample containing 300 µg Fe/L, 500 µg Al/L, 50 µg Cd/L, 110 µg Cr/L, 470 µg Cu/L, 70 µg Pb/L, 120 µg Mn/L, 150 µg Ag/L, and 650 µg Zn/L in distilled water was analyzed in 44 laboratories by the phenanthroline method, with a relative standard deviation of 25.5% and a relative error of 13.3%.

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3500-Pb A. Introduction

1. Occurrence and Significance

Lead (Pb) is the fifth element in Group IVA in the periodic table; it has an atomic number of 82, an atomic weight of 207.19, and valences of 2 and 4. The average abundance of Pb in the earth's crust is 13 ppm; in soils it ranges from 2.6 to 25 ppm; in streams it is 3 µg/L, and in groundwaters it is generally <0.1 mg/L. Lead is obtained chiefly from galena (PbS). It is used in batteries, ammunition, solder, piping, pigments, insecticides, and alloys. Lead also was used in gasoline for many years as an anti-knock agent in the form of tetraethyl lead.

The common aqueous species are Pb^{2+} and hydroxide and carbonate complexes. Lead in a water supply may come from industrial, mine, and smelter discharges or from the dissolution of plumbing and plumbing fixtures. Tap waters that are inherently noncorrosive or not suitably treated may contain lead resulting from an attack on lead service pipes, lead interior plumbing, brass fixtures and fittings, or solder pipe joints.

Lead is nonessential for plants and animals. It is toxic by ingestion and is a cumulative poison. The Food and Drug Administration regulates lead content in food and in house paints. Under the lead-copper rule, the U.S. EPA drinking water 90th percentile action level is 15 µg/L.

2. Selection of Method

The atomic absorption spectrometric method (Section 3111B) has a relatively high detection limit in the flame mode and requires an extraction procedure (Section 3111C) for the low concentrations common in potable water. The electrothermal atomic absorption (AA) method (Section 3113B) is more sensitive for low concentrations and does not require extraction. The inductively coupled plasma/mass spectrometric method (Section 3125) is even more sensitive than the electrothermal AA method. The inductively coupled plasma method (Section 3120) has a sensitivity similar to that of the flame atomic absorption method. Anodic stripping voltammetry (Section 3130B) can achieve superior detection limits, but is susceptible to interferences from copper, silver, gold, and organic compounds. The dithizone method (B) is sensitive and specific as a colorimetric procedure.

3500-Pb B. Dithizone Method

1. General Discussion

a. Principle: An acidified sample containing microgram quantities of lead is mixed with ammoniacal citrate-cyanide reducing solution and extracted with dithizone in chloroform (CHCl_3) to form a cherry-red lead dithizonate. The color of the mixed color solution is measured photometrically.^{1,2} Sample volume taken for analysis may be 2 L when digestion is used.

b. Interference: In a weakly ammoniacal cyanide solution (pH 8.5 to 9.5) dithizone forms

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colored complexes with bismuth, stannous tin, and monovalent thallium. In strongly ammoniacal citrate-cyanide solution (pH 10 to 11.5) the dithizonates of these ions are unstable and are extracted only partially.³ This method uses a high pH, mixed color, single dithizone extraction. Interference from stannous tin and monovalent thallium is reduced further when these ions are oxidized during preliminary digestion. A modification of the method allows detection and elimination of bismuth interference. Excessive quantities of bismuth, thallium, and tin may be removed.⁴

Dithizone in CHCl_3 absorbs at 510 nm; control its interference by using nearly equal concentrations of excess dithizone in samples, standards, and blank.

The method is without interference for the determination of 0.0 to 30.0 $\mu\text{g Pb}$ in the presence of 20 $\mu\text{g Tl}^+$, 100 $\mu\text{g Sn}^{2+}$, 200 $\mu\text{g In}^{3+}$, and 1000 μg each of Ba^{2+} , Cd^{2+} , Co^{2+} , Cu^{2+} , Mg^{2+} , Mn^{2+} , Hg^{2+} , Sr^{2+} , Zn^{2+} , Al^{3+} , Sb^{3+} , As^{3+} , Cr^{3+} , Fe^{3+} , V^{3+} , PO_4^{3-} , and SO_4^{2-} . Gram quantities of alkali metals do not interfere. A modification is provided to avoid interference from excessive quantities of bismuth or tin.

c. Preliminary sample treatment: At time of collection acidify with conc HNO_3 to pH <2 but avoid excess HNO_3 . Add 5 mL 0.1N iodine solution to avoid losses of volatile organo-lead compounds during handling and digesting of samples. Prepare a blank of lead-free water and carry through the procedure.

d. Digestion of samples: Unless digestion is shown to be unnecessary, digest all samples for dissolved or total lead as described in 3030H or K.

e. Minimum detectable concentration: 1.0 $\mu\text{g Pb}/10\text{ mL}$ dithizone solution.

2. Apparatus

a. Spectrophotometer for use at 510 nm, providing a light path of 1 cm or longer.

b. pH meter.

c. Separatory funnels: 250-mL Squibb type. Clean all glassware, including sample bottles, with 1 + 1 HNO_3 . Rinse thoroughly with reagent water.

d. Automatic dispensing burets: Use for all reagents to minimize indeterminate contamination errors.

3. Reagents

Prepare all reagents in lead-free water.

a. Stock lead solution: Dissolve 0.1599 g lead nitrate, $\text{Pb}(\text{NO}_3)_2$ (minimum purity 99.5%), in approximately 200 mL water. Add 10 mL conc HNO_3 and dilute to 1000 mL with water. Alternatively, dissolve 0.1000 g pure Pb metal in 20 mL 1 + 1 HNO_3 and dilute to 1000 mL with water; 1.00 mL = 100 $\mu\text{g Pb}$.

b. Working lead solution: Dilute 2.0 mL stock solution to 100 mL with water; 1 mL = 2.00

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µg Pb.

- c. *Nitric acid*, HNO_3 , 1 + 4: Dilute 200 mL conc HNO_3 to 1 L with water.
- d. *Ammonium hydroxide*, NH_4OH , 1 + 9: Dilute 10 mL conc NH_4OH to 100 mL with water.
- e. *Citrate-cyanide reducing solution*: Dissolve 400 g dibasic ammonium citrate, $(\text{NH}_4)_2\text{HC}_6\text{H}_5\text{O}_7$, 20 g anhydrous sodium sulfite, Na_2SO_3 , 10 g hydroxylamine hydrochloride, $\text{NH}_2\text{OH}\cdot\text{HCl}$, and 40 g potassium cyanide, KCN (CAUTION: *Poison*) in water and dilute to 1 L. Mix this solution with 2 L conc NH_4OH . *Do not pipet by mouth. Prepare solution in a fume hood.*
- f. *Stock dithizone solution*: The dithizone concentration in the stock dithizone solutions is based on having a 100% pure dithizone reagent. Some commercial grades of dithizone are contaminated with the oxidation product diphenylthiocarbodiazone or with metals. Purify dithizone as directed below. For dithizone solutions not stronger than 0.001% (w/v), calculate the exact concentration by dividing the absorbance of the solution in a 1.00-cm cell at 606 nm by 40.6×10^3 , the molar absorptivity.

In a fume hood, dissolve 100 mg dithizone in 50 mL CHCl_3 in a 150-mL beaker and filter through a 7-cm-diam paper.*#(109) Receive filtrate in a 500-mL separatory funnel or in a 125-mL erlenmeyer flask under slight vacuum; use a filtering device designed to handle the CHCl_3 vapor. Wash beaker with two 5-mL portions CHCl_3 , and filter. Wash the paper with three 5-mL portions CHCl_3 , adding final portion dropwise to edge of paper. If filtrate is in flask, transfer with CHCl_3 to a 500-mL separatory funnel.

Add 100 mL 1 + 99 NH_4OH to separatory funnel and shake moderately for 1 min; excessive agitation produces slowly breaking emulsions. Let layers separate, swirling funnel gently to submerge CHCl_3 droplets held on surface of aqueous layer. Transfer CHCl_3 layer to 250-mL separatory funnel, retaining the orange-red aqueous layer in the 500-mL funnel. Repeat extraction, receiving CHCl_3 layer in another 250-mL separatory funnel and transferring aqueous layer, using 1 + 99 NH_4OH , to the 500-mL funnel holding the first extract. Repeat extraction, transferring the aqueous layer to 500-mL funnel. Discard CHCl_3 layer.

To combined extracts in the 500-mL separatory funnel add 1 + 1 HCl in 2-mL portions, mixing after each addition, until dithizone precipitates and solution is no longer orange-red. Extract precipitated dithizone with three 25-mL portions CHCl_3 . Dilute combined extracts to 1000 mL with CHCl_3 ; 1.00 mL = 100 µg dithizone.

g. *Dithizone working solution*: Dilute 100 mL stock dithizone solution to 250 mL with CHCl_3 ; 1 mL = 40 µg dithizone.

h. *Special dithizone solution*: Dissolve 250 mg dithizone in 250 mL CHCl_3 . This solution may be prepared without purification because all extracts using it are discarded.

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- i. *Sodium sulfite solution:* Dissolve 5 g anhydrous Na_2SO_3 in 100 mL water.
- j. *Iodine solution:* Dissolve 40 g KI in 25 mL water, add 12.7 g resublimed iodine, and dilute to 1000 mL.

4. Procedure

a. *With sample digestion:* CAUTION: Perform the following procedure (excluding use of spectrophotometer) in a fume hood. To a digested sample containing not more than 1 mL conc acid add 20 mL 1 + 4 HNO_3 and filter through lead-free filter paper†#(110) and filter funnel directly into a 250-mL separatory funnel. Rinse digestion beaker with 50 mL water and add to filter. Add 50 mL ammoniacal citrate-cyanide solution, mix, and cool to room temperature. Add 10 mL dithizone working solution, shake stoppered funnel vigorously for 30 s, and let layers separate. Insert lead-free cotton in stem of separatory funnel and draw off lower layer. Discard 1 to 2 mL CHCl_3 layer, then fill absorption cell. Measure absorbance of extract at 510 nm, using dithizone working solution, ¶ 3g, to zero spectrophotometer.

b. *Without sample digestion:* To 100 mL acidified sample (pH 2) in a 250-mL separatory funnel add 20 mL 1 + 4 HNO_3 and 50 mL citrate-cyanide reducing solution; mix. Add 10 mL dithizone working solution and proceed as in ¶ 4a.

c. *Calibration curve:* Plot concentration of at least five standards and a blank against absorbance. Determine concentration of lead in extract from curve. All concentrations are $\mu\text{g Pb}/10\text{ mL final extract}$.

d. *Removal of excess interferences:* The dithizonates of bismuth, tin, and thallium differ from lead dithizonate in maximum absorbance. Detect their presence by measuring sample absorbance at 510 nm and at 465 nm. Calculate corrected absorbance of sample at each wavelength by subtracting absorbance of blank at same wavelength. Calculate ratio of corrected absorbance at 510 nm to corrected absorbance at 465 nm. The ratio of corrected absorbances for lead dithizonate is 2.08 and for bismuth dithizonate is 1.07. If the ratio for the sample indicates interference, i.e., is markedly less than 2.08, proceed as follows with a new 100-mL sample: If the sample has not been digested, add 5 mL Na_2SO_3 solution to reduce iodine preservative.

Adjust sample to pH 2.5 using a pH meter and 1 + 4 HNO_3 or 1 + 9 NH_4OH as required.

Transfer sample to 250-mL separatory funnel, extract with a minimum of three 10-mL portions special dithizone solution, or until the CHCl_3 layer is distinctly green. Extract with 20-mL portions CHCl_3 to remove dithizone (absence of green). Add 20 mL 1 + 4 HNO_3 , 50 mL citrate-cyanide reducing solution, and 10 mL dithizone working solution. Extract as in ¶ 4a and measure absorbance.

5. Calculation

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$$\text{mg Pb/L} = \frac{\mu\text{g Pb (in 10 mL, from calibration curve)}}{\text{mL sample}}$$

6. Precision and Bias

Single-operator precision in recovering 0.0104 mg Pb/L from Mississippi River water was 6.8% relative standard deviation and -1.4% relative error. At the level of 0.026 mg Pb/L, recovery was made with 4.8% relative standard deviation and 15% relative error.

7. References

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3500-Li LITHIUM*#(111)

3500-Li A. Introduction

1. Occurrence and Significance

Lithium (Li) is the second element in Group IA of the periodic table; it has an atomic number of 3, an atomic weight of 6.94, and a valence of 1. The average abundance of Li in the earth's crust is 18 ppm; in soils it is 14 to 32 ppm; in streams it is 3 µg/L, and in groundwaters it is <0.1 mg/L. The more important minerals containing lithium are lepidolite, spodumene, petalite, and amblygonite. Lithium compounds are used in pharmaceuticals, soaps, batteries, welding flux, ceramics, reducing agents (e.g., lithium aluminum hydride), and cosmetics.

Many lithium salts are only slightly soluble, and the metal's concentration in water is controlled by incorporation in clay minerals of soils. Lithium is considered nonessential for plants and animals, but it is essential for some microorganisms. Some lithium salts are toxic by ingestion. The United Nations Food and Agriculture Organization recommended maximum level for lithium in irrigation waters is 2.5 mg/L.

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2. Selection of Method

The atomic absorption spectrometric method (Section 3111B) and the inductively coupled plasma method (Section 3120) are preferred. The flame emission photometric method (B) also is available for laboratories not equipped to use preferred methods. The inductively coupled plasma/mass spectrometric method (Section 3125) may be applied successfully in most cases (with lower detection limits), even though lithium is not specifically listed as an analyte in the method.

3500-Li B. Flame Emission Photometric Method

1. General Discussion

a. Principle: Lithium can be determined in trace amounts by flame photometric methods at a wavelength of 670.8 nm.

b. Interference: A molecular band of strontium hydroxide with an absorption maximum at 671.0 nm interferes in the flame photometric determination of lithium. Ionization of lithium can be significant in both the air-acetylene and nitrous oxide-acetylene flames and can be suppressed by adding potassium. See Section 3500-NaB.1a for additional information on minimizing interferences in flame photometry.

c. Minimum detectable concentration: The minimum lithium concentration detectable is approximately 0.1 µg/L for reagent water analyzed on an atomic absorption spectrophotometer in the emission mode with an air-acetylene flame, or 0.03 µg/L with a nitrous oxide-acetylene flame.

d. Sampling and storage: Preferably collect sample in a polyethylene bottle, although borosilicate glass containers also may be used. At time of collection adjust sample to pH <2 with nitric acid (HNO₃).

2. Apparatus

Flame photometer: A flame photometer or an atomic absorption spectrometer operating in the emission mode using a lean air-acetylene flame is recommended.

3. Reagents

Use reagent water (see Section 3111B.3c) in reagent preparation and analysis.

a. Potassium ionization suppressant: Dissolve 95.35 g KCl dried at 110°C and dilute to 1000 mL with water; 1.00 mL = 50 mg K.

b. Stock lithium solution: Dissolve 152.7 mg anhydrous lithium chloride, LiCl, in water and dilute to 250 mL; 1.00 mL = 100 µg Li. Dry salt overnight in an oven at 105°C. Cool in a desiccator and weigh immediately after removal from desiccator. Alternatively, purchase prepared stock from a reputable supplier.

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c. *Standard lithium solution:* Dilute 10.00 mL stock LiCl solution to 500 mL with water; 1.00 mL = 2.0 µg Li.

4. Procedure

a. *Pretreatment of polluted water and wastewater samples:* Choose digestion method appropriate to matrix (see Section 3030).

b. *Suppressing ionization:* If necessary, filter sample through medium-porosity paper, add 1.0 mL potassium ionization suppressant to 50 mL volumetric flask, and dilute with sample for flame photometric determination. Sample solution will be in a 0.1% K matrix.

c. *Treatment of standard solutions:* Prepare dilutions of the Li standard solution to bracket sample concentration or to establish at least three points on a calibration curve of emission intensity against Li concentration. Prepare standards by adding appropriate volumes of standard lithium solution to 25 mL water + 1.0 mL potassium ionization suppressant reagent in a 50-mL volumetric flask. Dilute to 50.0 mL and mix. Both samples and standards will be in a 0.1% K matrix to suppress ionization of lithium.

d. *Flame photometric measurement:* Determine lithium concentration by direct intensity measurements at a wavelength of 670.8 nm. The bracketing method (Section 3500-NaB.4d) can be used with some photometric instruments, while the construction of a calibration curve is necessary with others. Run sample, water, and lithium standard as nearly simultaneously as possible. For best results, average several readings on each solution.

Follow the manufacturer's instructions for instrument operation.

5. Calculation

$$\mu\text{g Li/L} = (\mu\text{g Li/L in portion analyzed}) \times D$$

where:

$$D = \text{dilution ratio} \\ = \frac{\text{mL sample} + \text{mL water}}{\text{mL sample}}$$

6. Quality Control

Process a QC standard through entire analytical protocol as a way of determining systematic bias. The control limits for precision of duplicate determinations at concentrations (in water) of 4.0 µg/L and 10.0 µg/L were 4.09 ± 0.056 µg/L and 9.96 ± 0.094 µg/L, respectively. The single-operator RSD was 1.38% for a lithium solution containing 10 µg/L.

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3500-Mg MAGNESIUM*#(112)

3500-Mg A. Introduction

1. Occurrence and Significance

Magnesium (Mg) is the second element in Group IIA of the periodic table; it has an atomic number of 12, an atomic weight of 24.30, and a valence of 2. The average abundance of Mg in the earth's crust is 2.1%; in soils it is 0.03 to 0.84%; in streams it is 4 mg/L, and in groundwaters it is >5 mg/L. Magnesium occurs commonly in the minerals magnesite and dolomite. Magnesium is used in alloys, pyrotechnics, flash photography, drying agents, refractories, fertilizers, pharmaceuticals, and foods.

The common aqueous species is Mg^{2+} . The carbonate equilibrium reactions for magnesium are more complicated than for calcium, and conditions for direct precipitation of dolomite in natural waters are not common. Important contributors to the hardness of a water, magnesium salts break down when heated, forming scale in boilers. Chemical softening, reverse osmosis, or ion exchange reduces magnesium and associated hardness to acceptable levels.

Magnesium is an essential element in chlorophyll and in red blood cells. Some salts of magnesium are toxic by ingestion or inhalation. Concentrations greater than 125 mg/L also can have a cathartic and diuretic effect.

2. Selection of Method

The methods presented are applicable to waters and wastewaters. Direct determinations can

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be made with the atomic absorption spectrometric method (Section 3111B) and inductively coupled plasma method (Section 3120). The inductively coupled plasma mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though magnesium is not specifically listed as an analyte in the method. These methods can be applied to most concentrations encountered, although sample dilution may be required. Choice of method is largely a matter of personal preference and analyst experience. A calculation method (B) also is available.

3500-Mg B. Calculation Method

Magnesium may be estimated as the difference between hardness and calcium as CaCO_3 if interfering metals are present in noninterfering concentrations in the calcium titration (Section 3500-Ca.B) and suitable inhibitors are used in the hardness titration (Section 2340C).

$$\text{mg Mg/L} = [\text{total hardness (as mg CaCO}_3\text{/L)} - \text{calcium hardness (as mg CaCO}_3\text{/L)}] \times 0.243$$

3500-Mn MANGANESE*#(113)

3500-Mn A. Introduction

1. Occurrence and Significance

Manganese (Mn) is the first element in Group VIIB in the periodic table; it has an atomic number of 25, an atomic weight of 54.94, and common valences of 2, 4, and 7 (and more rarely, valences of 1, 3, 5, and 6). The average abundance of Mn in the earth's crust is 1060 ppm; in soils it is 61 to 1010 ppm; in streams it is 7 $\mu\text{g/L}$, and in groundwaters it is <0.1 mg/L. Manganese is associated with iron minerals, and occurs in nodules in ocean, fresh waters, and soils. The common ores are pyrolusite (MnO_2) and psilomelane. Manganese is used in steel alloys, batteries, and food additives.

The common aqueous species are the reduced Mn^{2+} and the oxidized Mn^{4+} . The aqueous chemistry of manganese is similar to that of iron. Since groundwater is often anoxic, any soluble manganese in groundwater is usually in the reduced state (Mn^{2+}). Upon exposure to air or other oxidants, groundwater containing manganese usually will precipitate black MnO_2 . Elevated manganese levels therefore can cause stains in plumbing/laundry, and cooking utensils. It is considered an essential trace element for plants and animals. The United Nations Food and Agriculture Organization recommended maximum level for manganese in irrigation waters is 0.2 mg/L. The U.S. EPA secondary drinking water standard MCL is 50 $\mu\text{g/L}$.

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2. Selection of Method

The atomic absorption spectrometric methods (Section 3111B and Section 3111C), the electrothermal atomic absorption method (Section 3113B), and the inductively coupled plasma methods (Section 3120 and Section 3125) permit direct determination with acceptable sensitivity and are the methods of choice. Of the various colorimetric methods, the persulfate method (B) is preferred because the use of mercuric ion can control interference from a limited chloride ion concentration.

3. Sampling and Storage

Manganese may exist in a soluble form in a neutral water when first collected, but it oxidizes to a higher oxidation state and precipitates or becomes adsorbed on the container walls. Determine manganese very soon after sample collection. When delay is unavoidable, total manganese can be determined if the sample is acidified at the time of collection with HNO_3 to $\text{pH} < 2$. See Section 3010B.

3500-Mn B. Persulfate Method

1. General Discussion

a. Principle: Persulfate oxidation of soluble manganous compounds to form permanganate is carried out in the presence of silver nitrate. The resulting color is stable for at least 24 h if excess persulfate is present and organic matter is absent.

b. Interference: As much as 0.1 g chloride (Cl^-) in a 50-mL sample can be prevented from interfering by adding 1 g mercuric sulfate (HgSO_4) to form slightly dissociated complexes. Bromide and iodide still will interfere and only trace amounts may be present. The persulfate procedure can be used for potable water with trace to small amounts of organic matter if the period of heating is increased after more persulfate has been added.

For wastewaters containing organic matter, use preliminary digestion with nitric and sulfuric acids (HNO_3 and H_2SO_4) (see Section 3030G). If large amounts of Cl^- also are present, boiling with HNO_3 helps remove it. Interfering traces of Cl^- are eliminated by HgSO_4 in the special reagent.

Colored solutions from other inorganic ions are compensated for in the final colorimetric step.

Samples that have been exposed to air may give low results due to precipitation of manganese dioxide (MnO_2). Add 1 drop 30% hydrogen peroxide (H_2O_2) to the sample, after adding the special reagent, to redissolve precipitated manganese.

c. Minimum detectable concentration: The molar absorptivity of permanganate ion is about $2300 \text{ L g}^{-1} \text{ cm}^{-1}$. This corresponds to a minimum detectable concentration (98% transmittance)

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of 210 µg Mn/L when a 1-cm cell is used or 42 µg Mn/L when a 5-cm cell is used.

2. Apparatus

Colorimetric equipment: One of the following is required:

- Spectrophotometer*, for use at 525 nm, providing a light path of 1 cm or longer.
- Filter photometer*, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 525 nm.
- Nessler tubes*, matched, 100-mL, tall form.

3. Reagents

a. *Special reagent:* Dissolve 75 g HgSO₄ in 400 mL conc HNO₃ and 200 mL distilled water. Add 200 mL 85% phosphoric acid (H₃PO₄), and 35 mg silver nitrate (AgNO₃). Dilute the cooled solution to 1 L.

b. *Ammonium persulfate*, (NH₄)₂S₂O₈, solid.

c. *Standard manganese solution:* Prepare a 0.1N potassium permanganate (KMnO₄) solution by dissolving 3.2 g KMnO₄ in distilled water and making up to 1 L. Age for several weeks in sunlight or heat for several hours near the boiling point, then filter through a fine fritted-glass filter crucible and standardize against sodium oxalate as follows:

Weigh several 100- to 200-mg samples of Na₂C₂O₄ to 0.1 mg and transfer to 400-mL beakers. To each beaker, add 100 mL distilled water and stir to dissolve. Add 10 mL 1 + 1 H₂SO₄ and heat rapidly to 90 to 95°C. Titrate rapidly with the KMnO₄ solution to be standardized, while stirring, to a slight pink end-point color that persists for at least 1 min. Do not let temperature fall below 85°C. If necessary, warm beaker contents during titration; 100 mg Na₂C₂O₄ will consume about 15 mL permanganate solution. Run a blank on distilled water and H₂SO₄.

$$\text{Normality of KMnO}_4 = \frac{\text{g Na}_2\text{C}_2\text{O}_4}{(A - B) \times 0.06701}$$

where:

A = mL titrant for sample and

B = mL titrant for blank.

Average results of several titrations. Calculate volume of this solution necessary to prepare 1 L of solution so that 1.00 mL = 50.0 µg Mn, as follows:

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$$\text{mL KMnO}_4 = \frac{4.55}{\text{normality KMnO}_4}$$

To this volume add 2 to 3 mL conc H_2SO_4 and NaHSO_3 solution dropwise, with stirring, until the permanganate color disappears. Boil to remove excess SO_2 , cool, and dilute to 1000 mL with distilled water. Dilute this solution further to measure small amounts of manganese.

d. Standard manganese solution (alternate): Dissolve 1.000 g manganese metal (99.8% min.) in 10 mL redistilled HNO_3 . Dilute to 1000 mL with 1% (v/v) HCl ; 1 mL = 1.000 mg Mn. Dilute 10 mL to 200 mL with distilled water; 1 mL = 0.05 mg Mn. Prepare dilute solution daily.

e. Hydrogen peroxide, H_2O_2 , 30%.

f. Nitric acid, HNO_3 , conc.

g. Sulfuric acid, H_2SO_4 , conc.

h. Sodium nitrite solution: Dissolve 5.0 g NaNO_2 in 95 mL distilled water.

i. Sodium oxalate, $\text{Na}_2\text{C}_2\text{O}_4$, primary standard.

j. Sodium bisulfite: Dissolve 10 g NaHSO_3 in 100 mL distilled water.

4. Procedure

a. Treatment of sample: If a digested sample has been prepared according to directions for reducing organic matter and/or excessive chlorides in Section 3030G, pipet a portion containing 0.05 to 2.0 mg Mn into a 250-mL conical flask. Add distilled water, if necessary, to 90 mL and proceed as in ¶ b.

b. To a suitable sample portion add 5 mL special reagent and 1 drop H_2O_2 . Concentrate to 90 mL by boiling or dilute to 90 mL. Add 1 g $(\text{NH}_4)_2\text{S}_2\text{O}_8$, bring to a boil, and boil for 1 min. Do not heat on a water bath. Remove from heat source, let stand 1 min, then cool under the tap. (Boiling too long results in decomposition of excess persulfate and subsequent loss of permanganate color; cooling too slowly has the same effect.) Dilute to 100 mL with distilled water free from reducing substances and mix. Prepare standards containing 0, 5.00, . . . 1500 μg Mn by treating various amounts of standard Mn solution in the same way.

c. Nessler tube comparison: Use standards prepared as in ¶ 4b and containing 5 to 100 μg Mn/100 mL final volume. Compare samples and standards visually.

d. Photometric determination: Use a series of standards from 0 to 1500 μg Mn/100mL final volume. Make photometric measurements against a distilled water blank. The following table shows light path length appropriate for various amounts of manganese in 100mL final volume:

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shows light path length appropriate for various amounts of manganese in 100mL final volume:

Mn Range μg	Light Path cm
5–200	15
20–400	5
50–1000	2
100–1500	1

Prepare a calibration curve of manganese concentration vs. absorbance from the standards and determine Mn in the samples from the curve. If turbidity or interfering color is present, make corrections as in 4e.

e. Correction for turbidity or interfering color: Avoid filtration because of possible retention of some permanganate on the filter paper. If visual comparison is used, the effect of turbidity only can be estimated and no correction can be made for interfering colored ions. When photometric measurements are made, use the following “bleaching” method, which also corrects for interfering color: As soon as the photometer reading has been made, add 0.05 mL H_2O_2 solution directly to the sample in the optical cell. Mix and, as soon as the permanganate color has faded completely and no bubbles remain, read again. Deduct absorbance of bleached solution from initial absorbance to obtain absorbance due to Mn.

5. Calculation

a. When all of the original sample is taken for analysis:

$$\text{mg Mn/L} = \frac{\mu\text{g Mn}/100 \text{ mL}}{\text{mL sample}} \times \frac{100}{\text{mL portion}}$$

b. When a portion of the digested sample (100 mL final volume) is taken for analysis:

$$\text{mg Mn/L} = \frac{\mu\text{g Mn (in 100 mL final volume)}}{\text{mL sample}}$$

6. Precision and Bias

A synthetic sample containing 120 $\mu\text{g Mn/L}$, 500 $\mu\text{g Al/L}$, 50 $\mu\text{g Cd/L}$, 110 $\mu\text{g Cr/L}$, 470 $\mu\text{g Cu/L}$, 300 $\mu\text{g Fe/L}$, 70 $\mu\text{g Pb/L}$, 150 $\mu\text{g Ag/L}$, and 650 $\mu\text{g Zn/L}$ in distilled water was analyzed in 33 laboratories by the persulfate method, with a relative standard deviation of 26.3% and a

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relative error of 0%.

A second synthetic sample, similar in all respects except for 50 µg Mn/L and 1000 µg Cu/L, was analyzed in 17 laboratories by the persulfate method, with a relative standard deviation of 50.3% and a relative error of 7.2%.

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3500-Hg MERCURY

Mercury (Hg) is the third element in Group IIB in the periodic table; it has an atomic number of 80, an atomic weight of 200.59, and valences of 1 and 2. The average abundance of Hg in the earth's crust is 0.09 ppm; in soils it is 30 to 160 ppb; in streams it is 0.07 µg/L, and in groundwaters it is 0.5 to 1 µg/L. Mercury occurs free in nature, but the chief source is cinnibar (HgS). Mercury is used in amalgams, mirror coatings, vapor lamps, paints, measuring devices (thermometers, barometers, manometers), pharmaceuticals, pesticides, and fungicides. It is often used in paper mills as a mold retardant for paper.

The common aqueous species are Hg^{2+} , $\text{Hg}(\text{OH})_2^0$, Hg^0 , and stable complexes with organic ligands. Inorganic mercury can be methylated in sediments when sulfides are present to form dimethyl mercury, $(\text{CH}_3)_2\text{Hg}$, which is very toxic and can concentrate in the aquatic food chain. Mercury poisoning occurred in Japan in the 1950s as the result of consumption of shellfish that had accumulated mercury. In times past, mercury was used in the haberdashery industry to block hats (the cause of the "mad hatter" syndrome).

Mercury is considered nonessential for plants and animals. The U.S. EPA primary drinking water standard MCL is 2 µg/L.

The cold-vapor atomic absorption method (Section 3112B) is the method of choice for all samples. The inductively coupled plasma mass spectrometric method (Section 3125) also may be applied successfully in some cases, even though mercury is not specifically listed as an analyte in the method. The dithizone method detailed in the 19th edition of *Standard Methods* can be used for determining high levels of mercury (>2 µg/L) in potable waters.

Because mercury can be lost readily from samples, preserve them by treating with HNO_3 to reduce the pH to <2 (see Section 1060). Glass storage containers are preferred to plastic, because they can extend the holding time to 30 d, rather than only the 14 d allowed in plastic containers.

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3500-Mo MOLYBDENUM

Molybdenum (Mo) is the second element in Group VIB in the periodic table; it has an atomic number of 42, and atomic weight of 95.95, and valences of 2, 3, 4, 5, and 6. The average abundance of Mo in the earth's crust is 1.2 ppm; in soils it is 2.5 ppm; in streams it is 1 µg/L, and in groundwaters it is <0.1 mg/L. Molybdenum occurs naturally as molybdenite (MoS_2) and wulfenite

(PbMoO_4). It is used in alloys, ink pigments, catalysts, and lubricants.

The common aqueous species are HMoO_4^- , MoO_4^{2-} , and organic complexes. It is considered an essential trace element for plants and animals. The United Nations Food and Agriculture Organization recommended maximum level for irrigation waters is 0.01 mg/L.

Use one of the flame atomic absorption spectrometric methods (Section 3111D or Section 3111E), the electrothermal atomic absorption spectrometric method (Section 3113B), or one of the inductively coupled plasma methods (Section 3120 or Section 3125).

3500-Ni NICKEL

Nickel (Ni) is the third element in Group VIII in the periodic table; it has an atomic number of 28, an atomic weight of 58.69, and a common valence of 2 and less commonly 1, 3, or 4. The average abundance of Ni in the earth's crust is 1.2 ppm; in soils it is 2.5 ppm; in streams it is 1 µg/L, and in groundwaters it is <0.1 mg/L. Nickel is obtained chiefly from pyrrhotite and garnierite. Nickel is used in alloys, magnets, protective coatings, catalysts, and batteries.

The common aqueous species is Ni^{2+} . In reducing conditions insoluble sulfides can form, while in aerobic conditions nickel complexes with hydroxide, carbonates, and organic ligands can form. It is suspected to be an essential trace element for some plants and animals. The United Nations Food and Agriculture Organization recommended maximum level for irrigation waters is 200 µg/L. The U.S. EPA primary drinking water standard MCL is 0.1 mg/L.

The atomic absorption spectrometric methods (Section 3111B and Section 3111C), the inductively coupled plasma methods (Section 3120 and Section 3125), and the electrothermal atomic absorption spectrometric method (Section 3113B) are the methods of choice for all samples.

3500-Os OSMIUM

Osmium (Os) is the seventh element in Group VIII in the periodic table; it has an atomic number of 76, an atomic weight of 190.2, and valences of 3, 4, and 6, and less commonly 1, 2, 5, 7, and 8. The average abundance of Os in the earth's crust is probably <0.005 ppm, and in groundwaters it is <0.01 mg/L. Osmium occurs in iridosime and in platinum-bearing river sands.

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Osmium is used as a hardener with iridium and as a catalyst with platinum.

The aqueous chemistry is controlled by complex compounds, although the solubility in natural waters is relatively unknown.

Analyze by flame atomic absorption methods (Section 3111D and Section 3111E).

3500-Pd PALLADIUM

Palladium (Pd) is the sixth element in Group VIII of the periodic table; it has an atomic number of 46, an atomic weight of 106.42, and valences of 2 and 4. Palladium occurs with platinum in nature. It is used in alloys to make electrical relays, catalysts, in the making of “white gold,” and in protective coatings.

Palladium has no known toxic effects. The United Nations Food and Agriculture Organization recommended maximum level for irrigation waters is 5 mg/L.

Preferably analyze by flame atomic absorption method (Section 3111B). The inductively coupled plasma mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though palladium is not specifically listed as an analyte in the method.

3500-Pt PLATINUM

Platinum (Pt) is the ninth element in Group VIII of the periodic table; it has an atomic number of 78, an atomic weight of 195.1, and valences of 2 and 4. The average abundance of Pt in the earth’s crust is probably <0.01 ppm, and in groundwaters it is <0.1 mg/L. Platinum is usually found in its native state, but also may be found as sperrylite (PtAs₂). Platinum is used as a catalyst and in laboratoryware, jewelry, and surgical wire.

The aqueous chemistry in natural waters is relatively unknown, although its solubility is probably controlled by complex compounds. In powder form, platinum can be flammable, and its soluble salts are toxic by inhalation.

Preferably analyze by flame atomic absorption method (Section 3111B). The inductively coupled plasma mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though platinum is not specifically listed as an analyte in the method.

3500-K POTASSIUM*(114)

3500-K A. Introduction

1. Occurrence and Significance

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Potassium (K) is the fourth element in Group IA of the periodic table; it has an atomic number of 19, an atomic weight of 39.10, and a valence of 1. The average abundance of K in the earth's crust is 1.84%; in soils it has a range of 0.1 to 2.6%; in streams it is 2.3 mg/L, and in groundwaters it has a range of 0.5 to 10 mg/L. Potassium is commonly associated with aluminosilicate minerals such as feldspars. ^{40}K is a naturally occurring radioactive isotope with a half-life of 1.3×10^9 years. Potassium compounds are used in glass, fertilizers, baking powder, soft drinks, explosives, electroplating, and pigments. Potassium is an essential element in both plant and human nutrition, and occurs in groundwaters as a result of mineral dissolution, from decomposing plant material, and from agricultural runoff.

The common aqueous species is K^+ . Unlike sodium, it does not remain in solution, but is assimilated by plants and is incorporated into a number of clay-mineral structures.

2. Selection of Method

Methods for the determination of potassium include flame atomic absorption (Section 3111B), inductively coupled plasma (Section 3120), flame photometry (B), and selective ion electrode (C). The inductively coupled plasma/mass spectrometric method (Section 3125) usually may be applied successfully (with lower detection limits), even though potassium is not specifically listed as an analyte in the method. The preferred methods are rapid, sensitive, and accurate; selection depends on instrument availability and analyst choice.

3. Storage of Samples

Do not store samples in soft-glass bottles because of the possibility of contamination from leaching of the glass. Use acid-washed polyethylene or borosilicate glass bottles. Adjust sample to $\text{pH} < 2$ with nitric acid. This will dissolve potassium salts and reduce adsorption on vessel walls.

3500-K B. Flame Photometric Method

1. General Discussion

a. Principle: Trace amounts of potassium can be determined in either a direct-reading or internal-standard type of flame photometer at a wavelength of 766.5 nm. Because much of the information pertaining to sodium applies equally to the potassium determination, carefully study the entire discussion dealing with the flame photometric determination of sodium (Section 3500-Na.B) before making a potassium determination.

b. Interference: Interference in the internal-standard method may occur at sodium-to-potassium ratios of 5:1 or greater. Calcium may interfere if the calcium-to-potassium ratio is 10:1 or more. Magnesium begins to interfere when the magnesium-to-potassium ratio exceeds 100:1.

c. Minimum detectable concentration: Potassium levels of approximately 0.1 mg/L can be

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determined.

2. Apparatus

See Section 3500-Na.B.2.

3. Reagents

To minimize potassium pickup, store all solutions in plastic bottles. Shake each container thoroughly to dissolve accumulated salts from walls before pouring.

a. Reagent water: See Section 1080. Use this water for preparing all reagents and calibration standards, and as dilution water.

b. Stock potassium solution: Dissolve 1.907 g KCl dried at 110°C and dilute to 1000 mL with water; 1 mL = 1.00 mg K.

c. Intermediate potassium solution: Dilute 10.0 mL stock potassium solution with water to 100 mL; 1.00 mL = 0.100 mg K.

Use this solution to prepare calibration curve in potassium range of 1 to 10 mg/L.

d. Standard potassium solution: Dilute 10.0 mL intermediate potassium solution with water to 100 mL; 1.00 mL = 0.010 mg K. Use this solution to prepare calibration curve in potassium range of 0.1 to 1.0 mg/L.

4. Procedure

Make determination as described in Section 3500-Na.B.4, but measure emission intensity at 766.5 nm.

5. Calculation

See Section 3500-Na.B.5.

6. Precision and Bias

A synthetic sample containing 3.1 mg K⁺/L, 108 mg Ca²⁺/L, 82 mg Mg²⁺/L, 19.9 mg Na⁺/L, 241 mg Cl⁻/L, 0.25 mg NO₂⁻-N/L, 1.1 mg NO₃⁻-N/L, 259 mg SO₄²⁻/L, and 42.5 mg total alkalinity/L (contributed by NaHCO₃) was analyzed in 33 laboratories by the flame photometric method, with a relative standard deviation of 15.5% and a relative error of 2.3%.

7. Bibliography

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Also see Section 3500-Na.B.7.

3500-K C. Potassium-Selective Electrode Method

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1. General Discussion

a. Principle: Potassium ion is measured potentiometrically by using a potassium ion-selective electrode and a double-junction, sleeve-type reference electrode. The analysis is performed with either a pH meter having an expanded millivolt scale capable of being read to the nearest 0.1 mV or a specific ion meter having a direct concentration scale for potassium.

Before measurement, an ionic strength adjustor reagent is added to both standards and samples to maintain a constant ionic strength. The electrode response is measured in standard solutions with potassium concentrations spanning the range of interest using a calibration line derived either by the instrument meter or manually. The electrode response in sample solutions is measured following the same procedure and potassium concentration determined from the calibration line or instrument direct readout.

b. Interferences: Although most sensitive to potassium, the potassium electrode will respond to other cations at high concentrations; this can result in a positive bias. Table 3500-K:I lists the concentration of common cations causing a 10% error at various concentrations of potassium chloride with a background ionic strength of 0.12N sodium chloride. Of the cations listed, ammonium ion is most often present in samples at concentrations high enough to result in a significant bias. It can be converted to gaseous ammonia by adjusting to pH > 10.

An electrode exposed to interfering cations tends to drift and respond sluggishly. To restore normal performance soak electrode for 1 h in distilled water and then for several hours in a standard potassium solution.

c. Detection limits: Samples containing from 0.1 to 1000 mg K⁺/L may be analyzed. To measure higher concentrations dilute the sample.

2. Apparatus

a. Expanded-scale or digital pH meter or ion-selective meter.

b. Potassium ion-selective electrode.

c. Sleeve-type double-junction reference electrode: Fill outer sleeve with reference electrode filling solution (see ¶ 3b). Fill inner sleeve with inner filling solution provided with the electrode.

d. pH electrode.

e. Mixer, magnetic, with a TFE-coated stirring bar.

3. Reagents

a. Ionic strength adjustor (ISA): Dissolve 29.22 g NaCl in reagent water and dilute to 100 mL.

b. Reference electrode outer sleeve filling solution: Dilute 2 mL ISA solution to 100 mL with reagent water.

c. Stock potassium solution: See Section 3500-K.B.3b.

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- d. *Sodium hydroxide*, NaOH, 6N.
- e. *Reagent water*: See Section 1080.

4. Procedure

a. *Preparation of standards*: Prepare a series of standards containing 100.0, 10.0, 1.0, and 0.1 mg K⁺/L by making serial dilutions of the stock potassium solution as in Section 3500-K.B.3c and Section 3500-K.B.3d.

b. *Instrument calibration*: Fill reference electrode according to the manufacturer's instructions using reference electrode filling solution. Transfer 100 mL 0.1 mg K⁺/L standard into a 150-mL beaker and add 2 mL ISA. Raise pH to about 11. Stir gently with magnetic mixer. Immerse electrodes, wait approximately 2 min for potential stabilization and record meter reading. Thoroughly rinse electrodes and blot dry. Repeat for each standard solution in order of increasing concentration. Prepare calibration curve on semilogarithmic graph paper by plotting observed potential in millivolts (linear scale) against concentration (log scale). Alternatively, calculate calibration line by regression analysis.

c. *Analysis of samples*: Transfer 100 mL sample into a 150-mL beaker and follow procedure applied to standards in ¶ 4b above. From the measured response, calculate K⁺ concentration from calibration curve.

5. Precision

Reproducibility of potential measured, over the method's range, can be expected to be ± 0.4 mV, corresponding to about $\pm 2.5\%$ in concentration.

6. Quality Assurance

The slope of the calibration line should be -56 mV/10-fold concentration change. If the slope is outside the range of -56 ± 3 mV, the electrode may require maintenance (replace filling solutions). If the proper electrode response cannot be obtained, replace electrode.

Analyze an independent check standard with a mid-range potassium concentration throughout analysis of a series, initially, every ten samples, and after final sample. If the value has changed by more than 5%, recalibrate electrode. Analyze a reagent blank at the same frequency. Readings must represent a lower concentration than the lowest concentration standard (0.1 mg/L).

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3500-Re RHENIUM

Rhenium (Re) is the third element in Group VIIB in the periodic table; it has an atomic number of 75, an atomic weight of 186.21, and valences of 1 through 7, with 7 being the most stable. The average abundance of Re in the earth's crust is 7 ppm, and in groundwaters it is <0.1 mg/L. Rhenium is found in columbite, tantalite, and wolframite, as well as in molybdenum ore concentrates. It is used in tungsten-molybdenum-based alloys, thermocouples, filaments, and flash bulbs. Rhenium in the powder form can be flammable.

For analysis methods, see flame atomic absorption methods (Section 3111D and Section 3111E). The inductively coupled plasma mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though rhenium is not specifically listed as an analyte in the method.

3500-Rh RHODIUM

Rhodium (Rh) is the fifth element in Group VIII in the periodic table; it has an atomic number of 45, an atomic weight of 102.91, and valences of 1 through 6, the most common being 1 and 3. Rhodium is found in its native state in platinum-bearing sands. It is used in platinum alloys for thermocouples, electrical contacts, and jewelry.

The aqueous chemistry in natural waters is relatively unknown. The metal is flammable in the powder form, and its salts are toxic by inhalation.

For analysis see flame atomic absorption method (Section 3111B). The inductively coupled plasma/mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though rhodium is not specifically listed as an analyte in the method.

3500-Ru RUTHENIUM

Ruthenium (Ru) is the fourth element in Group VIII in the periodic table; it has an atomic number of 44, an atomic weight of 101.07, and valences of 1 through 7, the most common being 2, 3, and 4. The average abundance of Ru in the earth's crust is probably <0.01 ppm, and in groundwaters it is <0.1 mg/L. It occurs in its native state in platinum-bearing river sands. It is used in jewelry with platinum, in electrical contacts, and as a catalyst.

The aqueous chemistry in natural waters is relatively unknown. Ruthenium has no known toxic effects.

For analysis see flame atomic absorption method (Section 3111B). The inductively coupled plasma mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though ruthenium is not specifically listed as an analyte in the method.

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3500-Se SELENIUM*#(115)

3500-Se A. Introduction

1. Occurrence and Significance

Selenium (Se) is the third element in Group VIA in the periodic table; it has an atomic number of 34, an atomic weight of 78.96, and valences of 2, 4, or 6. The average abundance of Se in the earth's crust is 0.2 ppm; in soils it is 0.27 to 0.74 ppm; in streams it is 0.2 µg/L, and in groundwaters it is <0.1 mg/L. Selenium is used in electronics, ceramics, and shampoos.

The inorganic fraction of dissolved selenium consists predominantly of selenium as the selenate ion (SeO_4^{2-}), designated here as Se(VI), and selenium as the selenite ion (SeO_3^{2-}), Se(IV). Other common aqueous species include Se^{2-} , HSe^- , and Se^0 . Selenium is considered a nonessential trace element for most plants, but is an essential trace nutrient for most animals, and selenium deficiency diseases are well known in veterinary medicine. Above trace levels, ingested selenium is toxic to animals and may be toxic to humans. While the selenium concentration of most natural waters is low, the pore water in seleniferous soils in semiarid areas may contain up to hundreds or thousands of micrograms dissolved selenium per liter. Certain plants that grow in such areas accumulate large concentrations of selenium and may poison livestock that graze on them. Water drained from such soil may cause severe environmental pollution and wildlife toxicity. Selenopolysulfide ions (SSe^{2-}) may occur in the presence of hydrogen sulfide in waterlogged, anoxic soils. Selenium derived from microbial degradation of seleniferous organic matter includes selenite, selenate, and the volatile organic compounds dimethylselenide and dimethyldiselenide. Nonvolatile organic selenium compounds may be released to water by microbial processes. Soluble selenium may be leached from coal ash and fly ash at electric power plants that burn seleniferous coal.

The United Nations Food and Agriculture Organization recommended maximum level for selenium in irrigation waters is 20 µg/L. The U.S. EPA primary drinking water standard MCL is 50 µg/L.

2. Selection of Method

The selenium methods using hydride generation atomic absorption (Section 3114B and Section 3114C), electrothermal atomic absorption (Section 3113B), and derivatization colorimetry (C) are the most sensitive currently available. For determination of selenium at higher concentrations, the inductively coupled plasma methods (Section 3120 and Section 3125) may be used.

By using suitable preparatory steps to convert other chemical species to Se(IV), it is possible to distinguish the chemical species in the sample. In drinking water and most surface and ground

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waters, Se(IV), Se(VI), and particulate selenium frequently are the only significant species. However, when speciation is important, for example, when a new matrix is being analyzed, the general analytical scheme shown in Figure 3500-Se:1 may be carried out as follows: Determine volatile selenium by stripping sample with nitrogen or air and collecting selenium in alkaline hydrogen peroxide (see Method D). To obtain an estimate of selenium in suspended particles, determine total selenium, filter sample, and make a second determination of total selenium. In any case, filter sample. Occasionally, a filtered sample may have the odor of hydrogen sulfide and a yellow color; such a sample may contain selenopolysulfides, which may be estimated by comparing results of total selenium analyses before and after acidification, stripping with nitrogen, settling for 10 min, and refiltration. Determine selenite, Se(IV), by analyzing filtered water sample directly by Methods 3114B or C, or by Section 3500-Se.C or Section 3500-Se.E. In principle, sample digestion with HCl will convert Se(VI) to Se(IV), and the value determined will equal the sum of the two species. In practice, samples frequently contain an unknown masking agent that produces an unduly low result. Test for this effect by analyzing samples with known additions of both species. If recovery is good, the HCl digestion followed by analyses will yield reliable results. If recovery is poor and organic selenium is to be determined subsequently, attempt to remove the interference by sample pretreatment with resin (B.1). Interference also can be eliminated by digestion with an oxidizing agent (B.2, 3, and 4), but these procedures prevent distinguishing of Se(VI) and organic selenium and also oxidize many organic selenium compounds. To measure nonvolatile organic selenium compounds, use Method E.

The choice of digestion method for oxidizing interferences and organic selenium depends on sample matrix. The methods described in B.2, 3, and 4, in order of increasing complexity and digestive ability, use ammonium (or potassium) persulfate, hydrogen peroxide, and potassium permanganate. Ammonium persulfate digestion is adequate for most filtered ground, drinking, and surface water. Hydrogen peroxide digestion may be required if organic selenium compounds are present, and potassium permanganate digestion may be needed with unfiltered samples or those containing refractory organic selenium compounds. Confirm results obtained with one digestion method by using a more rigorous method when characterizing a new matrix.

3. Interferences

Interferences are found in certain reagents, as well as in samples. Recognition of the presence of an interferant is critical, especially when unknown sample matrices are being analyzed. Routinely add Se(IV) and Se(VI) to test for interference. If present, characterize the interference and correct by the method of standard additions. A slope less than one indicates interference. In cases of mild interference (recoveries reduced by 25% or less), the standard additions method will largely correct determined values.

Because the hydride atomic absorption method is extremely sensitive, samples frequently need to be diluted to bring them within the linear range of the instrument. Diluting a filtered water sample frequently will eliminate sample-related interferences. Include full reagent blanks in each run to ensure absence of contamination from reagents. Hydride generator atomic absorption is susceptible to common interference problems related to nitrite in the sample or free

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chlorine in the reagent HCl (see Methods Section 3114B and Section 3114C).

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3500-Se B. Sample Preparation

1. Removal of Organic and Iron Interference by Resin Pretreatment

Interferences are common in selenium analysis, particularly when chemical speciation is attempted. Routine pretreatment of water samples as described here is not necessary. The methods in this section should be tried when poor recovery of standard additions indicates a problem.

Many waters contain iron and/or dissolved organic matter (humic acid) in quantities sufficient to interfere. Reduction of Se(VI) to Se(IV) usually is nonquantitative. When Se(VI) standard additions show poor recovery, treat the sample before analysis. To remove dissolved organic compounds, pass an acidified sample through a resin. Because dissolved organic selenium compounds also may be removed by this treatment, also determine total selenium in the untreated water sample (see 2, 3, or 4 below). To remove iron use a strong base ion exchange resin. *#(116) Iron is removed as the anionic chloro complex. In this treatment the acidity and ion exchanger do not alter speciation; complete speciation of selenium is possible.

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a. Apparatus:

- 1) *Chromatography column* for organics removal, glass, about 0.8 cm ID × 30 cm long, with fluorocarbon metering valve.
- 2) *Chromatography column* for ion exchange, disposable polyethylene.†#(117)
- 3) *pH meter*.

b. Reagents:

- 1) *Organics-removal resin*: Thoroughly rinse 16 to 50 mesh resin‡#(118) with deionized water and remove resin fines by decanting. Rinse three times with pH 12 solution. Store resin in pH 12 solution and refrigerate to prevent bacterial growth.
- 2) *Anion exchange resin*: Add 100 to 200 mesh anion exchange resin*#(119) to a beaker and thoroughly rinse with deionized water. Cover resin with 4*N* HCl, stir, and let settle. Decant and repeat acid rinse twice more. Store resin in 4*N* HCl.
- 3) *Hydrochloric acid*, conc: Before use, bubble helium through the acid for 3 h at rate of 100 mL/min. (CAUTION: *Use a fume hood.*)
- 4) *pH 1.6 solution*: Adjust pH of deionized water to 1.6 with HCl.
- 5) *pH 12 solution*: Adjust pH of deionized water to 12 with KOH.

c. Procedure:

1) *Organic removal*—Place 5 cm washed resin in a 0.8-cm-ID column. Precondition column, at 1 mL/min, with 30 mL pH 12 solution and 20 mL pH 1.6 solution. Using HCl and a pH meter adjust sample to pH 1.6 to 1.8. Pass sample through preconditioned column at rate of 1 mL/min. Discard first 10 mL and use next 11 to 50 mL collected for Se(IV) determinations by Methods 3114B or C, or 3500-Se.C preceded, if Se(VI) also is to be determined, by preparatory step B.5. If more than 50 mL sample are needed, use another column or use a column with twice as much resin.

2) *Iron removal*—Place 4 cm prepared resin in a small chromatographic column (add resin to column filled with 4*N* HCl to avoid air bubbles). Rinse column with 10 mL 4*N* HCl at flow rate < 6 mL/min. Let solution drain to top of resin, but do not let the column run dry. Adjust sample to 4*N* HCl and pour into column. Discard first 10 mL and collect the next 11 to 100 mL for Se(IV) analysis by Methods 3114B or C, or 3500-Se.C preceded, if necessary, by preparatory step B.2 and if Se(VI) also is to be determined, by preparatory step B.5 below.

2. Removal of Interference by Persulfate Digestion

The combination of this procedure with step B.5 below and Methods Section 3114B or Section 3114C, or Section 3500-Se.C is, in most cases, the preferred method for determining total selenium in filtered water. A small amount of ammonium or potassium persulfate is added to the mixture of sample and HCl to remove interference from reducing agents and to oxidize relatively labile organic selenium compounds such as selenoamino acids and methaneseleninic acid.

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If the sample contains hydrogen sulfide or a large concentration of organic matter or is otherwise suspect or to confirm method accuracy, reanalyze sample using digestion procedure 3 or 4 below.

Prepare 2% potassium or ammonium persulfate solution by dissolving 2.0 g in 100 mL deionized water (prepare weekly). Add 0.2 mL persulfate solution to the mixed sample and HCl of ¶ B.5c before heating and proceeding with pretreatment and analysis.

After completing analysis multiply concentration of selenium determined in the acidified sample by 2.04 to obtain total selenium in original sample.

3. Removal of Interference by Alkaline Hydrogen Peroxide Digestion

Occasionally, digestion with persulfate gives incomplete recovery of total selenium. In this case, digestion with hydrogen peroxide is used to remove all reducing agents that might interfere and to fully oxidize organic selenium to Se(VI). The resulting solution can be analyzed for total selenium after pretreatment according to step B.5 below.

This method is suitable for determining total selenium in unfiltered water samples, where particulate selenium is present. When working with a new matrix, confirm results obtained by reanalyzing the sample using digestion procedure 4, below.

a. Apparatus:

- 1) *Beakers*, 150-mL.
- 2) *Watch glasses*.
- 3) *Hot plate*.
- 4) *Pipetter*, 1-mL, and tips.
- 5) *Graduated cylinder*, 25-mL.

b. Reagents:

- 1) *Hydrogen peroxide*, H_2O_2 , 30%. Keep refrigerated.
- 2) *Sodium hydroxide*, NaOH, 1N.
- 3) *Hydrochloric acid*, HCl, 1.5N: Dilute 125 mL conc HCl to 1 L with deionized water.

c. Procedure: Add 2 mL 30% H_2O_2 and 1 mL 1 N NaOH to 25 mL sample in a beaker.

Cover beaker to control spattering and simmer on hot plate until fine bubbles characteristic of H_2O_2 decomposition subside and are replaced by ordinary boiling. Add 1 mL 1.5N HCl to redissolve any precipitate that may have formed, let cool, and pour into graduated cylinder. Rinse beaker with deionized water into graduated cylinder and make volume up to 25 mL. Proceed to B.5 and chosen analytical method.

4. Removal of Interference by Permanganate Digestion

This digestion method utilizes potassium permanganate to oxidize selenium and remove interfering organic compounds. Excess KMnO_4 and MnO_2 are removed by reaction with hydroxylamine. HCl digestion is included here, because it is conveniently performed in the same

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reaction vial. Selenite may then be determined directly by Methods Section 3114B or Section 3114C, or Section 3500-Se.C.

Verify recovery for the given matrix. This method gives good recovery even with heavily contaminated water samples that contain organic selenium compounds, dissolved organic matter, and visible suspended material.

Permanganate may oxidize chloride ion to free chlorine. Part of the chlorine (which interferes with hydride analysis) is removed by reaction with hydroxylamine, but the best way to eliminate free chlorine is by prolonged heating in an open vial during the digestion step. Excess hydroxylamine may reduce recovery by reducing selenium to Se(0).

a. Apparatus:

- 1) *Oven with thermostat*, for continuous operation at $110 \pm 5^{\circ}\text{C}$.
- 2) *Digestion vials*, 40-mL, with fluorocarbon-lined screw caps.
- 3) *Metal support rack* to hold 40 digestion vials.

b. Reagents:

- 1) *Hydrochloric acid*, HCl, conc. [See ¶ B.1b3)].
- 2) *Hydrochloric acid*, HCl, 10N: Dilute 1000 mL conc HCl to 1200 mL with deionized water.
- 3) *Sulfuric acid*, H_2SO_4 , conc and 25%. NOTE: Many brands of H_2SO_4 are contaminated with selenium. Run reagent blanks when starting a new bottle. Make 25% v/v solution by adding 250 mL conc H_2SO_4 to 500 mL deionized water, and diluting to 1 L.
- 4) *Potassium permanganate solution*, KMnO_4 , 5% (w/v): Dissolve 50 g KMnO_4 in 1000 mL deionized water.
- 5) *Hydroxylamine hydrochloride solution*: Dissolve 100 g $\text{NH}_2\text{OH}\cdot\text{HCl}$ in 1000 mL deionized water.

c. Procedure: Pipette 5 mL sample into digestion vial, add 5 mL 25% H_2SO_4 and 1 mL KMnO_4 solution. Screw cap on and place in preheated oven at 110°C for 1 h. Remove tray with vials from the oven and cool to room temperature. Open vial, carefully add a few drops hydroxylamine hydrochloride solution, mix, and wait until sample is decolorized and residual manganese dioxide is dissolved. Avoid excess hydroxylamine solution, which can cause a low reading. Add 10 mL conc HCl to the sample and heat vial 60 min at 95°C without cap. Let cool to room temperature. Transfer sample to a 25-mL volumetric flask or graduated cylinder, rinse vial into flask, dilute to mark, and mix well. Proceed to analyze by Methods Section 3114B or Section 3114C, or Section 3500-Se.C. If Method Section 3114B or Section 3114C is used, multiply spectrometer readings by the dilution factor as follows:

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$$\text{Concentration, } \mu\text{g/L} = \frac{\text{final volume}}{\text{volume of sample}} \times \text{reading}$$

5. Reduction of Se(VI) to Se(IV) by Hydrochloric Acid Digestion

Se(VI) is reduced to Se(IV) by digestion with HCl. Determine Se(IV) + Se(VI) by either hydride generation atomic absorption spectrometer (Method Section 3114B or Section 3114C) or as an organic derivative (Method C).

Test any given sample matrix to ensure recovery of added Se(VI). If recovery is poor, try to remove interference by the procedure of ¶ B.1, above, or if at least 75% recovery is achieved, use the method of standard additions. The method described here is of limited utility for direct analysis of water samples, but is useful as a step in determining total selenium in a sample where selenium has been oxidized to Se(VI).

a. Apparatus:

- 1) *Dispenser*, bottle type, 5-mL, suitable for dispensing concentrated HCl.
- 2) *Pipetters*, 0.2- and 5-mL.
- 3) *Screw-cap culture tubes*, borosilicate glass, 25- × 150-mm.
- 4) *Boiling water bath*, suitable for heating culture tubes; a 1-L beaker on a hot plate is suitable.

b. Reagents:

- 1) *Sodium selenate additions solution*: Dilute 1000 mg/L stock selenate solution with deionized water to prepare a solution of 1 to 10 mg/L, such that the concentration of the additions solution will be approximately 50 times greater than anticipated total selenium in the sample to be analyzed.

- 2) *Hydrochloric acid*, HCl, conc: See ¶ B.1b3).

c. *Procedure*: Calibrate acid dispenser using water. Preheat water bath. Pipet 5 mL filtered sample into a culture tube. Add 5 mL conc HCl. Loosely cap tube (do not tighten) and place in boiling water bath for 20 min. Let tube cool and tighten cap. Determine total Se(IV) by Methods Section 3114B or C, or Section 3500-Se.C.

Add 0.200 mL additions solution with a microliter pipet to sample and proceed as above. Analyze a deionized water blank and a blank with the addition to ensure absence of contamination and to determine the true value of the addition.

Multiply the concentration of selenium determined in the acidified sample by 2.00 to obtain total concentration of Se(IV) + Se(VI). Multiply reading obtained for sample with addition by 2.04.

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3500-Se C. Colorimetric Method

1. General Discussion

a. Principle: This method is specific to determining selenite ion in aqueous solution. Selenite ion reacts with 2,3-diaminonaphthalene to produce a brightly colored and strongly fluorescent piazselenol compound, which is extracted in cyclohexane and measured colorimetrically.

The optimum pH for formation of the piazselenol complex is approximately 1.5 but should not be above 2.5 because above pH 2, the rate of formation of the colored compound is critically dependent on pH. When indicators are used to adjust pH, results frequently are erratic; results can be improved when pH is monitored electrochemically.

b. Interference: No inorganic compounds are known to give a positive interference. Colored organic compounds extractable by cyclohexane may be encountered, but usually they are absent or can be removed by oxidizing the sample (see B.2, 3, or 4) or by treating it to remove dissolved organics (see B.1). Negative interference results from compounds that reduce the concentration of diaminonaphthalene by oxidizing it. Addition of EDTA eliminates negative interference from at least 2.5 mg Fe²⁺.

c. Minimum detectable quantity: 10 µg Se/L.

2. Apparatus

a. Colorimetric equipment: A spectrophotometer, for use at 480 nm, providing a light path of 1 cm or longer.

b. Separatory funnel, 250-mL, preferably with a fluorocarbon stopcock.

c. Thermostatically controlled water bath (50°C) with cover.

d. pH meter.

e. Centrifuge, with rotor for 50-mL tubes (optional).

f. Centrifuge bottles, 60-mL, screw-capped, fluorocarbon.

g. Shaker, suitable for separatory funnel (optional).

3. Reagents

Use reagent water (see Section 1080) in preparing reagents.

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- a. *Selenium standard reference solution*: Dissolve 2.190 g sodium selenite, Na_2SeO_3 , in water containing 10 mL HCl and dilute to 1 L. 1.00 mL = 1.00 mg Se(IV).
- b. *Working standard selenium solutions*: Dilute selenium reference standard solution with water or suitable background solution to produce a series of working standards spanning the concentration range of interest.
- c. *Hydrochloric acid*: HCl, conc and 0.1N.
- d. *Ammonium hydroxide*, NH_4OH , 50% v/v.
- e. *Cyclohexane*, C_6H_{12} .
- f. *2,3-Diaminonaphthalene (DAN) solution*: Dissolve 200 mg DAN in 200 mL 0.1N HCl. Shake 5 min. Extract three times with 25-mL portions of cyclohexane, retaining aqueous phase and discarding organic portions. Filter into opaque container*#(120) and store in cool, dark place for no longer than 8 h. CAUTION: *Toxic, handle with extreme care.*
- g. *Hydroxylamine-EDTA solution (HA-EDTA)*: Dissolve 4.5 g Na_2EDTA in approximately 450 mL water. Add 12.5 g hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$) and adjust volume to 500 mL.

4. Procedure

- a. *Formation of piaszelenol*: Add 2 mL HA-EDTA solution to 10 mL sample in 60-mL centrifuge bottle (filtered if Se(IV) is to be determined; oxidized using Method B.2, 3, or 4, then reduced using Method B.5 for total Se). Adjust to $\text{pH } 1.5 \pm 0.3$ with 0.1N HCl and 50% NH_4OH , using a pH meter. Add 5 mL DAN solution and heat in a covered water bath at 50°C for 30 min.
- b. *Extraction of piaszelenol*: Cool and add 2.0 mL cyclohexane. Cap container securely and shake vigorously for 5 min. Let solution stand for 5 min or until cyclohexane layer becomes well separated. If separation is slow, centrifuge for 5 min at 2000 rpm. Place bottle in a clamp on a ringstand at a 45° angle to the vertical. Remove aqueous phase using a disposable pipet attached to a vacuum line. Transfer organic phase to a small capped container using a clean disposable pipet, or to the spectrophotometer cuvette if absorbance is to be read immediately.
- c. *Determination of absorbance*: Read absorbance at 480 nm using a zero standard. The piaszelenol color is very stable but evaporation of the cyclohexane concentrates the color unless the container is capped. CAUTION: *Avoid inhaling cyclohexane vapors.* Beer's Law is obeyed up to 2 mg/L.

5. Calculation

Construct calibration curve using at least a three-point standard curve to bracket the expected sample concentration. Plot absorbance vs. concentration. Correct for digestion blank and any reagent blank.

6. Precision and Bias

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Three standard reference materials (wheat flour, water, and a commercial standard) were used to evaluate Se recovery.¹ The wheat flour sample was digested using HNO₃ and HClO₄ to convert total selenium to Se(VI), digested with HCl to convert Se(VI) to Se(IV) and finally, the colorimetric method was used. Results were as follows:

Standard	Selenium Concentration <i>μg Se/L</i>	
	Expected	Recovered*
NBS, SRM 1567, wheat flour†	1097 ± 197	1113 ± 8
NBS, SRM 1543ib, water	9.7 ± 0.5	8.7 ± 0
Fisher Certified AAS Standard	1002 ± 8	1002 ± 0

* Analyses in triplicate.

† Dry weight basis.

7. Reference

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3500-Se D. Determination of Volatile Selenium

1. General Discussion

Dimethylselenide and dimethyldiselenide are low boiling, extremely malodorous organic compounds sparingly soluble in water. They are produced by microbial processes in seleniferous soil and decaying seleniferous organic matter, and occasionally are present in natural waters. They are readily air stripped from a water sample and can be collected with high efficiency in an

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alkaline solution of hydrogen peroxide, which oxidizes them quantitatively to Se(VI). Total selenium is determined by digestion with HCl and analysis by the hydride atomic absorption (Section 3114B or Section 3114C) or colorimetric (Section 3500-Se.C) methods.

Either nitrogen or air may be used to strip the sample. Preferably use nitrogen if air-sensitive compounds (e.g., selenopolysulfides) are suspected.

Because volatile selenium can be lost in the course of sample collection and handling, preferably air-strip the sample in the field immediately after it is collected. After boiling to decompose H_2O_2 , return the alkaline peroxide solution to the laboratory for analysis.

2. Apparatus

All apparatus required for selenate reduction (§ B.5) and Methods Section 3114B or Section 3114C, or Section 3500-Se.C, plus:

- a. *Gas washing bottles*, borosilicate glass, 250-mL, with coarse porous glass gas dispersion frit. Mark 100-mL level on side of bottle.
- b. *Rotameter*, to measure 3 L/min air flow.
- c. *Gas flow regulator*
- d. *Hot plate*.
- e. *Graduated cylinder*, 100-mL.
- f. *Beakers*, 250-mL.
- g. *Rubber tubing*, to interconnect gas washing bottles and other gas equipment.
- h. *Rubber gloves*.

3. Reagents

All reagents required for selenate reduction (§ B.5) and Methods Section 3114B or Section 3114C, or Section 3500-Se.C, plus:

- a. *Hydrogen peroxide*, 30%. Refrigerate.
- b. *Sodium hydroxide solution*, NaOH, 1N.
- c. *Compressed air or nitrogen*.

4. Procedure

Set up air-flow train in this order: Regulated air supply→rotameter→gas washing bottle 1→gas washing bottle 2.

Prepare alkaline peroxide solution immediately before use by pouring 20 mL 30% H_2O_2 into a 100-mL graduated cylinder, adding 50 to 60 mL deionized water and 5 mL 1N NaOH, and making up to 100 mL. CAUTION: *Alkaline H_2O_2* is unstable. Do not keep in glass bottle; hold at about 0 °C in oversized plastic bottle. Solution is corrosive; protect eyes and skin. Pour into gas washing bottle 2. Pour approximately 100 mL freshly collected sample into gas washing bottle 1. Do not attempt to measure sample volume accurately before volatile selenium determination, as

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unnecessary handling may cause volatile selenium to be lost.

Connect and check all air lines, turn on air and adjust flow to 3 L/min. Strip for 30 min or more. After 30 min, turn off air, disconnect gas washing bottle 2, and place it on the hot plate. Adjust heat to produce a gentle simmering of oxygen bubbles from decomposition of H_2O_2 . Continue heating until the characteristic effervescence of oxygen subsides and is replaced by ordinary boiling. Remove from hot plate and let cool. Pour solution into beaker. (Volume will be very near to 100 mL, and correction will usually be unnecessary.) Analyze for total selenium using HCl digestion (B.5) and Methods Section 3114B or Section 3114C, or Section 3500-Se.C. Once boiled, this solution may be safely stored and transported in plastic bottles. Measure volume of sample in gas washing bottle 1.

5. Calculation

The concentration of volatile selenium compounds in the original water sample can be calculated as:

$$C = \frac{100}{\text{volume of original sample}} \times \text{conc of Se in solution}$$

6. Precision and Bias

Approximately 90% of dimethylselenide in samples will be recovered with 30 min air stripping. The recovery of dimethyldiselenide is not known. Loss of gases to the atmosphere during sampling and handling that precede analysis may cause a significant negative error.

3500-Se E. Determination of Nonvolatile Organic Selenium Compounds

1. General Discussion

In principle, the total amount of dissolved organic selenium plus polysulfidic selenium may be estimated by comparing “total Se,” determined by oxidation and HCl digestion (§ B.2 or § B.3 and § B.5, or § B.4), followed by Methods Section 3114B or Section 3114C, or Section 3500-Se.C, with Se(IV) + Se(VI) determined by HCl digestion (§ B.5) and Method Section 3114C, or Section 3500-Se.C. In practice, this will give a meaningful estimate only if a known addition of Se(VI) is fully recovered. Even if recovery is good, this estimate may be unreliable, because it is the difference of two (frequently larger) numbers determined by slightly different methods. Comparing total Se before and after treatment with resin (§ B.1c1)] gives a similarly unreliable estimate of nonvolatile organic Se.

It is preferable to separate and directly determine nonvolatile organic selenium. One method involves adsorption of dissolved organic matter onto a C-18 reverse phase HPLC resin, elution

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with an organic solvent, and determination of selenium in this fraction. While this technique is relatively simple, it is affected by pH and small organic molecules (e.g., individual selenium containing amino acids) are not retained by the resins. Adjust sample pH to 1.5 to 2.0 before using the column, but because the latter problem cannot be solved easily, the use of organic adsorbents provides only an estimate of organic selenium concentration.

Alternatively, isolate specific compounds and determine their selenium content. In some natural waters selenium may be associated with dissolved polypeptides or small proteins, and even small amounts of free selenoamino acids may be present. Because selenoamino acids are the most toxic form of the element, a direct determination is sometimes desirable.

To determine selenium in dissolved peptides, hydrolyze with acid and isolate the free amino acids via ligand exchange chromatography. Elute the selenoamino acids from the column and determine selenium. Selenoamino acids are unstable during acid hydrolysis and even using nonoxidizing methyl sulfonic acid and nitrogen-purged glass ampules, selenoamino acid recoveries are only 50 to 80%. This method is good only for estimating protein-bound selenium. A somewhat more reliable estimate of free selenoamino acids and selenium associated with small oligopeptides is obtained by performing a similar procedure without the hydrolysis step.

While these methods are too intricate for routine use, semiquantitative, and sensitive only to certain classes of organic compounds, at present they are the only ones available with any practical experience.

Imperfect separation of organic selenium compounds from inorganic forms of selenium may cause interference. In parallel with the actual determination, always perform the procedure using a solution compounded to resemble the actual matrix and containing a similar amount of selenium, but in the form of Se(IV) and Se(VI) to determine degree of interference.

2. Apparatus

- a. *Rotary evaporator*, with temperature-control bath and 30-mL pear-shaped flasks.
- b. *Glass ampule sealing apparatus*, or oxygen-gas torch.
- c. *Heating block*, 100°C, or pressure cooker.
- d. *Glass chromatography columns*, 15 cm long, 0.7 cm ID.*#(121)
- e. *Glass syringe*, 50-mL.
- f. *Glass ampules*, 10- or 20- mL. Clean by heating in a muffle furnace at 400°C for 24 h.
- g. *pH meter*.

3. Reagents

- a. *Hydrochloric acid*, HCl, 1N.
- b. *Methyl sulfonic acid*, conc.
- c. *Ammonium hydroxide*, NH₄OH, 1.5N: Dilute 100 mL conc NH₄OH to 1 L with deionized water.

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- d. *Sodium hydroxide*, NaOH pellets and 1N solution.
- e. *pH 1.6 solution*: Adjust pH of deionized water to 1.6 using HCl.
- f. *pH 9.0 solution*: Adjust pH of deionized water to 9.0 using NaOH.
- g. *Copper sulfate solution*, CuSO_4 , 1M: Dissolve 25 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in deionized water and dilute to 100 mL.
- h. *Methanol*, purified.
- i. *C-18 cartridges*†#(122) Using a glass syringe, pass the following sequence of reagents through the cartridge to clean the resin (6 mL/min or less): 10 mL deionized water; 20 mL 1N HCl; 10 mL deionized water; 20 mL methanol; 10 mL deionized water; and 20 mL pH 1.6 solution. Refrigerate but do not freeze cleaned cartridges.
- j. *Ligand-exchange chromatographic resin*, 100/200 mesh: Rinse resin‡#(123) with deionized water to remove fines. Then rinse resin three times in the following sequence: 1N HCl; 1.5N NH_4OH ; and deionized water. Store resin wet.
- k. *Copper-treated ligand-exchange chromatographic resin*, 100/200 mesh: Rinse resin‡ with deionized water to remove fines. Then rinse three times with 1N HCl, followed by deionized water. Using NaOH adjust pH of supernatant above the resin to about 7. Add CuSO_4 solution to the resin and stir. After settling, decant supernatant and add more CuSO_4 solution. Decant CuSO_4 solution and rinse with deionized water until no copper is noticeable in the supernatant. Rinse resin three times with 1.5N NH_4OH and three times with deionized water. Store resin wet.

4. Procedures

a. *Extractable organic selenium*: Adjust sample (5 to 50 mL) to pH 1.5 to 2.0 using HCl and place in a clean glass syringe with an attached cleaned C-18 cartridge. Push sample through the cartridge at a rate of 6 mL/min. After removing the cartridge, draw 2 mL pH 1.6 solution into syringe as a rinse, reattach cartridge, and push the rinse through the cartridge. Repeat two additional times. The cartridge can be refrigerated for storage. To elute organic selenium, push 10 mL methanol through the cartridge at rate of 2 mL/min and collect eluate in a 30 mL pear-shaped flask. Remove methanol by rotary evaporation, with the water bath temperature less than 40°C. Use deionized water to solubilize and transfer the residue into the vessel used for total selenium digestions. Determine total dissolved selenium by digestion with persulfate (pG; B.2) or peroxide (pG; B.3), reduction of Se(VI) (pG; B.5), and analysis by Methods Section 3114B or Section 3114C, or Section 3500-Se.C.

b. *Hydrolysis of protein-bound selenium*: Place filtered sample in a 10- or 20-mL glass ampule (depending on desired volume), and add conc methyl sulfonic acid to adjust concentration to 4M. Purge acidified sample with nitrogen for 10 min and seal top with a torch. Heat sealed vial at 100°C for 24 h in a heating block or pressure cooker. Transfer cooled hydrolysis solution with deionized water rinses to a 50-mL beaker and place in an ice bath. Using NaOH pellets and 1N NaOH, adjust to pH 9.0, taking care not to allow solution to heat to

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boiling.

c. *Determination of selenoamino acids*: If sample is not hydrolyzed as in ¶ 4b, filter, and adjust pH to 9.0 using 1N NaOH.

Fill an empty chromatographic column with deionized water and add ammonium-form resin to a depth of 2 cm. Add copper-treated resin to form a 12-cm length of resin. (The ammonium-form resin removes any copper that bleeds from the copper-treated resin above it). Rinse with deionized water until the pH of the effluent is 9.0; maintain flow through the column by gravity. Pass sample through column, rinse sample beaker with 5 mL pH 9 solution, and place the rinse on column (after the last of the sample reaches the top of the resin). Rinse beaker twice more. Discard flow through column.

Place clean beaker under column and add 20 mL 1.5N NH_4OH to the column. Neutralize NH_4OH eluate with 2.5 mL conc HCl. Determine total dissolved selenium by digestion with persulfate (¶ B.2) or peroxide (¶ B.3), reduction of Se(VI) (¶ B.5) and analysis by Methods Section 3114B or Section 3114C, or Section 3500-Se.C.

5. Precision and Bias

These procedures are only semiquantitative. Typical relative standard deviation is 12% for the C-18 isolation of dissolved organic selenium, and 15% for protein-bound selenium.

6. Bibliography

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3500-Ag SILVER

Silver (Ag) is the second element in Group IB of the periodic table; it has an atomic number of 47, an atomic weight of 107.87, and valences of 1 and 2. The average abundance of Ag in the earth's crust is 0.08 ppm; in soils it is <0.01 to 0.5 ppm; in streams it is 0.3 µg/L; in U.S. drinking waters it is 0.23 µg/L, and in groundwater it is <0.1 µg/L. Silver occurs in its native state and in combination with many nonmetallic elements such as argentite (Ag_2S) and horn silver (AgCl). Lead and copper ores also may yield considerable silver. Silver is widely used in photography, silverware, jewelry, mirrors, and batteries. Silver iodide has been used in the seeding of clouds, and silver oxide to a limited extent is used as a disinfectant for water.

In acidic water Ag^+ would predominate, and in high-chloride water a series of complexes would be expected. Silver is nonessential for plants and animals. Silver can cause argyria, a permanent, blue-gray discoloration of the skin and eyes that imparts a ghostly appearance. Concentrations in the range of 0.4 to 1 mg/L have caused pathological changes in the kidneys, liver, and spleen of rats. Toxic effects on fish in fresh water have been observed at concentrations as low as 0.17 µg/L. For freshwater aquatic life, total recoverable silver should

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not exceed 1.2 mg/L.

The atomic absorption spectrometric methods (Section 3111B and Section 3111C) and the inductively coupled plasma methods (Section 3120 and Section 3125) are preferred. The electrothermal atomization method (Section 3113B) is the most sensitive for determining silver in natural waters. The dithizone method detailed in the 19th edition of *Standard Methods* can be used when an atomic absorption spectrometer is unavailable. A method suitable for analysis of silver in industrial or other wastewaters at levels above 1 mg/L is available.¹

If total silver is to be determined, acidify sample with conc nitric acid (HNO₃) to pH <2 at time of collection. If sample contains particulate matter and only the “dissolved” metal content is to be determined, filter through a 0.45-μm membrane filter at time of collection. After filtration, acidify filtrate with HNO₃ to pH <2. Complete analysis as soon after collection as possible. Some samples may require special storage and digestion; see Section 3030D.

Reference

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3500-Na SODIUM*#(124)

3500-Na A. Introduction

1. Occurrence and Significance

Sodium (Na) is the third element in Group IA of the periodic table; it has an atomic number of 11, an atomic weight of 22.99, and a valence of 1. The average abundance of Na in the earth's crust is 2.5%; in soils it is 0.02 to 0.62%; in streams it is 6.3 mg/L, and in groundwaters it is generally >5 mg/L. Sodium occurs with silicates and with salt deposits. Sodium compounds are used in many applications, including caustic soda, salt, fertilizers, and water treatment chemicals.

Sodium is very soluble, and its monovalent ion Na⁺ can reach concentrations as high as 15 000 mg/L in equilibrium with sodium bicarbonate. The ratio of sodium to total cations is important in agriculture and human physiology. Soil permeability can be harmed by a high sodium ratio. In large concentrations it may affect persons with cardiac difficulties. A limiting concentration of 2 to 3 mg/L is recommended in feedwaters destined for high-pressure boilers. When necessary, sodium can be removed by the hydrogen-exchange process or by distillation. The U.S. EPA advisory limit for sodium in drinking water is 20 mg/L.

2. Selection of Method

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Method Section 3111B uses an atomic absorption spectrometer in the flame absorption mode. Method Section 3120B uses inductively coupled plasma; this method is not as sensitive as the other methods, but usually this is not important. Method Section 3500-Na.B uses either a flame photometer or an atomic absorption spectrometer in the flame emission mode. The inductively coupled plasma/mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though sodium is not specifically listed as an analyte in the method. When all of these instruments are available, the choice will depend on factors including relative quality of the instruments, precision and sensitivity required, number of samples and analytes per sample, matrix effects, and relative ease of instrument operation. If an atomic absorption spectrometer is used, operation in the emission mode is preferred.

3. Storage of Sample

Store alkaline samples or samples containing low sodium concentrations in polyethylene bottles to eliminate the possibility of sample contamination due to leaching of the glass container.

3500-Na B. Flame Emission Photometric Method

1. General Discussion

a. Principle: Trace amounts of sodium can be determined by flame emission photometry at 589 nm. Sample is nebulized into a gas flame under carefully controlled, reproducible excitation conditions. The sodium resonant spectral line at 589 nm is isolated by interference filters or by light-dispersing devices such as prisms or gratings. Emission light intensity is measured by a phototube, photomultiplier, or photodiode. The light intensity at 589 nm is approximately proportional to the sodium concentration. Alignment of the wavelength dispersing device and wavelength readout may not be precise. The appropriate wavelength setting, which may be slightly more or less than 589 nm, can be determined from the maximum emission intensity when aspirating a sodium standard solution, and then used for emission measurements. The calibration curve may be linear but has a tendency to level off or even reverse at higher concentrations. Work in the linear to near-linear range.

b. Interferences: Minimize interference by incorporation of one or more of the following:

- 1) Operate at the lowest practical concentration range.
- 2) Add releasing agents, such as strontium or lanthanum at 1000 mg/L, to suppress ionization and anion interference. Among common anions capable of causing interference are Cl^- , SO_4^{2-} and HCO_3^- in relatively large amounts.
- 3) Matrix-match standards and samples by adding identical amounts of interfering substances present in the sample to calibration standards.
- 4) Apply an experimentally determined correction in those instances where the sample

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contains a single important interference.

5) Remove interfering ions.

6) Remove burner-clogging particulate matter from the sample by filtration through a filter paper of medium retentiveness.

7) Use the standard addition technique as described in the flame photometric method for strontium (Section 3500-Sr.B). The method involves preparing a calibration curve using the sample matrix as a diluent, and determining the sample concentration either mathematically or graphically.

8) Use the internal standard technique. Potassium and calcium interfere with sodium determination by the internal-standard method if the potassium-to-sodium ratio is $\geq 5:1$ and the calcium-to-sodium ratio is $\geq 10:1$. When these ratios are exceeded, determine calcium and potassium concentrations and matrix-match sodium calibration standards by addition of approximately equivalent concentrations of interfering ions. Interference from magnesium is not significant until the magnesium-to-sodium ratio exceeds 100, a rare occurrence.

b. Minimum detectable concentration: Better flame photometers or atomic absorption spectrometers operating in the emission mode can be used to determine sodium levels approximating 5 $\mu\text{g/L}$.

2. Apparatus

a. Flame photometer (either direct-reading or internal-standard type) or atomic absorption spectrometer operating in the flame emission mode.

b. Glassware: Rinse all glassware with 1 + 15 HNO_3 followed by several portions of reagent water (¶ 3a).

3. Reagents

To minimize sodium contamination, store all solutions in plastic bottles. Use small containers to reduce the amount of dry element that may be picked up from the bottle walls when the solution is poured. Shake each container vigorously to wash accumulated salts from walls before pouring solution.

a. Reagent water: See Section 1080. Use reagent water to prepare all reagents and calibration standards, and as dilution water.

b. Stock sodium solution: Dissolve 2.542 g NaCl dried at 140°C to constant weight and dilute to 1000 mL with water; 1.00 mL = 1.00 mg Na.

c. Intermediate sodium solution: Dilute 10.00 mL stock sodium solution with water to 100.0 mL; 1.00 mL = 0.10 mg Na (1.00 mL = 100 μg Na). Use this intermediate solution to prepare calibration curve in sodium range of 1 to 10 mg/L.

d. Standard sodium solution: Dilute 10.00 mL intermediate sodium solution with water to 100 mL; 1.00 mL = 10.0 μg Na. Use this solution to prepare calibration curve in sodium range of

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0.1 to 1.0 mg/L.

4. Procedure

a. Pretreatment of polluted water and wastewater samples: Follow the procedures described in Section 3030.

b. Instrument operation: Because of differences between makes and models of instruments, it is impossible to formulate detailed operating instructions. Follow manufacturer's recommendation for selecting proper photocell and wavelength, adjusting slit width and sensitivity, appropriate fuel and oxidant gas pressures, and the steps for warm-up, correcting for interferences and flame background, rinsing of burner, igniting flame, and measuring emission intensity.

c. Direct-intensity measurement: Prepare a blank and sodium calibration standards in stepped amounts in any of the following applicable ranges: 0 to 1.0, 0 to 10, or 0 to 100 mg/L. Determine emission intensity at 589 nm. Aspirate calibration standards and samples enough times to secure a reliable average reading for each. Construct a calibration curve from the sodium standards. Determine sodium concentration of sample from the calibration curve. Where a large number of samples must be run routinely, the calibration curve provides sufficient accuracy. If greater precision and less bias are desired and time is available, use the bracketing approach described in ¶ 4d below.

d. Bracketing approach: From the calibration curve, select and prepare sodium standards that immediately bracket the emission intensity of the sample. Determine emission intensities of the bracketing standards (one sodium standard slightly less and the other slightly greater than the sample) and the sample as nearly simultaneously as possible. Repeat the determination on bracketing standards and sample. Calculate the sodium concentration by the equation in ¶ 5b and average the findings.

5. Calculation

a. For direct reference to the calibration curve:

$$\text{mg Na/L} = (\text{mg Na/L in portion}) \times D$$

b. For the bracketing approach:

$$\text{mg Na/L} = \left[\frac{(B - A)(s - a)}{(b - a)} + A \right] D$$

where:

B = mg Na/L in upper bracketing standard,

A = mg Na/L in lower bracketing standard,

b = emission intensity of upper bracketing standard,

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a = emission intensity of lower bracketing standard,

s = emission intensity of sample, and

D = dilution ratio,

$$= \frac{\text{mL sample} + \text{mL water}}{\text{mL sample}}$$

6. Precision and Bias

A synthetic sample containing 19.9 mg Na⁺/L, 108 mg Ca²⁺/L, 82 mg Mg²⁺/L, 3.1 mg K⁺/L, 241 mg Cl⁻/L, 0.25 mg NO₂⁻-N/L, 1.1 mg NO₃⁻-N/L, 259 mg SO₄²⁻/L, and 42.5 mg total alkalinity/L (as CaCO₃) was analyzed in 35 laboratories by the flame photometric method, with a relative standard deviation of 17.3% and a relative error of 4.0%.

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3500-Sr STRONTIUM*#(125)

3500-Sr A. Introduction

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1. Occurrence and Significance

Strontium (Sr) is the fourth element in Group IIA of the periodic table; it has an atomic number of 38, an atomic weight of 87.62, and a valence of 2. The average abundance of Sr in the earth's crust is 384 ppm; in soils Sr ranges from 3.6 to 160 ppm; in streams it averages 50 µg/L, and in groundwaters it ranges from 0.01 to 10 mg/L. Strontium is found chiefly in celestite (SrSO_4) and in strontianite (SrCO_3). Strontium compounds are used in pigments, pyrotechnics, ceramics, and flares. ^{90}Sr is a fission product of nuclear reactor fuels, and was widely distributed on the earth's surface as a result of fallout from nuclear weapons testing.

The common aqueous species is Sr^{2+} . The solubility of strontium is controlled by carbonate and sulfate. Some compounds are toxic by ingestion and inhalation. Although there is no U.S. EPA drinking water standard MCL for concentration of strontium, strontium-90 measurements are required when the gross beta activity of a water sample is greater than 50 pCi/L. The U.S. EPA primary drinking water standard MCL for ^{90}Sr is 8 pCi/L.

A method for determination of ^{90}Sr is found in Section 7500-Sr.

2. Selection of Method

The atomic absorption spectrometric method (Section 3111B) and inductively coupled plasma methods (Section 3120 and Section 3125) are preferred. The flame emission photometric method (B) also is available for those laboratories that do not have the equipment needed for one of the preferred methods.

3. Sampling and Storage

Polyethylene bottles are preferable for sample storage, although borosilicate glass containers also may be used. At time of collection adjust sample to pH <2 with nitric acid (HNO_3).

3500-Sr B. Flame Emission Photometric Method

1. General Discussion

a. Principle: The flame photometric method can be used for the determination of strontium in the concentration range prevalent in natural waters. The strontium emission is measured at a wavelength of 460.7 nm, while the background intensity is measured at a wavelength of 466 nm. The difference in readings obtained at these two wavelengths measures the light intensity emitted by strontium.

b. Interference: Emission intensity is a linear function of strontium concentration and concentration of other constituents. The standard addition technique distributes the same ions throughout the standards and the sample, thereby equalizing the radiation effect of possible interfering substances. A very low pH (<1) could produce an interference, but sample dilution should eliminate this interference.

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c. *Minimum detectable concentration:* Strontium levels of about 0.2 mg/L can be detected by the flame photometric method without prior sample concentration.

2. Apparatus

Spectrophotometer, equipped with photomultiplier tube and flame accessories; or an atomic absorption spectrophotometer capable of operation in flame emission mode.

3. Reagents

a. *Stock strontium solution:* Dissolve 2.415 g strontium nitrate, $\text{Sr}(\text{NO}_3)_2$, dried to constant weight at 140°C , in 1000 mL 1% (v/v) HNO_3 ; 1.00 mL = 1.00 mg Sr.

b. *Standard strontium solution:* Dilute 25.00 mL stock strontium solution to 1000 mL with water; 1.00 mL = 25.0 μg Sr. Use this solution for preparing Sr standards in the 0.2- to 25-mg/L range.

c. *Nitric acid*, HNO_3 , conc.

4. Procedure

a. *Pretreatment of polluted water and wastewater samples:* Select an appropriate procedure from Section 3030.

b. *Preparation of strontium standards:* Dilute samples, if necessary, to contain less than 400 mg Ca or Ba/L and less than 40 mg Sr/L. Add 25.0 mL sample (or a lesser but consistent volume to keep all standards in the linear range of the instrument) to 25.0 mL of each of a series of four or more strontium standards containing from 0 mg/L to a concentration exceeding that of the sample. For most natural waters 0, 2.0, 5.0, and 10.0 mg Sr/L standards are sufficient. A broader range curve might be preferable for brines. Dilute the brine sufficiently to eliminate burner splatter and clogging.

c. *Concentration of low-level strontium samples:* Concentrate samples containing less than 2 mg Sr/L. Polluted water or wastewater samples can be concentrated during digestion by starting with a larger volume (see Section 3030D). For other samples, add 3 to 5 drops conc HNO_3 to 250 mL sample and evaporate to about 25 mL. Cool and make up to 50.0 mL with distilled water. Proceed as in ¶ b. The HNO_3 concentration in the sample prepared for atomization can approach 0.4 mL/50 mL without producing interference.

d. *Flame photometric measurement:* Measure emission intensity of prepared samples (standards plus sample) at wavelengths of 460.7 and 466 nm. Follow manufacturer's instructions for correct instrument operation. Use a fuel-rich nitrous oxide-acetylene flame, if possible.

5. Calculation

a. Using a calculator or computer with linear regression capability, enter the net intensity (reading at 460.7 nm minus reading at 466 nm) versus concentration added to the sample and solve the equation for zero emissions. The negative of this number multiplied by any dilution

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factor is the sample concentration.

b. Plot net intensity (reading at 460.7 nm minus reading at 466 nm) against strontium concentration added to the sample. Because the plot forms a straight calibration line that intersects the ordinate, strontium concentration can be calculated from the equation:

$$\text{mg Sr/L} = \frac{A - B}{C} \times \frac{D}{E}$$

where:

A = sample emission-intensity reading of sample plus 0 mg/L at 460.7 nm,

B = background radiation reading at 466 nm, and

C = slope of calibration line.

Use the ratio D/E only when E mL of sample are concentrated to a final volume D mL (typically 50 mL).

c. *Graphical method:* Strontium concentration also can be evaluated by the graphical method illustrated in Figure 3500-Sr:1. Plot net intensity against strontium concentration added to sample. If the line intersects the ordinate at Y emissions, the strontium concentration is where the abscissa value of the point on the calibration line has an ordinate value of $2Y$ emissions due to the two-fold dilution with standards (if sample and standards are mixed in equal volumes). The calibration line in the example intersects the ordinate at 12. Thus, $Y = 12$ and $2Y = 24$. The strontium concentration of the sample is the abscissa value of the point on the calibration line having an ordinate value of 24. In the example, the strontium concentration is 9.0 mg/L.

d. Report strontium concentrations below 10 mg/L to the nearest 0.1 mg/L and above 10 mg/L to the nearest whole number.

6. Quality Control

See Part 1000 and Section 3020 for specific quality control procedures to be followed during sample preparation and analysis.

7. Precision and Bias

Strontium concentrations in the range 12.0 to 16.0 mg/L can be determined with an accuracy within ± 1 to 2 mg/L.

8. Bibliography

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water. U.S. Geol. Surv. Water Supply Pap. No. 1496A.

3500-Te TELLURIUM

Tellurium (Te) is the fourth element in Group VIA in the periodic table; it has an atomic number of 52, an atomic weight of 127.60, and valences of 2, 4, and 6. The average abundance of Te in the earth's crust is 0.002 ppm; in soils it is 0.001 to 0.01 ppm; and in groundwaters it is <0.1 mg/L. Tellurium is found in its native state and as the telluride of gold and other metals. It is used in alloys, catalysts, batteries, and as a coloring agent in glass and ceramics.

The common aqueous species is TeO_3^{2-} . The metal and its compounds are toxic by inhalation.

Perform analyses by the electrothermal atomic absorption method (Section 3113B). The inductively coupled plasma mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though tellurium is not specifically listed as an analyte in the method.

3500-Tl THALLIUM

Thallium (Tl) is the fifth element in Group IIIA in the periodic table; it has an atomic number of 81, an atomic weight of 204.38, and valences of 1 and 3. The average abundance in the earth's crust is 0.07 ppm, and in groundwaters it is <0.1 mg/L. The metal occurs chiefly in pyrites. Thallium is used in the production of glasses and rodenticides, in photoelectric applications, and in electrodes for dissolved oxygen meters.

The common aqueous species is Tl^+ . It is nonessential for plants and animals. Compounds of thallium are toxic on contact with moisture, and by inhalation. The U.S. EPA primary drinking water standard MCL is 2 µg/L.

For analysis, use one of the atomic absorption spectrometric methods (Section 3111B or Section 3113B), or one of the inductively coupled plasma methods (Section 3120 or Section 3125), depending upon sensitivity requirements.

3500-Th THORIUM

Thorium (Th) is the first element in the actinium series of the periodic table; it has an atomic number of 90, an atomic weight of 232.04, and a valence of 4. The average abundance in the earth's crust is 8.1 ppm; in soils it is 13 ppm; in streams it is 0.1 µg/L, and in groundwaters it is <0.1 mg/L. Thorium is a radioactive element, with ^{232}Th having a half-life of 1.4×10^{10} years. It is widely distributed in the earth, with the principal mineral being monazite. Thorium is used in sun lamps, photoelectric cells, incandescent lighting, and gas mantles.

The aqueous chemistry of thorium is controlled by the Th^{4+} ion, which forms a set of

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complex species with hydroxides. Thorium's radioactive decay isotopes are dangerous when inhaled or ingested as thorium dust particles.

Either of the flame atomic absorption spectrometric methods (Section 3111D or Section 3111E) may be used for analysis. The inductively coupled plasma mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though thorium is not specifically listed as an analyte in the method.

3500-Sn TIN

Tin (Sn) is the fourth element in Group IVA in the periodic table; it has an atomic number of 50, an atomic weight of 118.69, and valences of 2 and 4. The average abundance in the earth's crust is 2.1 ppm; in soils it is 10 ppm; in streams it is 0.1 µg/L, and in groundwaters it is <0.1 mg/L. Tin is found mostly in the mineral cassiterite (SnO_2), in association with granitic rocks. Tin is used in reducing agents, solder, bronze, pewter, and coatings for various metals.

The common aqueous species are Sn^{4+} , $\text{Sn}(\text{OH})_4$, $\text{SnO}(\text{OH})_2$, and $\text{SnO}(\text{OH})_3^-$. Tin is adsorbed to suspended solids, sulfides, and hydroxides. Tin can be methylated in sediments. Tributyl tin undergoes biodegradation quickly. Organo-tin compounds are toxic. Tin is considered nonessential for plants and animals.

Either the flame atomic absorption method (Section 3111B) or the electrothermal atomic absorption method (Section 3113B) may be used successfully for analyses, depending upon the sensitivity desired. The inductively coupled plasma/mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though tin is not specifically listed as an analyte in the method.

3500-Ti TITANIUM

Titanium (Ti) is the first element in Group IVB in the periodic table; it has an atomic number of 22, an atomic weight of 47.88, and valences of 2, 3, and 4. The average abundance of Ti in the earth's crust is 0.6%; in soils it is 1700 to 6600 ppm; in streams it is 3 µg/L, and in groundwaters it is <0.1 mg/L. The element is commonly associated with iron minerals. Titanium is used in alloys for aircraft, marine, and food-handling equipment. Compounds of the metal are used in pigments and as a reducing agent.

Titanium species are usually insoluble in natural waters, with the Ti^{4+} species being the most common ion when found. Some compounds are toxic by ingestion and the pure metal is flammable.

Either of the flame atomic absorption spectrometric methods (Section 3111D or Section 3111E) may be used. The inductively coupled plasma mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though titanium is not specifically listed as an analyte in the method.

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3500-U URANIUM

Uranium (U) is the third element in the actinide series of the periodic table; it has an atomic number of 92, an atomic weight of 238.04, and valences of 3, 4, and 6. The average abundance of U in the earth's crust is 2.3 ppm, and in soils it is 1.8 ppm. Concentrations of uranium in drinking waters usually are expressed in terms of picocuries per liter, but that is now being replaced by Becquerel per liter (Bq/L). The approximate conversion factor, assuming equilibrium between ^{234}U and ^{238}U , is 1 μg uranium equals 0.67 pCi. The mean concentration of uranium in drinking water is 1.8 pCi/L. The chief ore is uraninite, or pitchblende, uranous uranate [$\text{U}(\text{UO}_4)_2$]. Uranium is known mainly for its use in the nuclear industry, but has also been used in glass, ceramics, and photography.

Uranium compounds are radioactive and are thereby toxic by inhalation and ingestion. There are three natural radioisotopes of uranium. Uranium-238 has a half-life of 4.5×10^9 years, represents 99% of uranium's natural abundance, and is not fissionable, but can be used to form plutonium-239, which is fissionable. Uranium-235 has a half-life of 7.1×10^8 years, represents 0.75% of uranium's natural abundance, is readily fissionable, and was the energy source in the original atomic bombs. Uranium-234 has a half-life of 2.5×10^5 years and represents only 0.006% of uranium's natural abundance.

The common forms in natural water are U^{4+} and UO_2^{2+} . In natural waters below pH 5, UO_2^{2+} would dominate; in the pH range of 5 to 10, soluble carbonate complexes predominate. Although there is no U.S. EPA drinking water standard MCL for uranium, an analysis for uranium is required if the gross alpha activity of a water sample is greater than 15 pCi/L.

Perform analyses by the inductively coupled plasma/mass spectrometry method (Section 3125) or by one of the methods in Section 7500-U (for regulatory compliance purposes).

3500-V VANADIUM*#(126)

3500-V A. Introduction

1. Occurrence and Significance

Vanadium (V) is the first element in Group VB in the periodic table; it has an atomic number of 23, an atomic weight of 50.94, and valences of 2, 3, 4, and 5. The average abundance of V in the earth's crust is 136 ppm; in soils it ranges from 15 to 110 ppm; in streams it averages about 0.9 $\mu\text{g/L}$, and in groundwaters it is generally <0.1 mg/L. Though relatively rare, vanadium is found in a variety of minerals; most important among these are vanadinite [$\text{Pb}_5(\text{VO}_4)_3\text{Cl}$], and patronite (possibly VS_4), occurring chiefly in Peru. Vanadium complexes have been noted in

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coal and petroleum deposits. Vanadium is used in steel alloys and as a catalyst in the production of sulfuric acid and synthetic rubber.

The dominant form in natural waters is V^{5+} . It is associated with organic complexes and is insoluble in reducing environments. It is considered nonessential for most higher plants and animals, although it may be an essential trace element for some algae and microorganisms. Laboratory and epidemiological evidence suggests that vanadium may play a beneficial role in the prevention of heart disease. In water supplies in New Mexico, which has a low incidence of heart disease, vanadium has been found in concentrations of 20 to 150 $\mu\text{g/L}$. In a state where incidence of heart disease is high, vanadium was not found in water supplies. However, vanadium pentoxide dust causes gastrointestinal and respiratory disturbances. The United Nations Food and Agriculture Organization recommended maximum level for irrigation waters is 0.1 mg/L.

2. Selection of Method

The atomic absorption spectrometric methods (Section 3111D and Section 3111E), the electrothermal atomic absorption method (Section 3113B), the inductively coupled plasma methods (Section 3120 and Section 3125), and gallic acid method (Section 3500-V.B) are suitable for potable water samples. The atomic absorption spectrometric and inductively coupled plasma methods are preferred for polluted samples. The electrothermal atomic absorption method also may be used successfully with an appropriate matrix modifier.

3500-V B. Gallic Acid Method

1. General Discussion

a. Principle: The concentration of trace amounts of vanadium in water is determined by measuring the catalytic effect it exerts on the rate of oxidation of gallic acid by persulfate in acid solution. Under the given conditions of concentrations of reactants, temperature, and reaction time, the extent of oxidation of gallic acid is proportional to the concentration of vanadium. Vanadium is determined by measuring the absorbance of the sample at 415 nm and comparing it with that of standard solutions treated identically.

b. Interference: The substances listed in Table 3500-V:I will interfere in the determination of vanadium if the specified concentrations are exceeded. This is not a serious problem for Cr^{6+} , Co^{2+} , Mo^{6+} , Ni^{2+} , Ag^+ , and U^{6+} because the tolerable concentration is greater than that commonly encountered in fresh water. However, in some samples the tolerable concentration of Cu^{2+} , Fe^{2+} , and Fe^{3+} may be exceeded. Because of the high sensitivity of the method, interfering substances in concentrations only slightly above tolerance limits can be rendered harmless by dilution.

Traces of Br^- and I^- interfere seriously and dilution alone will not always reduce the concentration below tolerance limits. Mercuric ion may be added to complex these halides and

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minimize their interference; however, mercuric ion itself interferes if in excess. Adding 350 µg mercuric nitrate, $\text{Hg}(\text{NO}_3)_2$, per sample permits determination of vanadium in the presence of up to 100 mg Cl^-/L , 250 µg Br^-/L , and 250 µg I^-/L . Dilute samples containing high concentrations of these ions to concentrations below the values given above and add $\text{Hg}(\text{NO}_3)_2$.

c. Minimum detectable concentration: 0.025 µg V in approximately 13 mL final volume or approximately 2 µg V/L.

2. Apparatus

- a. Water bath,* capable of being operated at $25 \pm 0.5^\circ\text{C}$.
- b. Colorimetric equipment:* One of the following is required:
 - 1) *Spectrophotometer,* for measurements at 415 nm, with a light path of 1 to 5 cm.
 - 2) *Filter photometer,* providing a light path of 1 to 5 cm and equipped with a violet filter with maximum transmittance near 415 nm.

3. Reagents

Use reagent water (see Section 1080) in preparation of reagents, for dilutions, and as blanks.

a. Stock vanadium solution: Dissolve 229.6 mg ammonium metavanadate, NH_4VO_3 , in a volumetric flask containing approximately 800 mL water and 15 mL 1 + 1 nitric acid (HNO_3). Dilute to 1000 mL; 1.00 mL = 100 µg V.

b. Intermediate vanadium solution: Dilute 1.00 mL stock vanadium solution with water to 100 mL; 1.00 mL = 1.00 µg V.

c. Standard vanadium solution: Dilute 1.00 mL intermediate vanadium solution with water to 100 mL; 1.00 mL = 0.010 µg V.

d. Mercuric nitrate solution: Dissolve 350 mg $\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$ in 1000 mL water.

e. Ammonium persulfate-phosphoric acid reagent: Dissolve 2.5 g $(\text{NH}_4)_2\text{S}_2\text{O}_8$ in 25 mL water. Bring just to a boil, remove from heat, and add 25 mL conc H_3PO_4 . Let stand approximately 24 h before use. Discard after 48 h.

f. Gallic acid solution: Dissolve 2 g $\text{H}_6\text{C}_7\text{O}_5$ in 100 mL warm water, heat to a temperature just below boiling, and filter through filter paper. ¹²⁷ Prepare a fresh solution for each set of samples.

4. Procedure

a. Preparation of standards and sample: Prepare both blank and sufficient standards by diluting 0- to 8.0-mL portions (0 to 0.08 µg V) of standard vanadium solution to 10 mL with water. Pipet sample (10.00 mL maximum) containing less than 0.08 µg V into a suitable container and adjust volume to 10.0 mL with water. Filter colored or turbid samples. Add 1.0 mL $\text{Hg}(\text{NO}_3)_2$ solution to each blank, standard, and sample. Place containers in a water bath

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regulated to $25 \pm 0.5^{\circ}\text{C}$ and allow 30 to 45 min for samples to come to the bath temperature.

b. Color development and measurement: Add 1.0 mL ammonium persulfate-phosphoric acid reagent (temperature equilibrated), swirl to mix thoroughly, and return to water bath. Add 1.0 mL gallic acid solution (temperature equilibrated), swirl to mix thoroughly, and return to water bath. Add gallic acid to successive samples at intervals of 30 s or longer to permit accurate control of reaction time. Exactly 60 min after adding gallic acid, remove sample from water bath and measure its absorbance at 415 nm, using water as a reference. Subtract absorbance of blank from absorbance of each standard and sample. Construct a calibration curve by plotting absorbance values of standards versus micrograms vanadium. Determine amount of vanadium in a sample by referring to the corresponding absorbance on the calibration curve. Prepare a calibration curve with each set of samples.

5. Calculation

$$\text{mg V/L} = \frac{\mu\text{g V (in 13 mL final volume)}}{\text{original sample volume, mL}}$$

6. Precision and Bias

In a synthetic sample containing 6 $\mu\text{g V/L}$, 40 $\mu\text{g As/L}$, 250 $\mu\text{g Be/L}$, 240 $\mu\text{g B/L}$, and 20 $\mu\text{g Se/L}$ in distilled water, vanadium was measured in 22 laboratories with a relative standard deviation of 20% and no relative error.

7. Bibliography

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3500-Zn ZINC*#(128)

3500-Zn A. Introduction

1. Occurrence and Significance

Zinc (Zn) is the first element in Group IIB in the periodic table; it has an atomic number of 30, an atomic weight of 65.38, and a valence of 2. The average abundance of Zn in the earth's crust is 76 ppm; in soils it is 25 to 68 ppm; in streams it is 20 $\mu\text{g/L}$, and in groundwaters it is $<0.1 \text{ mg/L}$. The solubility of zinc is controlled in natural waters by adsorption on mineral surfaces, carbonate equilibrium, and organic complexes. Zinc is used in a number of alloys such as brass and bronze, and in batteries, fungicides, and pigments. Zinc is an essential growth element for plants and animals but at elevated levels it is toxic to some species of aquatic life.

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The United Nations Food and Agriculture Organization recommended level for zinc in irrigation waters is 2 mg/L. The U.S. EPA secondary drinking water standard MCL is 5 mg/L. Concentrations above 5 mg/L can cause a bitter astringent taste and an opalescence in alkaline waters. Zinc most commonly enters the domestic water supply from deterioration of galvanized iron and dezincification of brass. In such cases lead and cadmium also may be present because they are impurities of the zinc used in galvanizing. Zinc in water also may result from industrial waste pollution.

2. Selection of Method

The atomic absorption spectrometric methods (Section 3111B and Section 3111C) and inductively coupled plasma methods (Section 3120 and Section 3125) are preferred. The zincon method (B), suitable for analysis of both potable and polluted waters, may be used if instrumentation for the preferred methods is not available..

3. Sampling and Storage

See Section 3010B.2 for sample handling and storage.

3500-Zn B. Zincon Method

1. General Discussion

a. Principle: Zinc forms a blue complex with 2-carboxy-2'-hydroxy-5'-sulfoformazyl benzene (zincon) in a solution buffered to pH 9.0. Other heavy metals likewise form colored complexes with zincon. Cyanide is added to complex zinc and heavy metals. Cyclohexanone is added to free zinc selectively from its cyanide complex so that it can be complexed with zincon to form a blue color. Sodium ascorbate reduces manganese interference. The developed color is stable except in the presence of copper (see table below).

b. Interferences: The following ions interfere at concentrations exceeding those listed:

Ion	mg/L	Ion	mg/L
Cd ²⁺	1	Cr ³⁺	10
Al ³⁺	5	Ni ²⁺	20
Mn ²⁺	5	Cu ²⁺	30
Fe ³⁺	7	Co ²⁺	30
Fe ²⁺	9	CrO ₄ ²⁻	50

c. Minimum detectable concentration: 0.02 mg Zn/L.

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2. Apparatus

- a. *Colorimetric equipment*: One of the following is required:
 - 1) *Spectrophotometer*, for measurements at 620 nm, providing a light path of 1 cm or longer.
 - 2) *Filter photometer*, providing a light path of 1 cm or longer and equipped with a red filter having maximum transmittance near 620 nm. Deviation from Beer's Law occurs when the filter band pass exceeds 20 nm.
- b. *Graduated cylinders*, 50-mL, with ground-glass stoppers, Class B or better.
- c. *Erlenmeyer flasks*, 50-mL.
- d. *Filtration apparatus*: 0.45- μ m filters and filter holders.

3. Reagents

- a. *Metal-free water*: See Section 3111B.3c. Use water for rinsing apparatus and preparing solutions and dilutions.
- b. *Stock zinc solution*: Dissolve 1000 mg (1.000 g) zinc metal in 10 mL 1 + 1 HNO₃. Dilute and boil to expel oxides of nitrogen. Dilute to 1000 mL; 1.00 mL = 1.00 mg Zn.
- c. *Standard zinc solution*: Dilute 10.00 mL stock zinc solution to 1000 mL; 1.00 mL = 10.00 μ g Zn.
- d. *Sodium ascorbate*, fine granular powder, USP.
- e. *Potassium cyanide solution*: Dissolve 1.00 g KCN in approximately 50 mL water and dilute to 100 mL. CAUTION: *Potassium cyanide is a deadly poison. Avoid skin contact or inhalation of vapors. Do not pipet by mouth or bring in contact with acids.*
- f. *Buffer solution*, pH 9.0: Dissolve 8.4 g NaOH pellets in about 500 mL water. Add 31.0 g H₃BO₃ and swirl or stir to dissolve. Dilute to 1000 mL with water and mix thoroughly.
- g. *Zincon reagent*: Dissolve 100 mg zincon (2-carboxy-2'-hydroxy-5'-sulfoformazyl benzene) in 100 mL methanol. Because zincon dissolves slowly, stir and/or let stand overnight.
- h. *Cyclohexanone*, purified.
- i. *Hydrochloric acid*, HCl, conc and 1N.
- j. *Sodium hydroxide*, NaOH, 6N and 1N.

4. Procedure

- a. *Preparation of colorimetric standards*: Accurately deliver 0, 0.5, 1.0, 3.0, 5.0, 10.0, and 14.0 mL standard zinc solution to a series of 50-mL graduated mixing cylinders. Dilute each to 20.0 mL to yield solutions containing 0, 0.25, 0.5, 1.5, 2.5, 5.0, and 7.0 mg Zn/L, respectively. (Lower-range standards may be prepared to extend the quantitation range. Longer optical path cells can be used. Verify linearity of response in this lower concentration range.) Add the following to each solution in sequence, mixing thoroughly after each addition: 0.5 g sodium ascorbate, 5.0 mL buffer solution, 2.0 mL KCN solution, and 3.0 mL zincon solution. Pipet 20.0

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mL of the solution into a clean 50-mL erlenmeyer flask. Reserve remaining solution to zero the instrument. Add 1.0 mL cyclohexanone to the erlenmeyer flask. Swirl for 10 s and note time. Transfer portions of both solutions to clean sample cells. Use solution without cyclohexanone to zero colorimeter. Read and record absorbance for solution with cyclohexanone after 1 min. The calibration curve does not pass through zero because of the color enhancement effect of cyclohexanone on zincon.

b. Treatment of samples: To determine readily acid-extractable total zinc, add 1 mL conc HCl to 50 mL sample and mix thoroughly. Filter and adjust to pH 7. To determine dissolved zinc, filter sample through a 0.45- μ m membrane filter. Adjust to pH 7 with 1N NaOH or 1N HCl if necessary after filtering.

c. Sample analysis: Cool samples to less than 30°C if necessary. Analyze 20.0 mL of prepared sample as described in ¶ 4a above, beginning with “Add the following to each solution . . .” If the zinc concentration exceeds 7 mg Zn/L prepare a sample dilution and analyze a 20.0-mL portion.

5. Calculation

Read zinc concentration (in milligrams per liter) directly from the calibration curve.

6. Precision and Bias

A synthetic sample containing 650 μ g Zn/L, 500 μ g Al/L, 50 μ g Cd/L, 110 μ g Cr/L, 470 μ g Cu/L, 300 μ g Fe/L, 70 μ g Pb/L, 120 μ g Mn/L, and 150 μ g Ag/L in doubly demineralized water was analyzed in a single laboratory. A series of 10 replicates gave a relative standard deviation of 0.96% and a relative error of 0.15%. A wastewater sample from an industry in Standard Industrial Classification (SIC) No. 3333, primary smelting and refining of zinc, was analyzed by 10 different persons. The mean zinc concentration was 3.36 mg Zn/L and the relative standard deviation was 1.7%. The relative error compared to results from an atomic absorption analysis of the same sample was -1.0%.

7. Bibliography

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Figures

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Figure 3112:1. Schematic arrangement of equipment for measurement of mercury by cold-vapor atomic absorption technique.

Figure 3114:1. Manual reaction cell for producing As and Se hydrides.

Figure 3114:2. Schematic of a continuous hydride generator.

Figure 3500-Al:1. Correction curves for estimation of aluminum in the presence of fluoride. Above the mg F⁻/L present, locate the point corresponding to the apparent mg Al/L measured. From this point interpolate between the curves shown, if the point does not fall directly on one of the curves, to read the true mg Al/L on the ordinate, which corresponds to 0.00 mg F⁻/L. For example, an apparent 0.20 mg Al/L in a sample containing 1.00 mg F⁻/L would actually be 0.30 mg Al/L if no fluoride were present to interfere.

Figure 3500-As:1. Arsine generator and absorber assembly.

Figure 3500-Se:1. General scheme for speciation of selenium in water.

Figure 3500-Sr:1. Graphical method of computing strontium concentration.

Tables

TABLE 3010:I. APPLICABLE METHODS FOR ELEMENTAL ANALYSIS

Element	Flame Atomic Absorption (Direct)	Flame Atomic Absorption (Extracted)	Flame Photometry	Electrothermal Atomic Absorption	Hydride/Cold Vapor Atomic Absorption	Inductively Coupled Plasma (ICP)	ICP/ Spect (ICF)
Aluminum	3111D	3111E		3113B		3120A	31
Antimony	3111B			3113B		3120A	31
Arsenic				3113B	3114B	3120A	31
Barium	3111D	3111E		3113B		3120A	31
Beryllium	3111D	3111E		3113B		3120A	31
Bismuth	3111B			3113B			31
Boron						3120A	31
Cadmium	3111B	3111C		3113B		3120A	31
Calcium	3111B,D	3111E				3120A	31
Cesium	3111B						31
Chromium	3111B	3111C		3113B		3120A	31
Cobalt	3111B	3111C		3113B		3120A	31
Copper	3111B	3111C		3113B		3120A	31
Gallium				3113B			31
Germanium				3113B			31
Gold	3111B			3113B			31
Indium				3113B			31

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Element	Flame Atomic Absorption (Direct)	Flame Atomic Absorption (Extracted)	Flame Photometry	Electrothermal Atomic Absorption	Hydride/Cold Vapor Atomic Absorption	Inductively Coupled Plasma (ICP)	ICP/ Spect (ICF)
Iridium	3111B						31
Iron	3111B	3111C		3113B		3120A	31
Lead	3111B	3111C		3113B		3120A	31
Lithium	3111B		3500-Li.B			3120A	31
Magnesium	3111B					3120A	31
Manganese	3111B	3111C		3113B		3120A	31
Mercury					3112B		31
Molybdenum	3111D	3111E		3113B		3120A	31
Nickel	3111B	3111C		3113B		3120A	31
Osmium	3111D	3111E					31
Palladium	3111B						31
Platinum	3111B						31
Potassium	3111B		3500-K.B			3120A	31
Rhenium	3111D	3111E					31
Rhodium	3111B						31
Ruthenium	3111B						31
Selenium				3113B	3114B,C	3120A	31
Silicon	3111D	3111E				3120A	31
Silver	3111B	3111C		3113B		3120A	31
Sodium	3111B		3500-Na.B			3120A	31
Strontium	3111B		3500-Sr.B			3120A	31
Tellurium				3113B			31
Thallium	3111B			3113B		3120A	31
Thorium	3111D	3111E					31
Tin	3111B			3113B			31
Titanium	3111D	3111E					31
Uranium							31
Vanadium	3111D	3111E		3113B		3120A	31
Zinc	3111B	3111C		3113B		3120A	31

* Metal is not specifically mentioned in the method, but 3125 may be used successfully in most cases.

† Additional alternative methods for aluminum, beryllium, cadmium, mercury, selenium, silver, and zinc may be found in the 19th Edition of *Standard Methods*.

TABLE 3030:I. ACIDS USED IN CONJUNCTION WITH HNO₃ for SAMPLE PREPARATION

Standard Methods for the Examination of Water and Wastewater

TABLE 3030:I. ACIDS USED IN CONJUNCTION WITH HNO₃ for SAMPLE PREPARATION

Acid	Recommended for	May Be Helpful for	Not Recommended for
HCl	Ag	Sb, Ru, Sn	Th, Pb
H ₂ SO ₄	Ti	—	Ag, Pb, Ba
HClO ₄	—	Organic materials	—
HF	—	Siliceous materials	—

TABLE 3111:I. ATOMIC ABSORPTION CONCENTRATION RANGES WITH DIRECT ASPIRATION
ATOMIC ABSORPTION

Element	Wave-length <i>nm</i>	Flame Gases*	Instrument Detection Limit <i>mg/L</i>	Sensitivity <i>mg/L</i>	Optimum Concentration Range <i>mg/L</i>
Ag	328.1	A-Ac	0.01	0.06	0.1–4
Al	309.3	N-Ac	0.1	1	5–100
Au	242.8	A-Ac	0.01	0.25	0.5–20
Ba	553.6	N-Ac	0.03	0.4	1–20
Be	234.9	N-Ac	0.005	0.03	0.05–2
Bi	223.1	A-Ac	0.06	0.4	1–50
Ca	422.7	A-Ac	0.003	0.08	0.2–20
Cd	228.8	A-Ac	0.002	0.025	0.05–2
Co	240.7	A-Ac	0.03	0.2	0.5–10
Cr	357.9	A-Ac	0.02	0.1	0.2–10
Cs	852.1	A-Ac	0.02	0.3	0.5–15
Cu	324.7	A-Ac	0.01	0.1	0.2–10
Fe	248.3	A-Ac	0.02	0.12	0.3–10
Ir	264.0	A-Ac	0.6	8	—
K	766.5	A-Ac	0.005	0.04	0.1–2
Li	670.8	A-Ac	0.002	0.04	0.1–2
Mg	285.2	A-Ac	0.0005	0.007	0.02–2
Mn	279.5	A-Ac	0.01	0.05	0.1–10
Mo	313.3	N-Ac	0.1	0.5	1–20
Na	589.0	A-Ac	0.002	0.015	0.03–1
Ni	232.0	A-Ac	0.02	0.15	0.3–10

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Element	Wave-length nm	Flame Gases*	Instrument Detection Limit mg/L	Sensitivity mg/L	Optimum Concentration Range mg/L
Os	290.9	N–Ac	0.08	1	—
Pb†	283.3	A–Ac	0.05	0.5	1–20
Pt	265.9	A–Ac	0.1	2	5–75
Rh	343.5	A–Ac	0.5	0.3	—
Ru	349.9	A–Ac	0.07	0.5	—
Sb	217.6	A–Ac	0.07	0.5	1–40
Si	251.6	N–Ac	0.3	2	5–150
Sn	224.6	A–Ac	0.8	4	10–200
Sr	460.7	A–Ac	0.03	0.15	0.3–5f
Ti	365.3	N–Ac	0.3	2	5–100
V	318.4	N–Ac	0.2	1.5	2–100
Zn	213.9	A–Ac	0.005	0.02	0.05–2

* A–Ac = air-acetylene; N–Ac = nitrous oxide-acetylene.

† The more sensitive 217.0 nm wavelength is recommended for instruments with background correction capabilities. Copyright ASTM. Reprinted with permission.

TABLE 3111:II. INTERLABORATORY PRECISION AND BIAS DATA FOR ATOMIC ABSORPTION METHODS—DIRECT ASPIRATION AND EXTRACTED METALS

Metal	Conc.*	SD*	Relative SD %	Relative Error %	No. of Participants
Direct determination:					
Aluminum ¹	4.50	0.19	4.2	8.4	5
Barium ²	1.00	0.089	8.9	2.7	11
Beryllium ¹	0.46	0.0213	4.6	23.0	11
Cadmium ³	0.05	0.0108	21.6	8.2	26
Cadmium ¹	1.60	0.11	6.9	5.1	16
Calcium ¹	5.00	0.21	4.2	0.4	8
Chromium ¹	3.00	0.301	10.0	3.7	9

Standard Methods for the Examination of Water and Wastewater

Metal	Conc.*	SD*	Relative SD %	Relative Error %	No. of Participants
Cobalt ¹	4.00	0.243	6.1	0.5	14
Copper ³	1.00	0.112	11.2	3.4	53
Copper ¹	4.00	0.331	8.3	2.8	15
Iron ¹	4.40	0.260	5.8	2.3	16
Iron ³	0.30	0.0495	16.5	0.6	43
Lead ¹	6.00	0.28	4.7	0.2	14
Magnesium ³	0.20	0.021	10.5	6.3	42
Magnesium ¹	1.10	0.116	10.5	10.0	8
Manganese ¹	4.05	0.317	7.8	1.3	16
Manganese ³	0.05	0.0068	13.5	6.0	14
Nickel ¹	3.93	0.383	9.8	2.0	14
Silver ³	0.05	0.0088	17.5	10.6	7
Silver ¹	2.00	0.07	3.5	1.0	10
Sodium ¹	2.70	0.122	4.5	4.1	12
Strontium ¹	1.00	0.05	5.0	0.2	12
Zinc ³	0.50	0.041	8.2	0.4	48

Extracted determination:

Aluminum ²	300	32	10.7	0.7	15
Beryllium ²	5	1.7	34.0	20.0	9
Cadmium ³	50	21.9	43.8	13.3	12
Cobalt ¹	300	28.5	9.5	1.0	6
Copper ¹	100	71.7	71.7	12.0	8
Iron ¹	250	19.0	7.6	3.6	4
Manganese ¹	21.5	2.4	11.2	7.4	8
Molybdenum ¹	9.5	1.1	11.6	1.3	5
Nickel ¹	56.8	15.2	26.8	13.6	14
Lead ³	50	11.8	23.5	19.0	8

Standard Methods for the Examination of Water and Wastewater

Metal	Conc.*	SD*	Relative SD %	Relative Error %	No. of Participants
Silver ¹	5.2	1.4	26.9	3.0	7

* For direct determinations, mg/L; for extracted determinations, µg/L.

Superscripts refer to reference numbers.

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TABLE 3111:III. SINGLE-OPERATOR PRECISION AND RECOMMENDED CONTROL RANGES FOR ATOMIC ABSORPTION METHODS—DIRECT ASPIRATION AND EXTRACTED METALS

Metal	Conc.*	SD*	Relative SD %	No. of Participants	QC Std.*	Acceptable Range ^a
Direct determination:						
Aluminum ¹	4.50	0.23	5.1	15	5.00	4.3–5.7
Beryllium ¹	0.46	0.012	2.6	10	0.50	0.46–0.54
Calcium ¹	5.00	0.05	1.0	8	5.00	4.8–5.2
Chromium ¹	7.00	0.69	9.9	9	5.00	3.3–6.7
Cobalt ¹	4.00	0.21	5.3	14	4.00	3.4–4.6
Copper ¹	4.00	0.115	2.9	15	4.00	3.7–4.3
Iron ¹	5.00	0.19	3.8	16	5.00	4.4–5.6
Magnesium ¹	1.00	0.009	0.9	8	1.00	0.97–1.03
Nickel ⁴	5.00	0.04	0.8	—	5.00	4.9–5.1
Silver ¹	2.00	0.25	12.5	10	2.00	1.2–2.8
Sodium ⁴	8.2	0.1	1.2	—	5.00	4.8–5.2
Strontium ¹	1.00	0.04	4.0	12	1.00	0.87–1.13
Potassium ⁴	1.6	0.2	12.5	—	1.6	1.0–2.2
Molybdenum ⁴	7.5	0.07	0.9	—	10.0	9.7–10.3
Tin ⁴	20.0	0.5	2.5	—	20	.018.5–21.5
Titanium ⁴	50.0	0.4	0.8	—	50.0	48.8–51.2
Vanadium	50.0	0.2	0.4	—	50.0	49.4–50.6

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Metal	Conc.*	SD*	Relative SD %	No. of Participants	QC Std.*	Acceptable Range [†]
Extracted determination:						
Aluminum ¹	300	12	4.0	15	300	264–336
Cobalt ¹	300	20	6.7	6	300	220–380
Copper ¹	100	21	21	8	100	22–178
Iron ¹	250	12	4.8	4	250	180–320
Manganese ¹	21.5	202	10.2	8	25	17–23
Molybdenum ¹	9.5	1.0	10.5	5	10	5.5–14.5
Nickel ¹	56.8	9.2	16.2	14	50	22–78
Silver ¹	5.2	1.2	23.1	7	5.0	0.5–9.5

* For direct determinations, mg/L; for extracted determinations, µg/L.

Superscripts refer to reference numbers.

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TABLE 3112:I. INTERLABORATORY PRECISION AND BIAS OF COLD-VAPOR ATOMIC ABSORPTION SPECTROMETRIC METHOD FOR MERCURY¹

Form	Conc. µg/L	SD µg/L	Relative SD %	Relative Error %	No. of Participants
Inorganic	0.34	0.077	22.6	21.0	23
Inorganic	4.2	0.56	13.3	14.4	21
Organic	4.2	0.36	8.6	8.4	21

TABLE 3113:I. POTENTIAL MATRIX MODIFIERS FOR ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY*

Modifier	Analyses for Which Modifier May Be Useful

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Modifier	Analyses for Which Modifier May Be Useful
1500 mg Pd/L + 1000 mg Mg(NO ₃) ₂ /L ¹	Ag, As, Au, Bi, Cu, Ge, Mn, Hg, In, Sb, Se, Sn, Te, Tl
500–2000 mg Pd/L + reducing agent ^{2†}	Ag, As, Bi, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb
5000 mg Mg(NO ₃) ₂ /L ¹	Be, Co, Cr, Fe, Mn, V
100–500 mg Pd/L ²	As, Ga, Ge, Sn
50 mg Ni/L ²	As, Se, Sb
2% PO ₄ ³⁻ + 1000 mg Mg (NO ₃) ₂ /L ¹	Cd, Pb

*Assumes 10 µL modifier/10 µL sample.†Citric acid (1–2%) preferred; ascorbic acid or H₂ acceptable.

TABLE 3113:II. DETECTION LEVELS AND CONCENTRATION RANGES FOR ELECTROTHERMAL ATOMIZATION ATOMIC ABSORPTION SPECTROMETRY

Element	Wavelength nm	Estimated Detection Limit µg/L	Optimum Concentration Range µg/L
Al	309.3	3	20–200
Sb	217.6	3	20–300
As	193.7	1	5–100
Ba	553.6	2	10–200
Be	234.9	0.2	1–30
Cd	228.8	0.1	0.5–10
Cr	357.9	2	5–100
Co	240.7	1	5–100
Cu	324.7	1	5–100
Fe	248.3	1	5–100
Pb*	283.3	1	5–100
Mn	279.5	0.2	1–30
Mo	313.3	1	3–60
Ni	232.0	1	5–100

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Element	Wavelength <i>nm</i>	Estimated Detection Limit $\mu\text{g/L}$	Optimum Concentration Range $\mu\text{g/L}$
Se	196.0	2	5–100
Ag	328.1	0.2	1–25
Sn	224.6	5	20–300

*The more sensitive 217.0-nm wavelength is recommended for instruments with background correction capabilities.

TABLE 3113:III. INTERLABORATORY SINGLE-ANALYST PRECISION DATA FOR ELECTROTHERMAL ATOMIZATION METHODS¹

Single-Analyst Precision % <i>RSD</i>							
Element	Concentration $\mu\text{g/L}$	Lab Pure Water	Drinking Water	Surface Water	Effluent 1	Effluent 2	Effluent 3
Al	28	66	108	70	—	—	66
	125	27	35	24	—	—	34
	11 000	11	—	—	22	—	—
	58 300	27	—	—	19	—	—
	460	9	—	—	—	30	—
	2 180	28	—	—	—	4	—
	10.5	20	13	13	13	56	18
As	230	10	18	13	21	94	14
	9.78	40	25	15	74	23	11
	227	10	6	8	11	15	6
Ba	56.5	36	21	29	59	23	27
	418	14	12	20	24	24	18
Be	0.45	18	27	15	30	2	11
	10.9	14	4	9	7	12	12
Cd	0.43	72	49	1	121	35	27
	12	11	17	22	14	11	15
Cr	9.87	24	33	10	23	15	10
	236	16	7	11	13	16	7

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Single-Analyst Precision % RSD							
Element	Concentration μg/L	Lab Pure Water	Drinking Water	Surface Water	Effluent 1	Effluent 2	Effluent 3
Co	29.7	10	17	10	19	24	12
	420	8	11	13	14	9	5
Cu	10.1	49	47	17	17	—	30
	234	8	15	6	21	—	11
	300	6	—	—	—	11	—
	1 670	11	—	—	—	6	—
Fe	26.1	144	52	153	—	—	124
	455	48	37	45	—	—	31
	1 030	17	—	—	30	—	—
	5 590	6	—	—	32	—	—
	370	14	—	—	—	19	—
	2 610	9	—	—	—	18	—
Pb	10.4	6	19	17	21	19	33
	243	17	7	17	18	12	16
Mn	0.44	187	180	—	—	—	275
	14.8	32	19	—	—	—	18
	91.0	15	—	—	48	—	—
	484.0	4	—	—	12	—	—
	111.0	12	—	—	—	21	—
	666.0	6	—	—	—	20	—
Ni	26.2	20	26	25	24	18	9
	461.0	15	11	9	8	11	4
Se	10.0	12	27	16	35	41	13
	235.0	6	6	15	6	13	14
Ag	8.48	10	—	—	15	27	16
	56.5	14	—	—	7	16	23
	0.45	27	166	48	—	—	—
	13.6	15	4	10	—	—	—

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TABLE 3113:IV. INTERLABORATORY OVERALL PRECISION DATA FOR ELECTROTHERMAL ATOMIZATION METHODS¹

Overall Precision% <i>RSD</i>							
Element	Concentration µg/L	Lab Pure Water	Drinking Water	Surface Water	Effluent 1	Effluent 2	Effluent 3
Al	28	99	114	124	—	—	131
	125	45	47	49	—	—	40
	11 000	19	—	—	43	—	—
	58 300	31	—	—	32	—	—
	460	20	—	—	—	47	—
	2 180	30	—	—	—	15	—
	10.5	37	19	22	50	103	39
	230	26	16	16	17	180	21
As	9.78	43	26	37	72	50	39
	227	18	12	13	20	15	14
Ba	56.5	68	38	43	116	43	65
	418	35	35	28	38	48	16
Be	0.45	28	31	15	67	50	35
	10.9	33	15	26	20	9	19
Cd	0.43	73	60	5	88	43	65
	12	19	25	41	26	20	27
Cr	9.87	30	53	24	60	41	23
	236	18	14	24	20	14	20
Co	29.7	13	26	17	18	21	17
	420	21	21	17	18	13	13
Cu	10.1	58	82	31	32	—	74
	234	12	33	19	21	—	26
	300	13	—	—	—	14	—
	1 670	12	—	—	—	13	—
Fe	26.1	115	93	306	—	—	204
	455	53	46	53	—	—	44
	1 030	32	—	—	25	—	—

Standard Methods for the Examination of Water and Wastewater

Overall Precision% <i>RSD</i>							
Element	Concentration µg/L	Lab Pure Water	Drinking Water	Surface Water	Effluent 1	Effluent 2	Effluent 3
Pb	5 590	10	—	—	43	—	—
	370	28	—	—	—	22	—
	2 610	13	—	—	—	22	—
	10.4	27	42	31	23	28	47
	243	18	19	17	19	19	25
Mn	0.44	299	272	—	—	—	248
	14.8	52	41	—	—	—	29
	91.0	16	—	—	45	—	—
Ni	484.0	5	—	—	17	—	—
	111.0	15	—	—	—	17	—
	666.0	8	—	—	—	24	—
	26.2	35	30	49	35	37	43
Se	461.0	23	22	15	12	21	17
	10.0	17	48	32	30	44	51
	235.0	16	18	18	17	22	34
Ag	8.48	23	—	—	16	35	34
	56.5	15	—	—	24	32	28
	0.45	57	90	368	—	—	—
	13.6	19	19	59	—	—	—

TABLE 3113:V. INTERLABORATORY RELATIVE ERROR DATA FOR ELECTROTHERMAL ATOMIZATION METHODS¹

Relative Error %							
Element	Concentration µg/L	Lab Pure Water	Drinking Water	Surface Water	Effluent 1	Effluent 2	Effluent 3
Al	28.0	86	150	54	—	—	126
	125.0	4	41	39	—	—	30
	11 000.0	2	—	—	14	—	—

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Element	Concentration µg/L	Relative Error %					
		Lab Pure Water	Drinking Water	Surface Water	Effluent 1	Effluent 2	Effluent 3
Sb	58 300.0	12	—	—	7	—	—
	460.0	2	—	—	—	11	—
	2 180.0	11	—	—	—	9	—
	10.5	30	32	28	24	28	36
	230.0	35	14	19	13	73	39
As	9.78	36	1	22	106	13	16
	227.0	3	7	10	19	6	13
Ba	56.5	132	54	44	116	59	40
	418.0	4	0	0	13	6	60
Be	0.45	40	16	11	16	10	15
	10.9	13	2	9	7	8	8
Cd	0.43	58	45	37	66	16	19
	12.0	4	6	5	22	18	3
Cr	9.87	10	9	4	2	5	15
	236.0	11	0	9	13	5	8
Co	29.7	7	7	1	6	3	13
	420.0	12	8	8	11	5	18
Cu	10.1	16	48	2	5	—	15
	234.0	8	7	0	4	—	19
	300.0	4	—	—	—	21	—
Fe	1 670.0	6	—	—	—	2	—
	26.1	85	60	379	—	—	158
	455.0	43	22	31	—	—	18
	1 030.0	8	—	—	8	—	—
	5 590.0	2	—	—	12	—	—
Pb	370.0	4	—	—	—	11	—
	2 610.0	35	—	—	—	2	—
	10.4	16	10	17	1	34	14
	243.0	5	15	8	18	15	29
	0.44	332	304	—	—	—	556

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		Relative Error %					
Element	Concentration µg/L	Lab					
		Pure Water	Drinking Water	Surface Water	Effluent 1	Effluent 2	Effluent 3
Ni	14.8	10	1	—	—	—	36
	91.0	31	—	—	10	—	—
	484.0	42	—	—	4	—	—
	111.0	1	—	—	—	29	—
	666.0	6	—	—	—	23	—
	26.2	9	16	10	7	33	54
	461.0	15	19	18	31	16	18
	10.0	12	9	6	36	17	37
	235.0	7	7	0	13	10	17
	8.48	12	—	—	1	51	20
Ag	56.5	16	—	—	8	51	22
	0.45	34	162	534	—	—	—
	13.6	3	12	5	—	—	—

TABLE 3120:I. SUGGESTED WAVELENGTHS, ESTIMATED DETECTION LIMITS, ALTERNATE WAVELENGTHS, CALIBRATION CONCENTRATIONS, AND UPPER LIMITS

Element	Suggested Wavelength <i>nm</i>	Estimated Detection Limit µg/L	Alternate Wavelength* <i>nm</i>	Calibration Concentration <i>mg/L</i>	Upper Limit Concentration† <i>mg/L</i>
Aluminum	308.22	40	237.32	10.0	100
Antimony	206.83	30	217.58	10.0	100
Arsenic	193.70	50	189.04‡	10.0	100
Barium	455.40	2	493.41	1.0	50
Beryllium	313.04	0.3	234.86	1.0	10
Boron	249.77	5	249.68	1.0	50
Cadmium	226.50	4	214.44	2.0	50
Calcium	317.93	10	315.89	10.0	100
Chromium	267.72	7	206.15	5.0	50
Cobalt	228.62	7	230.79	2.0	50

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Element	Suggested Wavelength <i>nm</i>	Estimated Detection Limit $\mu\text{g/L}$	Alternate Wavelength* <i>nm</i>	Calibration Concentration <i>mg/L</i>	Upper Limit Concentration† <i>mg/L</i>
Copper	324.75	6	219.96	1.0	50
Iron	259.94	7	238.20	10.0	100
Lead	220.35	40	217.00	10.0	100
Lithium	670.78	4 [§]	—	5.0	100
Magnesium	279.08	30	279.55	10.0	100
Manganese	257.61	2	294.92	2.0	50
Molybdenum	202.03	8	203.84	10.0	100
Nickel	231.60	15	221.65	2.0	50
Potassium	766.49	100 [§]	769.90	10.0	100
Selenium	196.03	75	203.99	5.0	100
Silica (SiO ₂)	212.41	20	251.61	21.4	100
Silver	328.07	7	338.29	2.0	50
Sodium	589.00	30 [§]	589.59	10.0	100
Strontium	407.77	0.5	421.55	1.0	50
Thallium	190.86‡	40	377.57	10.0	100
Vanadium	292.40	8	—	1.0	50
Zinc	213.86	2	206.20	5.0	100

* Other wavelengths may be substituted if they provide the needed sensitivity and are corrected for spectral interference.† Defines the top end of the effective calibration range. Do not extrapolate to concentrations beyond highest standard.‡ Available with vacuum or inert gas purged optical path. § Sensitive to operating conditions.

TABLE 3120:II. ICP PRECISION AND BIAS DATA

Element	Concentration Range $\mu\text{g/L}$	Total Digestion* $\mu\text{g/L}$	Recoverable Digestion* $\mu\text{g/L}$
Aluminum	4792 69–	$X = 0.9273C + 3.6$	$X = 0.9380C + 22.1$
		$S = 0.0559X + 18.6$	$S = 0.0873X + 31.7$
		$SR = 0.0507X + 3.5$	$SR = 0.0481X + 18.8$
Antimony	1406 77–	$X = 0.7940C - 17.0$	$X = 0.8908C + 0.9$

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Element	Concentration Range $\mu\text{g/L}$	Total Digestion* $\mu\text{g/L}$	Recoverable Digestion* $\mu\text{g/L}$
Arsenic	1887 69–	$S = 0.1556X - 0.6$	$S = 0.0982X + 8.3$
		$SR = 0.1081X + 3.9$	$SR = 0.0682X + 2.5$
		$X = 1.0437C - 12.2$	$X = 1.0175C + 3.9$
Barium	377 9–	$S = 0.1239X + 2.4$	$S = 0.1288X + 6.1$
		$SR = 0.0874X + 6.4$	$SR = 0.0643X + 10.3$
		$X = 0.7683C + 0.47$	$X = 0.8380C + 1.68$
Beryllium	1906 3–	$S = 0.1819X + 2.78$	$S = 0.2540X + 0.30$
		$SR = 0.1285X + 2.55$	$SR = 0.0826X + 3.54$
		$X = 0.9629C + 0.05$	$X = 1.0177C - 0.55$
Boron	5189 19–	$S = 0.0136X + 0.95$	$S = 0.0359X + 0.90$
		$SR = 0.0203X - 0.07$	$SR = 0.0445X - 0.10$
		$X = 0.8807C + 9.0$	$X = 0.9676C + 18.7$
Cadmium	1943 9–	$S = 0.1150X + 14.1$	$S = 0.1320X + 16.0$
		$SR = 0.0742X + 23.2$	$SR = 0.0743X + 21.1$
		$X = 0.9874C - 0.18$	$X = 1.0137C - 0.65$
Calcium	47 170 17–	$S = 0.0557X + 2.02$	$S = 0.0585X + 1.15$
		$SR = 0.0300X + 0.94$	$SR = 0.0332X + 0.90$
		$X = 0.9182C - 2.6$	$X = 0.9658C + 0.8$
Chromium	1406 13–	$S = 0.1228X + 10.1$	$S = 0.0917X + 6.9$
		$SR = 0.0189X + 3.7$	$SR = 0.0327X + 10.1$
		$X = 0.9544C + 3.1$	$X = 1.0049C - 1.2$
Cobalt	2340 17–	$S = 0.0499X + 4.4$	$S = 0.0698X + 2.8$
		$SR = 0.0009X + 7.9$	$SR = 0.0571X + 1.0$
		$X = 0.9209C - 4.5$	$X = 0.9278C - 1.5$
		$S = 0.0436X + 3.8$	$S = 0.0498X + 2.6$

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Element	Concentration Range $\mu\text{g/L}$	Total Digestion* $\mu\text{g/L}$	Recoverable Digestion* $\mu\text{g/L}$
Copper	1887 8–	$SR = 0.0428X + 0.5$	$SR = 0.0407X + 0.4$
		$X = 0.9297C - 0.30$	$X = 0.9647C - 3.64$
Iron	9359 13–	$S = 0.0442X + 2.85$	$S = 0.0497X + 2.28$
		$SR = 0.0128X + 2.53$	$SR = 0.0406X + 0.96$
Lead	4717 42–	$X = 0.8829C + 7.0$	$X = 0.9830C + 5.7$
		$S = 0.0683X + 11.5$	$S = 0.1024X + 13.0$
Magnesium	13 868 34–	$SR = -0.0046X + 10.0$	$SR = 0.0790X + 11.5$
		$X = 0.9699C - 2.2$	$X = 1.0056C + 4.1$
Manganese	1887 4–	$S = 0.0558X + 7.0$	$S = 0.0799X + 4.6$
		$SR = 0.0353X + 3.6$	$SR = 0.0448X + 3.5$
Molybdenum	1830 17–	$X = 0.9881C - 1.1$	$X = 0.9879C + 2.2$
		$S = 0.0607X + 11.6$	$S = 0.0564X + 13.2$
Nickel	47 170 17–	$SR = 0.0298X + 0.6$	$SR = 0.0268X + 8.1$
		$X = 0.9417C + 0.13$	$X = 0.9725C + 0.07$
Potassium	347–14 151	$S = 0.0324X + 0.88$	$S = 0.0557X + 0.76$
		$SR = 0.0153X + 0.91$	$SR = 0.0400X + 0.82$
		$X = 0.9682C + 0.1$	$X = 0.9707C - 2.3$
		$S = 0.0618X + 1.6$	$S = 0.0811X + 3.8$
		$SR = 0.0371X + 2.2$	$SR = 0.0529X + 2.1$
		$X = 0.9508C + 0.4$	$X = 0.9869C + 1.5$
		$S = 0.0604X + 4.4$	$S = 0.0526X + 5.5$
		$SR = 0.0425X + 3.6$	$SR = 0.0393X + 2.2$
		$X = 0.8669C - 36.4$	$X = 0.9355C - 183.1$
		$S = 0.0934X + 77.8$	$S = 0.0481X + 177.2$
		$SR = -0.0099X + 144.2$	$SR = 0.0329X + 60.9$

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Element	Concentration Range $\mu\text{g/L}$	Total Digestion* $\mu\text{g/L}$	Recoverable Digestion* $\mu\text{g/L}$
Selenium	1415 69–	$X = 0.9363C - 2.5$ $S = 0.0855X + 17.8$ $SR = 0.0284X + 9.3$	$X = 0.9737C - 1.0$ $S = 0.1523X + 7.8$ $SR = 0.0443X + 6.6$
Silicon	189–9434	$X = 0.5742C - 35.6$ $S = 0.4160X + 37.8$ $SR = 0.1987X + 8.4$	$X = 0.9737C - 60.8$ $S = 0.3288X + 46.0$ $SR = 0.2133X + 22.6$
Silver	189 8–	$X = 0.4466C + 5.07$ $S = 0.5055X - 3.05$ $SR = 0.2086X - 1.74$	$X = 0.3987C + 8.25$ $S = 0.5478X - 3.93$ $SR = 0.1836X - 0.27$
Sodium	47 170 35–	$X = 0.9581C + 39.6$ $S = 0.2097X + 33.0$ $SR = 0.0280X + 105.8$	$X = 1.0526C + 26.7$ $S = 0.1473X + 27.4$ $SR = 0.0884X + 50.5$
Thallium	1434 79–	$X = 0.9020C - 7.3$ $S = 0.1004X + 18.3$ $SR = 0.0364X + 11.5$	$X = 0.9238C + 5.5$ $S = 0.2156X + 5.7$ $SR = -0.0106X + 48.0$
Vanadium	4698 13–	$X = 0.9615C - 2.0$ $S = 0.0618X + 1.7$ $SR = 0.0220X + 0.7$	$X = 0.9551C + 0.4$ $S = 0.0927X + 1.5$ $SR = 0.0472X + 0.5$
Zinc	7076 7–	$X = 0.9356C - 0.30$ $S = 0.0914X + 3.75$ $SR = -0.0130X + 10.07$	$X = 0.9500C + 1.22$ $S = 0.0597X + 6.50$ $SR = 0.0153X + 7.78$

*X=mean recovery, $\mu\text{g/L}$, C=true value, $\mu\text{g/L}$, S=multi-laboratory standard deviation, $\mu\text{g/L}$, SR=single-an

TABLE 3125:I. RECOMMENDED ANALYTE MASSES, INSTRUMENTAL DETECTION LIMITS (IDL), and INTERNAL STANDARDS

Standard Methods for the Examination of Water and Wastewater

TABLE 3125:I. RECOMMENDED ANALYTE MASSES, INSTRUMENTAL DETECTION LIMITS (IDL), and INTERNAL STANDARDS

Element	Analytical Mass	IDL $\mu\text{g/L}$	Recommended Internal Standard
Be	9	0.025	Li
Al	27	0.03	Sc
V	51	0.02	Sc
Cr	52	0.04	Sc
Cr	53	0.03	Sc
Mn	55	0.002	Sc
Co	59	0.002	Sc
Ni	60	0.004	Sc
Ni	62	0.025	Sc
Cu	63	0.003	Sc
Cu	65	0.004	Sc
Zn	66	0.017	Ge
Zn	68	0.020	Ge
As	75	0.025	Ge
Se	77	0.093	Ge
Se	82	0.064	Ge
Ag	107	0.003	In
Ag	109	0.002	In
Cd	111	0.006	In
Cd	114	0.003	In
Sb	121	0.07	In
Sb	123	0.07	In
Tl	203	0.03	Th
Tl	205	0.03	Th
Pb	208	0.005	Th
U	235	0.032	Th
U	238	0.001	Th
Mo	98	0.003†	In
Ba	135	0.008†	In
Sr	88	0.001‡	In

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* IDLs were determined on a Perkin Elmer Elan 6000 ICP/MS using seven replicate analyses of a 1% nitric acid solution, at Manchester Environmental Laboratory, July 1996.† From EPA Method 200.8 for the Analysis of Drinking Waters-Application Note, Order No. ENVA-300A, The Perkin Elmer Corporation, 1996.‡ From Perkin Elmer Technical Summary TSMS-12.

TABLE 3125:II. ELEMENTAL ABUNDANCE EQUATIONS AND COMMON MOLECULAR ION CORRECTION EQUATIONS

Elemental and Molecular Equations*†	
Li 6	= C 6
Be 9	= C 9
Al 27	= C 27
Sc 45	= C 45
V 51	= C 51 – (3.127)[(C 53) – (0.113 × C 52)]
Cr 52	= C 52
Cr 53	= C 53
Mn 55	= C 55
Co 59	= C 59
Ni 60	= C 60
Ni 62	= C 62
Cu 63	= C 63
Cu 65	= C 65
Zn 66	= C 66
Zn 68	= C 68
As 75	= C 75 – (3.127)[(C 77) – (0.815 × C 82)]
Se 77	= C 77
Se 82	= C 82 – (1.008696 × C 83)
Sr 88	= C 88
Mo 98	= C 98 – (0.110588 × C 101)
Rh 103	= C 103
Ag 107	= C 107
Ag 109	= C 109
Cd 111	= C 111 – (1.073)[(C 108) – (0.712 × C 106)]
Cd 114	= C 114 – (0.02686 × C 118)
Sb 121	= C 121
Sb 123	= C 123 – (0.127189 × C 125)

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Elemental and Molecular Equations*†
Ba 135 = C 135
Ho 165 = C 165
Tl 203 = C 203
Tl 205 = C 205
Pb 208 = C 208 + (1 × C 206) + (1 × C 207)
Th 232 = C 232
U 238 = C 238

* C = calibration blank corrected counts at indicated mass.† From EPA Method 200.8 for the Analysis of Drinking Waters – Application Note, Order No. ENVA-300A, The Perkin Elmer Corporation, 1996.

TABLE 3125:III. COMMON MOLECULAR ION INTERFERENCES IN ICP/MS¹

Molecular Ion	Mass	Element Measurement Affected by Interference
Background molecular ions:		
NH ⁺	15	—
OH ⁺	17	—
OH ₂ ⁺	18	—
C ₂ ⁺	24	Mg
CN ⁺	26	Mg
CO ⁺	28	Si
N ₂ ⁺	28	Si
N ₂ H ⁺	29	Si
NO ⁺	30	—
NOH ⁺	31	P
O ₂ ⁺	32	S
O ₂ H ⁺	33	—
³⁶ ArH ⁺	37	Cl
³⁸ ArH ⁺	39	K
⁴⁰ ArH ⁺	41	—
CO ₂ ⁺	44	Ca

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Molecular Ion	Mass	Element Measurement Affected by Interference
CO_2^+H	45	Sc
$\text{ArC}^+, \text{ArO}^+$	52	Cr
ArN^+	54	Cr
ArNH^+	55	Mn
ArO^+	56	Fe
ArH^+	57	Fe
$^{40}\text{Ar}^{36}\text{Ar}^+$	76	Se
$^{40}\text{Ar}^{38}\text{Ar}$	78	Se
$^{40}\text{Ar}_2^+$	80	Se
Matrix molecular ions:		
Bromide:		
$^{81}\text{BrH}^+$	82	Se
$^{79}\text{BrO}^+$	95	Mo
$^{81}\text{BrO}^+$	97	Mo
$^{81}\text{BrOH}^+$	09	Mo
$\text{Ar}^{81}\text{Br}^+$	121	Sb
Chloride:		
$^{35}\text{ClO}^+$	51	V
$^{35}\text{ClOH}^+$	52	Cr
$^{37}\text{ClO}^+$	53	Cr
$^{37}\text{ClOH}^+$	54	Cr
$\text{Ar}^{35}\text{Cl}^+$	75	As
$\text{Ar}^{37}\text{Cl}^+$	77	Se
Sulfate:		
$^{32}\text{SO}^+$	48	Ti
$^{32}\text{SOH}^+$	49	—
$^{34}\text{SO}^+$	50	V, Cr
$^{34}\text{SOH}^+$	51	V
$\text{SO}_2^+, \text{S}_2^+$	64	Zn

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Molecular Ion	Mass	Element Measurement Affected by Interference
Ar ³² S ⁺	72	Ge
Ar ³⁴ S ⁺	74	Ge
Phosphate:		
PO ⁺	47	Ti
POH ⁺	48	Ti
PO ₂ ⁺	63	Cu
ArP ⁺	71	Ga
Group I II metals:		
pArNa ⁺	63	Cu
ArK ⁺	79	Br
ArCa ⁺	80	Se
Matrix oxides*		
TiO	62–66	Ni, Cu, Zn
ZrO	106–112	Ag, Cd
MoO	108–116	Cd
NbO	109	Ag

* Oxide interferences normally will be very small and will affect the method elements only when oxide-producing elements are present at relatively high concentrations, or when the instrument is improperly tuned or maintained. Preferably monitor Ti and Zr isotopes for soil, sediment, or solid waste samples, because these samples potentially contain high levels of these interfering elements.

TABLE 3125:IV. SUGGESTED ANALYTICAL RUN SEQUENCE

Sample Type	Comments
Tuning/optimization standard	Check mass calibration and resolution
Tuning/optimization standard	Optimize instrument for maximum rhodium counts while keeping oxides, double charged ions, and background within instrument specifications
Rinse	—
Reagent blank	Check for contamination

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Sample Type	Comments
Reagent blank	Calibration standard blank
5- $\mu\text{g/L}$ standard	—
10- $\mu\text{g/L}$ standard	—
20- $\mu\text{g/L}$ standard	—
50- $\mu\text{g/L}$ standard	—
100- $\mu\text{g/L}$ standard	—
Rinse	—
Initial calibration verification, 50 $\mu\text{g/L}$	—
Initial calibration blank	—
0.30- $\mu\text{g/L}$ standard	Low-level calibration verification
1.0- $\mu\text{g/L}$ standard	Low-level calibration verification
External reference material	NIST 1643c or equivalent
Continuing calibration verification	—
Continuing blank calibration	—
Project sample method blank	—
Project sample laboratory-fortified blank	—
Project sample 1–4	—
Project sample 5	—
Project sample 5 with known addition	—
Project sample 5 duplicate with known addition	—
Continuing calibration verification	—
Continuing calibration blank	—

TABLE 3125:V. SUMMARY OF PERFORMANCE CRITERIA

Performance Characteristic	Criteria
Mass resolution	Manufacturer's specification
Mass calibration	Manufacturer's specification
Ba ²⁺ /Ba ⁺	Manufacturer's specification
CeO/Ce	Manufacturer's specification
Background counts at mass 220	Manufacturer's specification
Correlation coefficient	≥0.995

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Performance Characteristic	Criteria
Calibration blanks	< Reporting limit
Calibration verification standards	±10% of true value
Laboratory fortified blank (control sample)	±30% of true value
Precision	±20% relative percent difference for lab duplicates
Known-addition recovery	75–125%
0.3 and 1.0 µg/L standards	Dependent on data quality objectives
Reference materials	Dependent on data quality objectives
Internal standard response	70–125% of response in calibration blank with known addition

TABLE 3125:VI. QUALITY CONTROL ANALYSES FOR ICP/MS METHOD

Analysis	Frequency	Acceptance Criteria
Reference material [¶ 3c9]	Greater of: once per sample batch, or 5%	Dependent on data quality objectives
Preparatory/method blank [¶ 3c4]	Greater of: once per sample batch, or 5%	± Absolute value of instrument detection limit; ± absolute value of laboratory reporting limit or MDL is acceptable
Laboratory fortified blank [¶ 3c7]	Greater of: once per sample batch, or 5%	± 30% of true value
Duplicate known-addition samples	Greater of: once per sample batch, or 5%	± 20% relative percent difference
Continuing calibration verification standards [¶ 3c5]	10%	± 10% of known concentration

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Analysis	Frequency	Acceptance Criteria
Continuing calibration verification blank [¶ 3c6)]	10%	± Absolute value of instrument detection limit; ± absolute value of laboratory reporting limit or MDL is acceptable

TABLE 3125:VII. METHOD PERFORMANCE WITH CALIBRATION VERIFICATION STANDARDS*

Element	Mass	Continuing Calibration Verification Standard (N = 44)				Initial Calibration Verifi (N = 12)		
		Mean Recovery %	Mean	Standard Deviation	Relative Standard Deviation %	Mean Recovery %	Mean	Stan Devi
Be	9	98.71	49.35	3.43	6.94	100.06	50.03	1.
Al	27	99.62	49.81	2.99	6.01	98.42	49.21	1.
V	51	100.97	50.48	1.36	2.68	99.91	49.96	1.
Cr	52	101.39	50.70	1.86	3.66	99.94	49.97	1.
Cr	53	100.68	50.34	1.91	3.79	99.13	49.56	1.
Mn	55	101.20	50.60	1.98	3.91	99.48	49.74	1.
Co	59	101.67	50.83	2.44	4.79	99.44	49.72	1.
Ni	60	99.97	49.99	2.14	4.28	97.98	48.99	1.
Ni	62	99.79	49.89	2.09	4.18	97.57	48.79	1.
Cu	63	100.51	50.25	2.19	4.36	97.87	48.93	1.
Cu	65	100.39	50.19	2.26	4.51	98.34	49.17	1.
Zn	66	101.07	50.53	1.93	3.82	98.75	49.38	0.
Zn	68	100.42	50.21	1.89	3.77	97.75	48.87	0.
As	75	100.76	50.38	1.15	2.28	98.83	49.41	0.
Se	77	101.71	50.85	1.43	2.81	99.54	49.77	1.
Se	82	101.97	50.98	1.50	2.95	99.76	49.88	0.
Ag	107	101.50	50.75	1.68	3.30	99.27	49.63	1.
Ag	109	101.65	50.83	1.68	3.31	99.66	49.83	1.
Cd	111	100.92	50.46	1.94	3.84	98.61	49.30	1.
Cd	114	100.90	50.45	2.07	4.10	99.20	49.60	1.

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Element	Mass	Continuing Calibration Verification Standard (N = 44)				Initial Calibration Verifi (N = 12)		
		Mean Recovery %	Mean	Standard Deviation	Relative Standard Deviation %	Mean Recovery %	Mean	Stan Devi
Sb	121	100.14	50.07	2.39	4.77	99.38	49.69	1.
Sb	123	99.98	49.99	2.48	4.97	99.09	49.54	1.
Tl	203	101.36	50.68	1.64	3.23	100.05	50.02	1.
Tl	205	102.40	51.20	1.93	3.78	101.23	50.62	1.
Pb	208	101.21	50.61	1.65	3.25	99.33	49.67	0.
U	238	101.54	50.77	1.93	3.80	99.80	49.90	1.

* Single-laboratory, single-operator, single-instrument data, determined using a 50- μ g/L standard prepared from sources independent of calibration standard source. Data acquired January-November 1996 during actual sample determinations. Performance of continuing calibration verification standards at different levels may vary. Perkin-Elmer Elan 6000 ICP/MS used for determination.

TABLE 3125:VIII. METHOD PERFORMANCE FOR RECOVERY OF KNOWN ADDITION IN NATURAL WATERS*

Element	Mass	Total Recoverable Metals†		Dissolved Metals‡	
		Mean Recovery %	Relative Standard Deviation %	Mean Recovery %	Relative Standard Deviation %
Be	9	89.09	5.77	—	—
V	51	87.00	8.82	—	—
Cr	52	87.33	8.42	88.38	6.43
Cr	53	86.93	7.90	88.52	5.95
Mn	55	91.81	10.12	—	—
Co	59	87.67	8.92	—	—
Ni	60	85.07	8.42	89.31	5.70
Ni	62	84.67	8.21	89.00	5.82
Cu	63	84.13	8.46	88.55	8.33
Cu	65	84.37	8.05	88.26	7.80
Zn	66	86.14	23.01	95.59	13.81
Zn	68	81.95	20.31	91.94	13.27
As	75	90.43	4.46	97.30	8.84

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Element	Mass	Total Recoverable Metals†		Dissolved Metals‡	
		Mean Recovery %	Relative Standard Deviation %	Mean Recovery %	Relative Standard Deviation %
Se	77	83.09	4.76	105.36	10.80
Se	82	83.42	4.73	105.36	10.75
Ag	107	—	—	91.98	5.06
Ag	109	—	—	92.25	4.96
Cd	111	91.37	5.47	96.91	6.03
Cd	114	91.47	6.04	97.03	5.42
Sb	121	94.40	5.24	—	—
Sb	123	94.56	5.36	—	—
Tl	203	97.24	5.42	—	—
Tl	205	98.14	6.21	—	—
Pb	208	96.09	7.08	100.69	7.28

* Single-laboratory, single-operator, single-instrument data. Samples were Washington State surface waters from various locations. Data acquired January-November 1996 during actual sample determinations. Performance of known additions at different levels may vary. Perkin-Elmer Elan 6000 ICP/MS used for determination.

† Known-addition level 20 µg/L. Additions made before preparation according to Section 3030E (modified by cleanhood digestion in TFE beakers). *N* = 20.

‡ Known-addition level for Cd and Pb 1 µg/L; for other analytes 10 µg/L. Additions made after filtration through 1;NO1 HNO₃ precleaned 0.45-µm filters. *N* = 28.

TABLE 3125:IX. METHOD PERFORMANCE WITH LOW-LEVEL CHECK STANDARDS*

Element	Mass	1.0-µg/L Standard				0.3-µg/L Standard		
		Mean Recovery %	Mean	Standard Deviation	Relative Standard Deviation %	Mean Recovery %	Mean	Standard Deviation
Be	9	97	0.97	0.06	6.24	95	0.284	0.06
Al	27	121	1.21	0.32	26.49	196	0.588	0.06
V	51	104	1.04	0.06	5.83	111	0.332	0.06
Cr	52	119	1.19	0.34	28.62	163	0.490	0.06
Cr	53	102	1.02	0.36	35.54	113	0.338	0.06
Mn	55	103	1.03	0.07	6.55	110	0.329	0.06
Co	59	103	1.03	0.07	6.42	102	0.307	0.06

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Element	Mass	1.0- μ g/L Standard				0.3- μ g/L Standard		
		Mean Recovery %	Mean	Standard Deviation	Relative Standard Deviation %	Mean Recovery %	Mean	Standard Deviation
Ni	60	101	1.01	0.05	5.24	107	0.321	0.01
Ni	62	102	1.02	0.06	5.42	109	0.326	0.01
Cu	63	107	1.07	0.09	8.78	118	0.355	0.01
Cu	65	107	1.07	0.10	9.05	117	0.352	0.01
Zn	66	117	1.17	0.51	43.52	182	0.547	0.01
Zn	68	116	1.16	0.50	42.90	179	0.537	0.01
As	75	97	0.97	0.05	5.23	101	0.302	0.01
Se	77	89	0.89	0.08	8.72	88	0.265	0.01
Se	82	92	0.92	0.14	15.50	106	0.317	0.01
Ag	107	101	1.01	0.05	4.53	94	0.282	0.01
Ag	109	103	1.03	0.07	6.57	92	0.277	0.01
Cd	111	98	0.98	0.04	3.80	96	0.288	0.01
Cd	114	100	1.00	0.03	3.39	98	0.293	0.01
Sb	121	94	0.94	0.05	5.28	93	0.280	0.01
Sb	123	94	0.94	0.05	5.36	93	0.278	0.01
Tl	203	101	1.01	0.04	3.57	98	0.294	0.01
Tl	205	104	1.04	0.05	5.15	100	0.300	0.01
Pb	208	104	1.04	0.04	3.65	104	0.312	0.01
U	238	106	1.06	0.05	4.64	102	0.307	0.01

* Single-laboratory, single-operator, single-instrument data. $N = 24$ for both standards.

TABLE 3130:I. PRECISION OF Cd, Pb, AND Zn ANALYSIS BY ASV

Sample	Electrode	ASV Mode	Metal Concentration μ g/L			RSD %		
			Cd	Pb	Zn	Cd	Pb	Zn
Tap water #1 ⁴	HMDE	SW	0.068	0.57	—	4.2	4.8	—
Tap water #2 ⁴	HMDE	SW	—	2.50	—	—	5.1	—
Seawater #1 ⁵	TFME	DP	0.0121	0.0086	—	10.7	8.1	—

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Sample	Electrode	ASV Mode	Metal Concentration $\mu\text{g/L}$			RSD %		
			Cd	Pb	Zn	Cd	Pb	Zn
Seawater #2 ⁵	TFME	DP	0.032	0.032	—	6.3	6.3	—
Soil extract #1 ⁶	HMDE	SW	189	11.8	—	2.5	5.6	—
Soil extract #1 ⁶	HMDE	DP	186	11.9	—	2.5	4.0	—
Deionized water ⁴	HMDE	SW	0.13	0.79	—	5.5	2.2	—
Wastewater #1 ⁷	HMDE	DP	—	74	26	—	4.3	4.5
Wastewater #2 ⁷	HMDE	DP	—	47	86	—	5.2	6.3
Wastewater #3 ⁷	HMDE	DP	—	46	65	—	4.6	6.2
Wastewater #4 ⁸	TFME	SW	5.2	60	12	5.2	6.1	7.4

TABLE 3500-Cr:I. ION CHROMATOGRAPHIC CONDITIONS

Variable	Value
Guard column	Dionex IonPac NG1
Separator column	Dionex IonPac AS7
Eluent	250 mM (NH ₄) ₂ SO ₄ 100 mM NH ₄ OH
Eluent flow rate	1.5 mL/min
Post-column reagent	2 mM diphenylcarbohydrazide 10% v/v CH ₃ OH 1N H ₂ SO ₄
Post-column reagent flow rate	0.5 mL/min
Detector	Visible 530 nm
Retention time	3.8 min

TABLE 3500-Cr:II. SINGLE-LABORATORY PRECISION AND BIAS

Simple Type	Conc.* $\mu\text{g/L}$	Mean Recovery %	RPD†

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Simple Type	Conc.* µg/L	Mean Recovery %	RPD†
Reagent water	100	100	0.8
	1000	100	0.0
Drinking water	100	105	6.7
	1000	98	1.5
Groundwater	100	98	0.0
	1000	96	0.8
Primary wastewater effluent	100	100	0.7
	1000	104	2.7
Electroplating effluent	100	99	0.4
	1000	101	0.4

*Sample fortified at this concentration level.

†RPD - relative percent difference between fortified duplicates.

TABLE 3500-Cr:III. MULTILABORATORY DETERMINATION OF BIAS FOR HEXAVALENT CHROMIUM*

Water	Amount Added µg/L	Amount Found µg/L	S _t	S _o	Bias %
Reagent	6.00	6.68	1.03	0.53	+11.3
	8.00	8.64	1.10		+8.0
	16.0	17.4	2.25	0.77	+8.8
	20.0	21.4	2.31		+7.0
	100	101	1.91	3.76	+1.0
	140	143	5.52		+2.1
	800	819	24.3	12.7	+2.4
	960	966	18.5		+7.3
Waste	6.0	5.63	1.17	0.55	-6.2
	8.0	7.31	1.91		-8.6
	16.0	15.1	2.70	1.85	-5.6
	20.0	19.8	1.01		-1.0
	100	98.9	4.36	3.31	-1.1
	140	138	8.39		-1.4

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Water	Amount Added $\mu\text{g/L}$	Amount Found $\mu\text{g/L}$	S_t	S_o	Bias %
	800	796	60.6	27.1	-0.5
	960	944	72.1		-1.7

*Each Youden pair was used to calculate one laboratory data point (S_o).

TABLE 3500-Fe:I. SELECTION OF LIGHT PATH LENGTH FOR VARIOUS IRON CONCENTRATIONS

Fe μg		Light Path <i>cm</i>
50-mL Final Volume	100-mL Final Volume	
50–200	100–400	1
25–100	50–200	2
10–40	20–80	5
5–20	10–40	10

TABLE 3500-K:I. CONCENTRATION OF CATIONS INTERFERING AT VARIOUS CONCENTRATIONS OF POTASSIUM

Cation	Concentration Causing 10% Error <i>mg/L</i>		
	K conc = 1 mg/L	K conc = 10 mg/L	K conc = 100 mg/L
Cs^+	1.0	10	100
NH_4^+	2.7	27	270
Ti^+	31.4	314	3 140
Ag^+	2 765	27 650	276 500
Tris^+	3 105	31 050	310 500
Li^+	356	3 560	35 600
Na^+	1 179	11 790	117 900

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Cation	Concentration Causing 10% Error <i>mg/L</i>		
	K conc = 1 mg/L	K conc = 10 mg/L	K conc = 100 mg/L
H ⁺	3.6*	2.6*	1.6*

* pH.

TABLE 3500-V:1. CONCENTRATION AT WHICH VARIOUS IONS INTERFERE IN THE DETERMINATION OF VANADIUM

Ion	Concentration <i>mg/L</i>
Cr ⁶⁺	1.0
Co ²⁺	1.0
Cu ²⁺	0.05
Fe ²⁺	0.3
Fe ³⁺	0.5
Mo ⁶⁺	0.1
Ni ²⁺	3.0
Ag ⁺	2.0
U ⁶⁺	3.0
Br ⁻	0.1
Cl ⁻	100.0
I ⁻	0.001

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Endnotes

1 (Popup - Footnote)

* Note that these definitions are not the same as false-positive or false-negative instrument readings, where similar terms commonly are used by laboratory or field personnel to describe a fault in a single result; false-positive and false-negative decision errors are defined in the context of hypothesis testing, where the terms are defined with respect to the null hypothesis.

2 (Popup - Footnote)

* As CaCO_3 .

3 (Popup - Footnote)

* Pyrex or equivalent.

4 (Popup - Footnote)

† DQO-PRO, available (free) by downloading from American Chemical Society Division of Environmental Chemistry home page at <http://acs.environmental.duq.edu/acsenv/envchem.htm>, and also as part of the tutorial, Reliable Environmental Sampling and Analysis, Instant Reference Sources, Inc., <http://instantref.com/inst.ref.htm>.

5 (Popup - Footnote)

* Ascarite II, Fisher Scientific Co., or equivalent.

6 (Popup - Footnote)

*APPROVED BY STANDARD METHODS COMMITTEE, 1993.

7 (Popup - Footnote)

*APPROVED BY STANDARD METHODS COMMITTEE, 1993.

8 (Popup - Footnote)

* Celite No. 505, Manville Corp., or equivalent.

9 (Popup - Footnote)

* Fisher Electrophotometer or equivalent.

10 (Popup - Footnote)

† General Electric lamp No. 1719 (at 6 V) or equivalent.

11 (Popup - Footnote)

‡ General Electric photovoltaic cell, Type PV-1, or equivalent.

12 (Popup - Footnote)

§ Corning CS-3-107 (No. 1), CS-4-98 (No. 2), and CS-5-70 (No. 3), or equivalent.

13 (Popup - Footnote)

* Fisher Electrocolorimeter, Model 181, or equivalent.

14 (Popup - Footnote)

† Instrumental Colour Systems, Ltd., 7 Bucklebury Place, Upper Woolhampton, Berkshire RG7 5UD, England.

15 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1994.

16 (Popup - Footnote)

† Nephelometers that instrument manufacturers claim meet the design specifications of this method may not give the same reading for a given suspension, even when each instrument has

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been calibrated using the manufacturer's manual. This differential performance is especially important when measurements are made for regulatory purposes. Consult regulatory authorities when selecting a nephelometer to be used for making measurements that will be reported for regulatory purposes.

17 (Popup - Footnote)

* Nuclepore Corp., 7035 Commerce Circle, Pleasanton, CA, or equivalent.

18 (Popup - Footnote)

† AMCO-AEPA-1 Standard, Advanced Polymer Systems, 3696 Haven Ave., Redwood City, CA, or equivalent.

19 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

20 (Popup - Footnote)

* For approximate metric dimensions in centimeters multiply dimensions in inches by 2.54.

21 (Popup - Footnote)

† Nuchar WV-G, Westvaco, Covington, VA; Filtrasorb 200, Calgon Carbon Corporation, Pittsburgh, PA; or equivalent.

22 (Popup - Footnote)

‡ There are numerous methods of arranging and presenting samples for odor determinations. The methods offered here are practical and economical of time and personnel. If extensive tests are planned and statistical analysis of data is required, become familiar with the more accurate methods that have been used extensively by flavor and allied industries.⁵

23 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

24 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

25 (Popup - Footnote)

* Nochromix, Godax Laboratories, Inc., New York, NY, or equivalent.

26 (Popup - Footnote)

† Plastic cup, 7- or 8-ounce, Solo or equivalent, any color except clear.

27 (Popup - Footnote)

‡ Keebler Sea Toast or equivalent.

28 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

29 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

30 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

31 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

32 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

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33 (Popup - Footnote)

* The number of sample portions can be increased when working with samples of unknown demand and may be decreased when working with samples of familiar origin.

34 (Popup - Footnote)

† Size of sample portions is not critical, but must be large enough to ensure reproducible results as well as provide volume sufficient to measure chlorine residual, pH, and temperature.

35 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

36 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

37 (Popup - Footnote)

* Available from Standard Seawater Services, Institute of Oceanographic Services, Warmley, Godalming, Surrey, England.

38 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

39 (Popup - Footnote)

* Teflon or equivalent.

40 (Popup - Footnote)

† Whatman GF/C or equivalent.

41 (Popup - Footnote)

* Freon or equivalent.

42 (Popup - Footnote)

† Whatman No. 40 or equivalent.

43 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

44 (Popup - Footnote)

* Vycor, product of Corning Glass Works, Corning, N.Y., or equivalent.

45 (Popup - Footnote)

† Kimble Nos. 37005 or 37034B, or equivalent.

46 (Popup - Footnote)

‡ Class B or better.

47 (Popup - Footnote)

* Whatman grade 934AH; Gelman type A/E; Millipore type AP40; E-D Scientific Specialties grade 161; Environmental Express Pro Weigh; or other products that give demonstrably equivalent results. Practical filter diameters are 2.2 to 12.5 cm.

48 (Popup - Footnote)

† Gelman No. 4201 or equivalent.

49 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

50 (Popup - Footnote)

* Some commercial thermometers may be as much as 3°C in error.

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51 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

52 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

53 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

54 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

55 (Popup - Footnote)

* Venture Innovations, P.O. Box 53631, Lafayette, LA 70505; or Triton Electronics Ltd., Bigods Hall, Dunmow, Essex, England, CM63BE; or equivalent.

56 (Popup - Footnote)

† Available from CST apparatus supplier or use Whatman No. 17 chromatography grade paper cut into 7- × 9-cm sections with grain parallel to long side.

57 (Popup - Footnote)

* Whatman No. 1 or 2 or equivalent.

58 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

59 (Popup - Footnote)

* Gas chromatographic methods are extremely sensitive to the materials used. Use of trade names in *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped products that give *demonstrably* equivalent results.

60 (Popup - Footnote)

† Commercially available columns and gases they will separate include: Silica gel and activated alumina: Silica gel: H₂, air (O₂ + N₂), CO, CH₄, C₂H₆.

Activated alumina: air (O₂ + N₂), CH₄, C₂H₆, C₃H₈. Molecular sieves (zeolites):

Molecular sieve 5A: H₂, O₂ – Ar, N₂, CH₄, CO.

Molecular sieve 13X: O₂, N₂, CH₄, CO. Porous polymers:

Chromosorb 102: air (H₂, O₂, N₂, CO), CH₄, CO₂. Porapak Q: air - CO, CO₂.

HayeSep Q: H₂, air (O₂ + N₂), CH₄, CO₂, C₂H₆, H₂S. Carbon molecular sieves:

Carbosphere: O₂, N₂, CO, CH₄, CO₂, C₂H₆ (these gases can be eluted isothermally at various temperatures or by temperature programming).

Carbosieve S-II: H₂, air (O₂ + N₂), CO, CH₄, CO₂, C₂H₆ (temperature programming required).

61 (Popup - Footnote)

† Commercially available columns and gases they will separate include: Silica gel and activated alumina: Silica gel: H₂, air (O₂ + N₂), CO, CH₄, C₂H₆.

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Activated alumina: air ($O_2 + N_2$), CH_4 , C_2H_6 , C_3H_8 . Molecular sieves (zeolites):

Molecular sieve 5A: H_2 , O_2 – Ar, N_2 , CH_4 , CO.

Molecular sieve 13X: O_2 , N_2 , CH_4 , CO. Porous polymers:

Chromosorb 102: air (H_2 , O_2 , N_2 , CO), CH_4 , CO_2 . Porapak Q: air - CO, CO_2 .

HayeSep Q: H_2 , air ($O_2 + N_2$), CH_4 , CO_2 , C_2H_6 , H_2S . Carbon molecular sieves:

Carbosphere: O_2 , N_2 , CO, CH_4 , CO_2 , C_2H_6 (these gases can be eluted isothermally at various temperatures or by temperature programming).

Carbosieve S-II: H_2 , air ($O_2 + N_2$), CO, CH_4 , CO_2 , C_2H_6 (temperature programming required).

62 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1996.

63 (Popup - Footnote)

* Common Sensing, Clark Fork, ID; Eco Enterprises, Seattle, WA; Novatech, Vancouver, B.C., Canada; and Sweeney Aquametrics, Stony Creek, CT

64 (Popup - Footnote)

† Methods for these variables may be found in [Section 4500-O](#), 2550, and [Section 2520](#), respectively.

65 (Popup - Footnote)

* Ultrex, J.T. Baker, or equivalent.

66 (Popup - Footnote)

* Nochromix, Godax Laboratories, or equivalent.

67 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

68 (Popup - Footnote)

* Falcon tubes or equivalent.

69 (Popup - Footnote)

† Ultrex, Optima grade or equivalent.

70 (Popup - Footnote)

* Or equivalent.

71 (Popup - Footnote)

† Such as TFMTM or equivalent.

72 (Popup - Footnote)

‡ At temperatures greater than 80°C, but not boiling.

73 (Popup - Footnote)

§ Or equivalent.

74 (Popup - Footnote)

|| Or equivalent.

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75 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993

76 (Popup - Footnote)

* GFS Chemicals, Inc., Columbus, OH, Cat. No. 64, or equivalent.

77 (Popup - Footnote)

† Alpha Ventron, P.O. Box 299, 152 Andover St., Danvers, MA 01923, or equivalent.

78 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

79 (Popup - Footnote)

* Tygon or equivalent.

80 (Popup - Footnote)

† Use specially prepared reagents low in mercury.

81 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

82 (Popup - Footnote)

* Chelex 100, or equivalent, available from Bio-Rad Laboratories, Richmond, CA.

83 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

84 (Popup - Footnote)

* Dow Corning or equivalent.

85 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

86 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

87 (Popup - Footnote)

* Or equivalent.

88 (Popup - Footnote)

† Cambridge Isotope Laboratories or equivalent.

89 (Popup - Footnote)

‡ Performance data for the method were obtained with these concentrations.

90 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

91 (Popup - Footnote)

* Ultrex, Suprapur, Aristar, or equivalent.

92 (Popup - Footnote)

* Ultrex, Suprapur, Aristar, or equivalent.

93 (Popup - Footnote)

* Ultrex, Suprapur, Aristar, or equivalent.

94 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

95 (Popup - Footnote)

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* Arnold Hoffman & Co., Providence, RI.

96 (Popup - Footnote)

† K & K Laboratories, K & K Lab. Div., Life Sciences Group, Plainview, NY.

97 (Popup - Footnote)

‡ Pfaltz & Bauer, Inc., Stamford, CT.

98 (Popup - Footnote)

§ EM Science, Gibbstown, NJ.

99 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

100 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

101 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

102 (Popup - Footnote)

* IonPac NGL, Dionex, 4700 Lakeside Drive, Sunnyvale, CA 94086, or equivalent.

103 (Popup - Footnote)

† IonPac AS7, Dionex, or equivalent.

104 (Popup - Footnote)

‡ The multilaboratory precision and bias data cited in this method were the result of a collaborative study carried out jointly between U.S. EPA Environmental Monitoring Systems Laboratory (Cincinnati) and Committee D-19 of ASTM.

105 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

106 (Popup - Footnote)

* GFS Chemicals, Inc., Columbus, OH, or equivalent.

107 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

108 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

109 (Popup - Footnote)

* Whatman No. 42 or equivalent.

110 (Popup - Footnote)

† Whatman No. 541 or equivalent.

111 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

112 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

113 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

114 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

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115 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

116 (Popup - Footnote)

* Bio-Rad AG1-X8 or equivalent.

117 (Popup - Footnote)

† Bio-Rad Econo-Columns or equivalent.

118 (Popup - Footnote)

‡ Amberlite XAD-8, Supelco, or equivalent.

119 (Popup - Footnote)

* Bio-Rad AG1-X8 or equivalent.

120 (Popup - Footnote)

* Use Whatman No. 42 filter paper, or equivalent.

121 (Popup - Footnote)

* Bio-Rad glass Econo-Columns or equivalent.

122 (Popup - Footnote)

† Sep-Pak, Waters Associates, or equivalent.

123 (Popup - Footnote)

‡ Chelex 100 (ammonium form) Bio Rad, or equivalent.

124 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

125 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

126 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

127 (Popup - Footnote)

* Whatman No. 42 or equivalent.

128 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

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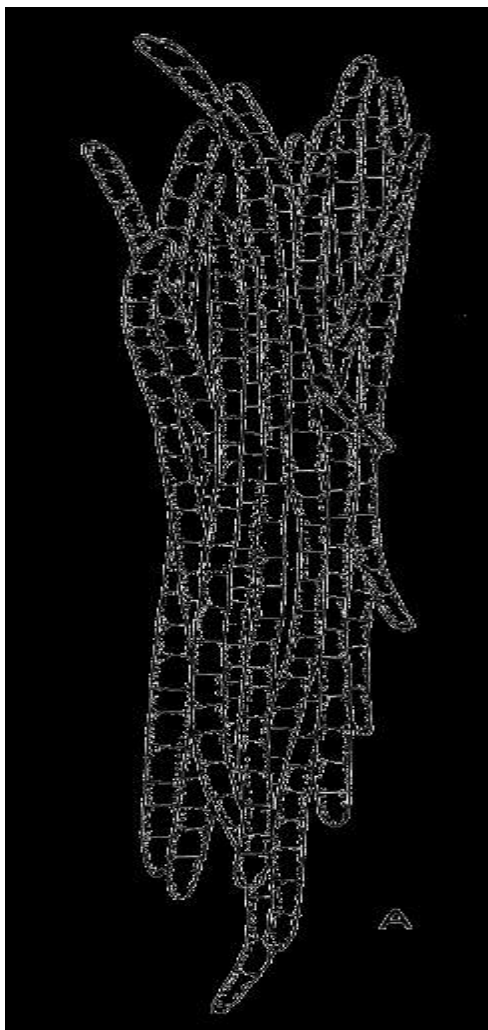
AQUATIC ORGANISMS PLATES

Plate 1A. Cyanobacteria and green algae. Dimensions refer to individual cells or filaments.
All organisms inhabit fresh water.

Cyanobacteria (Phylum Cyanophyta):

A

Aphanizomenon, aggregate of
filaments (3–6
 μm)



B

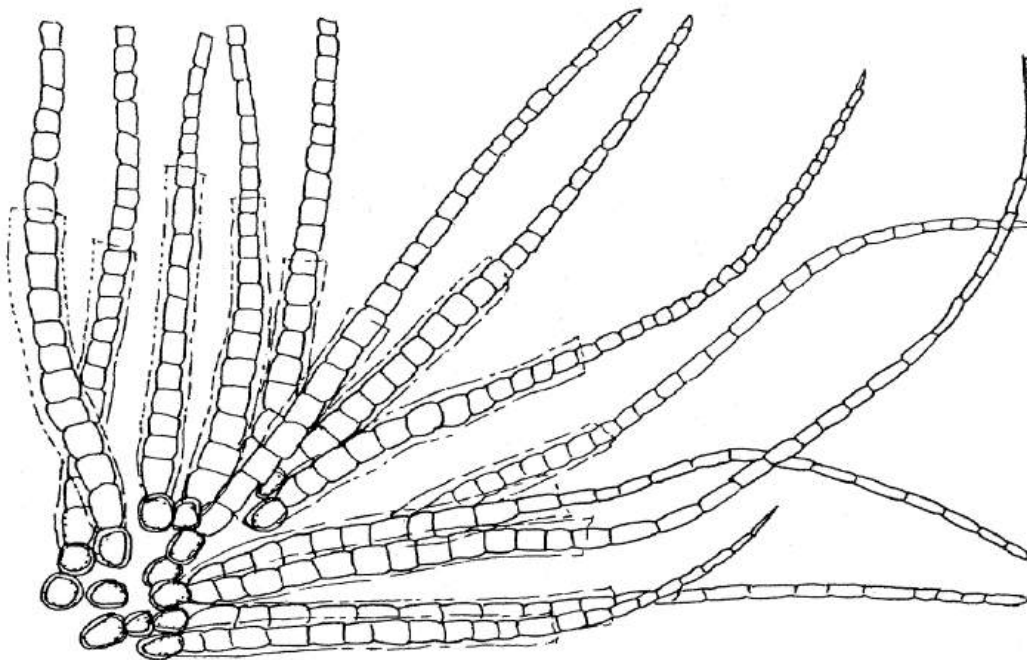
Aphanizomenon, detail

Standard Methods for the Examination of Water and Wastewater



C

Gloeotrichia, portion of colony
(cells 7–9 μm diameter)

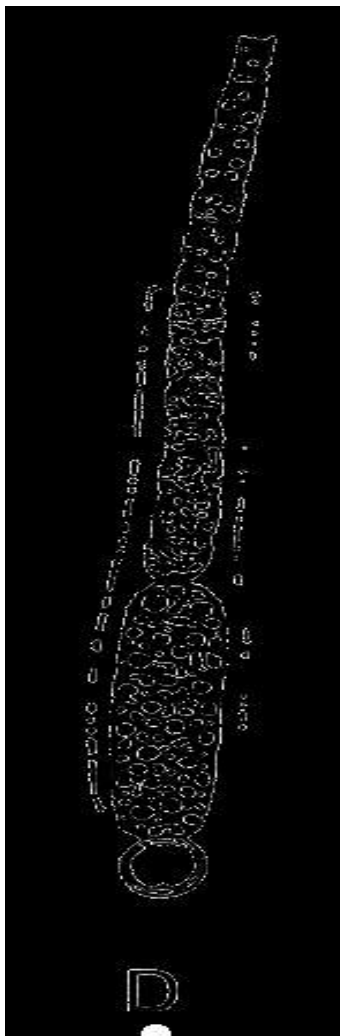


C

Standard Methods for the Examination of Water and Wastewater

D

Gloeotrichia, detail

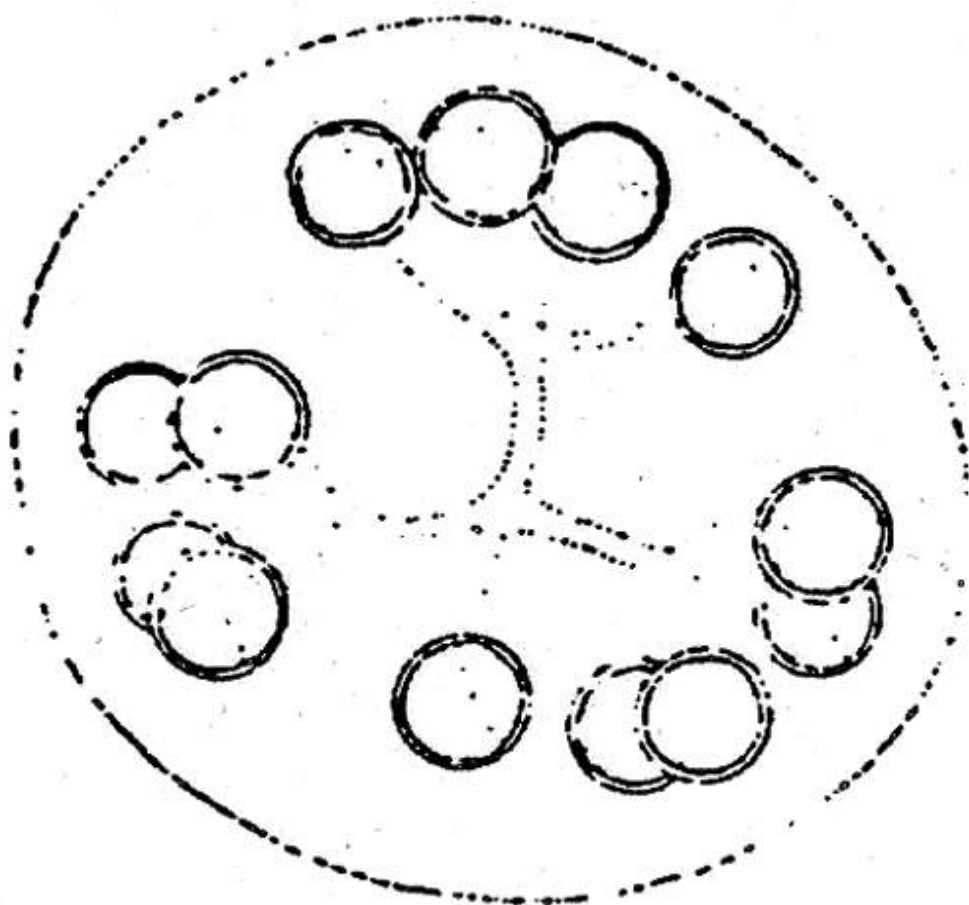


Green algae (Phylum Chlorophyta):

E

Dictyosphaerium
(8–14 μm)

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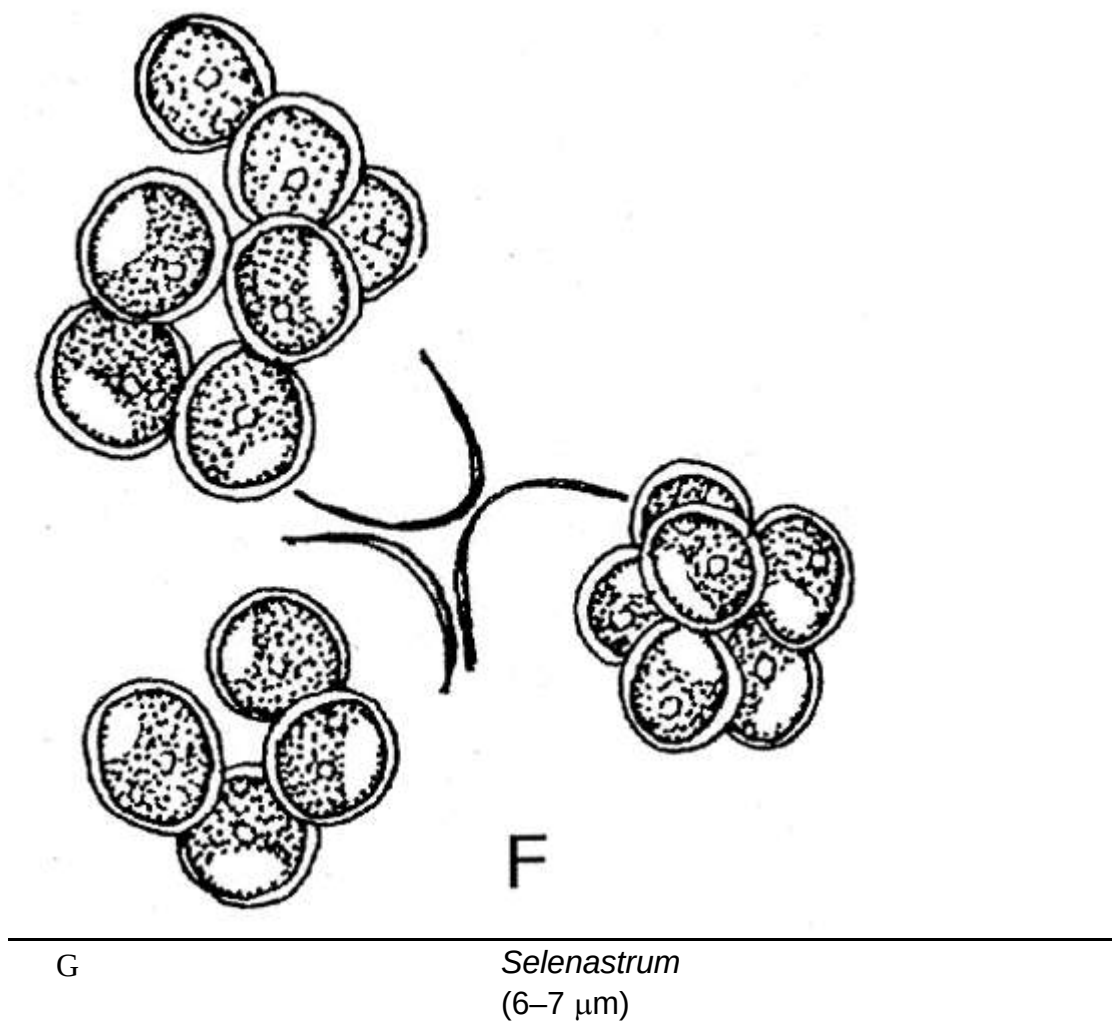


E

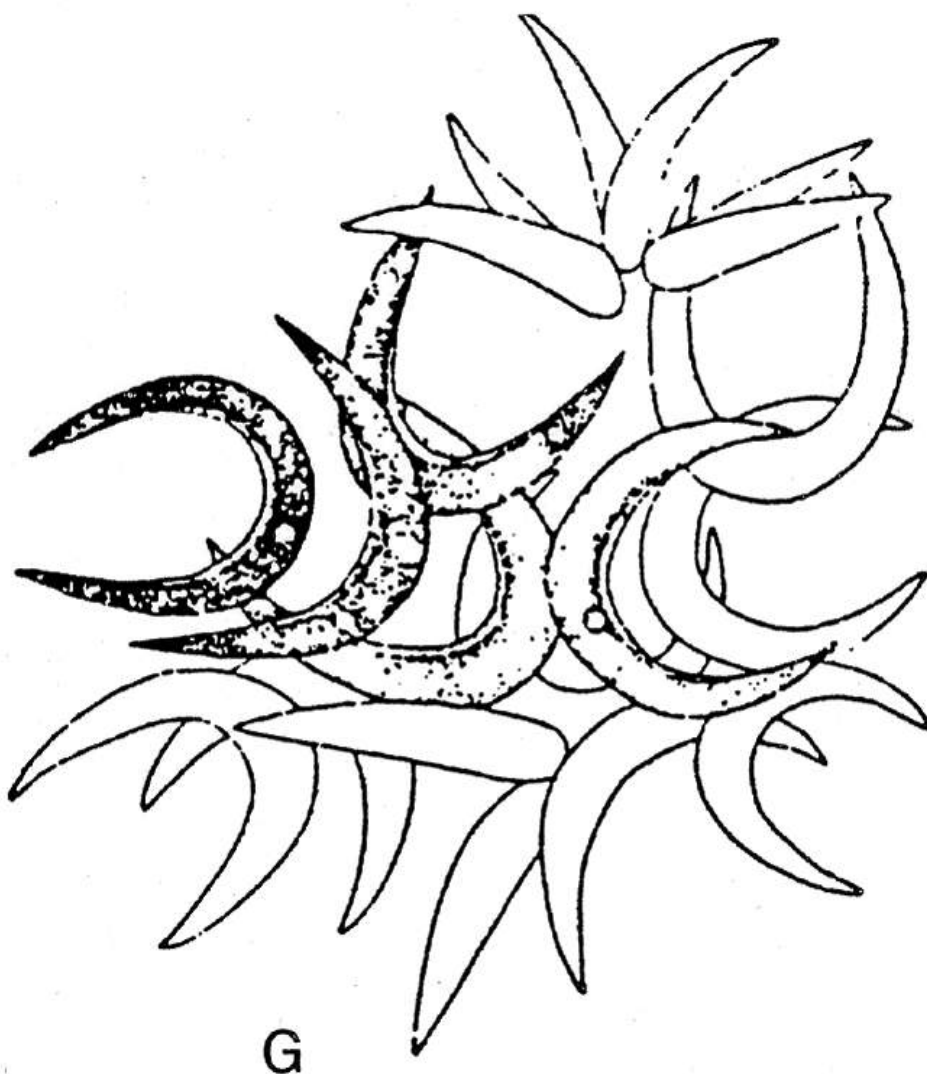
F

Westella
(5–7 μm)

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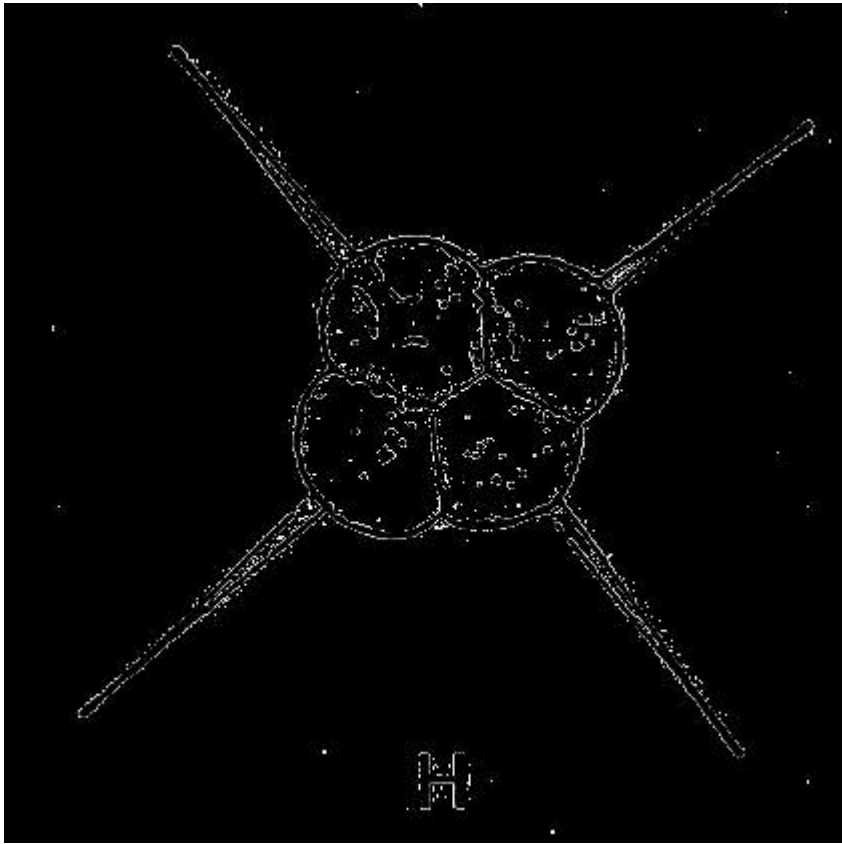
Standard Methods for the Examination of Water and Wastewater



H

Tetrastrum
(5–9 μm)

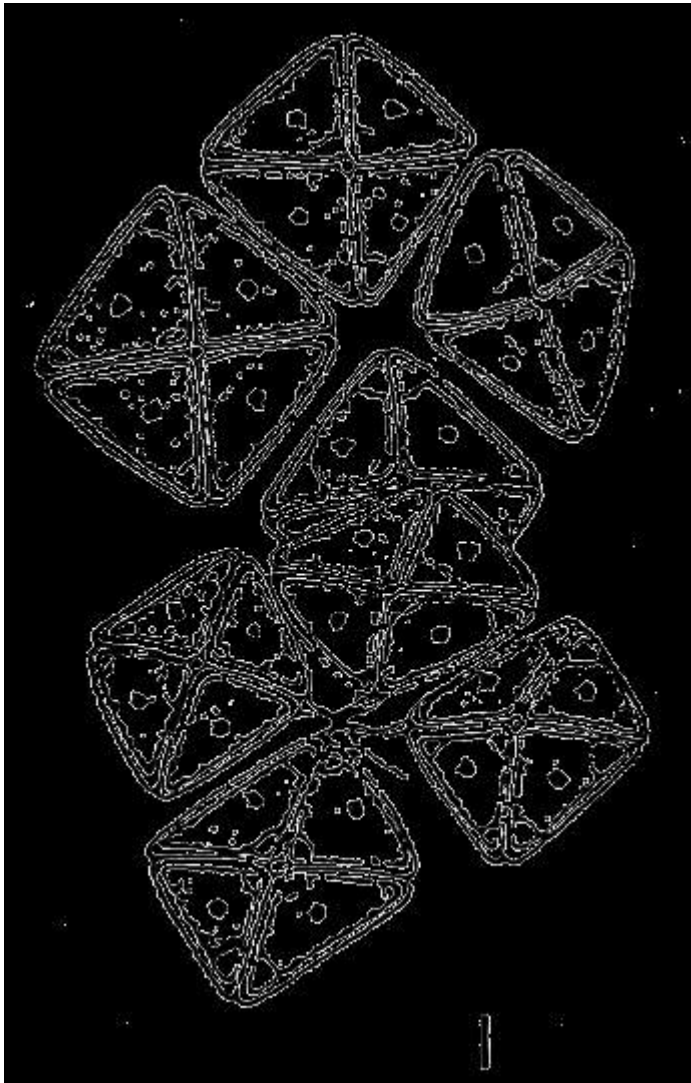
Standard Methods for the Examination of Water and Wastewater



I

Crucigenia
(5–8 μm)

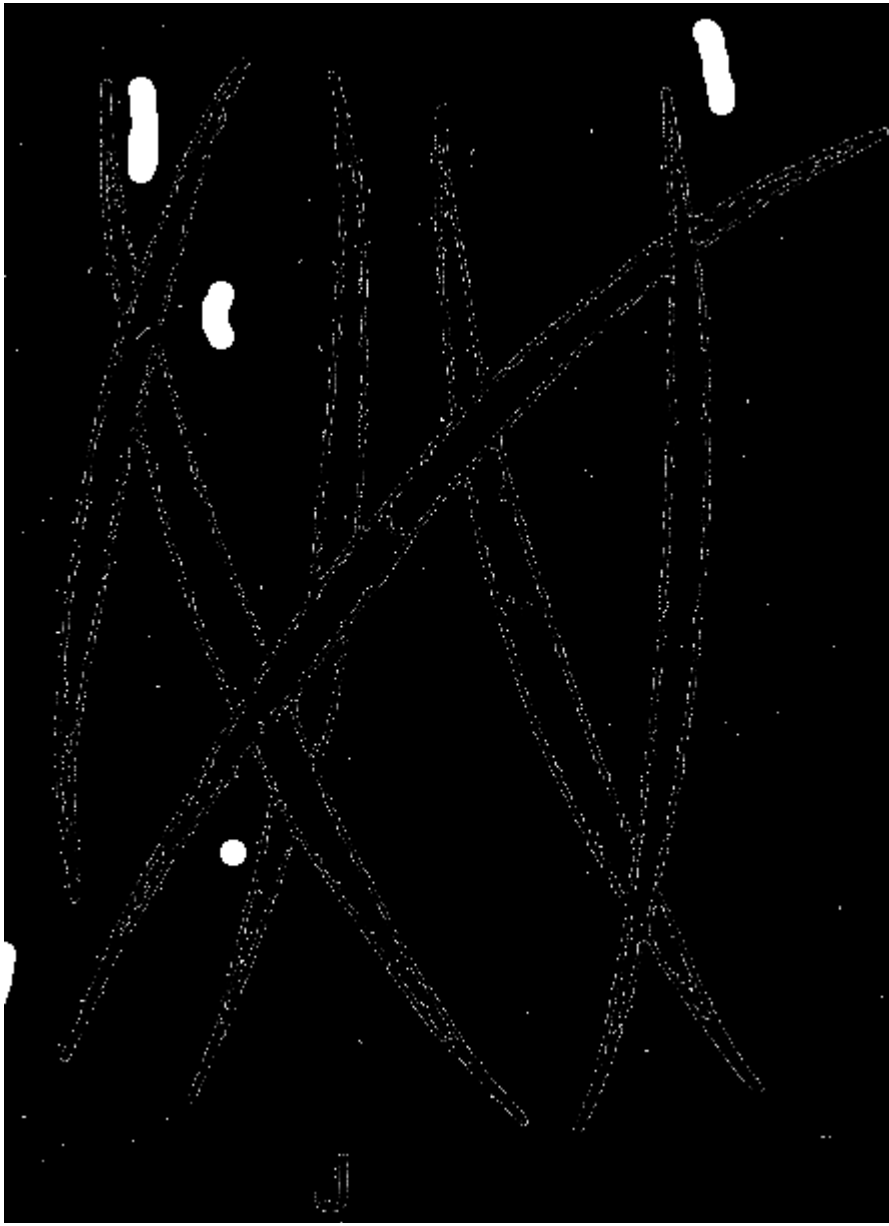
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J

Ankistrodesmus
(2–3 μm)

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K

Schizomeris(12–18 μm)

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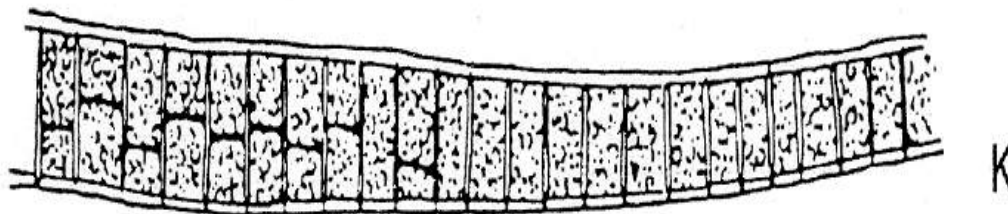


Plate 1B. Yellow-green, green, and golden-brown algae. Dimensions refer to individual cells or filaments. All organisms inhabit fresh water.

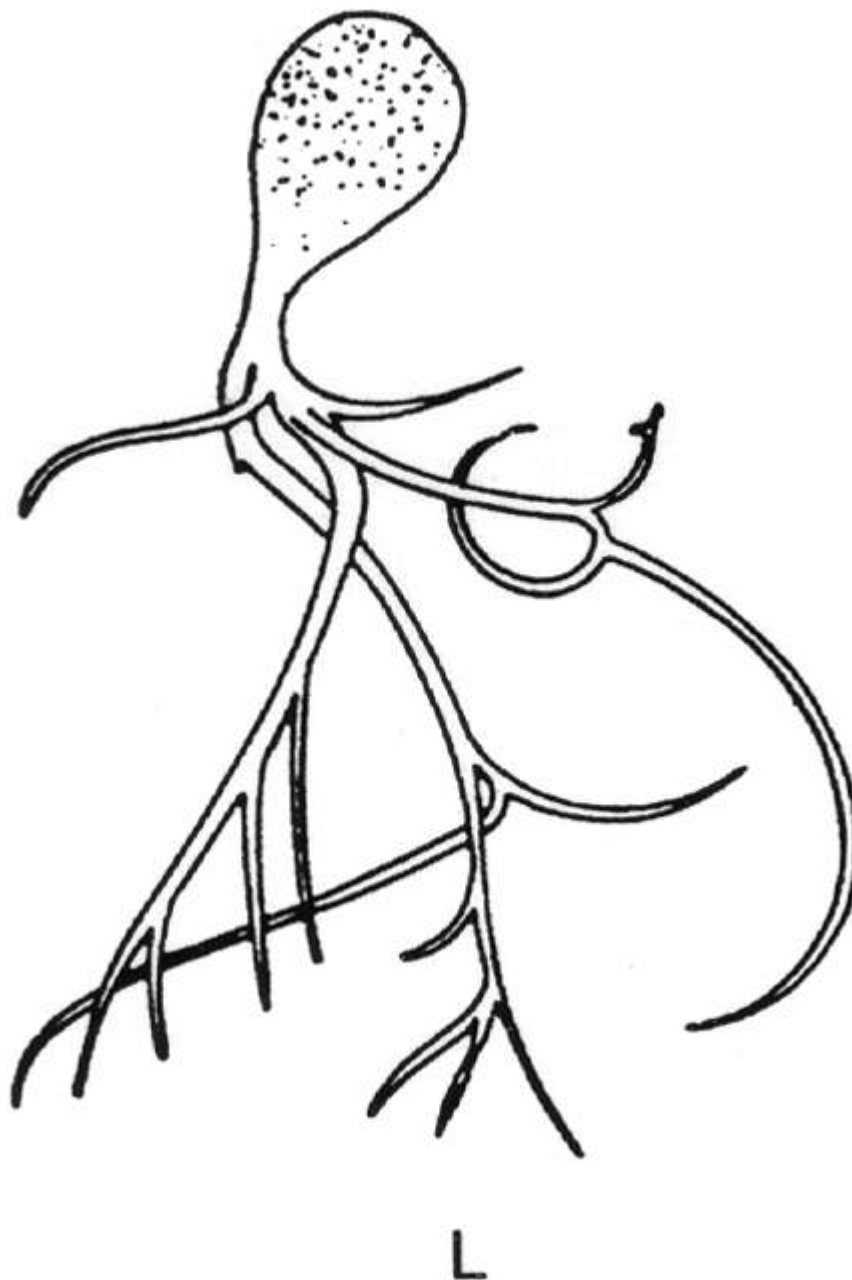
Yellow-green algae (Phylum Xanthophyta):

L

Botrydium

(1–2 μm)

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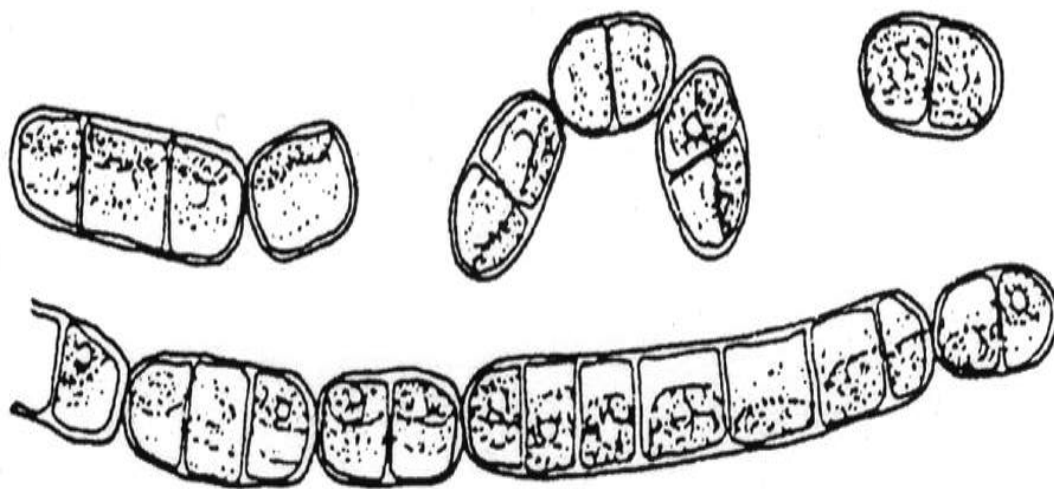


Green algae (Phylum Chlorophyta):

M

Stichococcus (3 μm)

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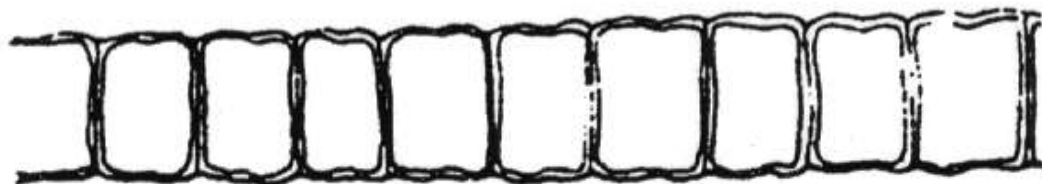


M

N

Hyalotheca

(12–30
μm)



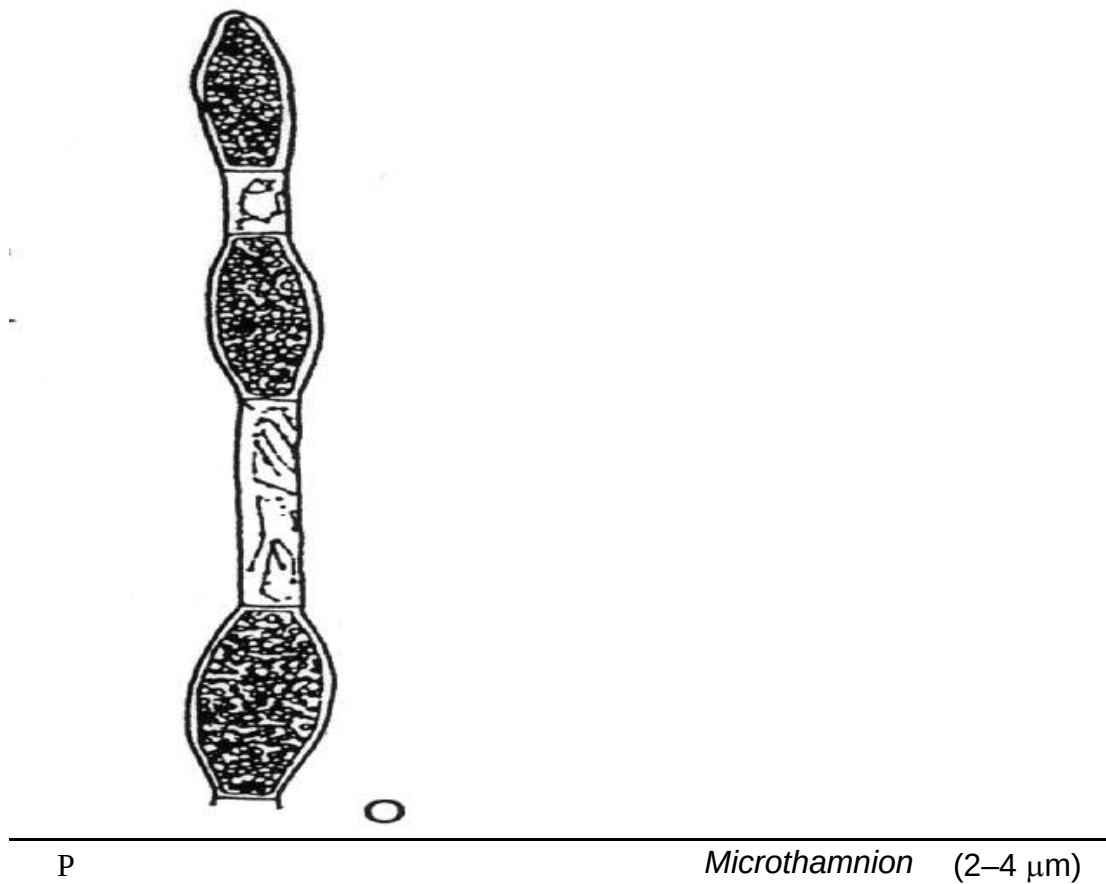
N

O

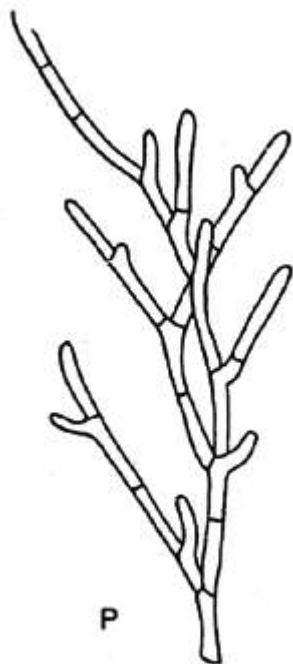
Pithopora

(50–100
μm)

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Q

Dichotomosiphon (50–100
μm)



Golden-brown algae (Phylum Chrysophyta) (diatoms):

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R

Cocconeis

(10 μm)



R

S

Achnanthes

(10 μm)



S

T

Cyclotella

(10 μm)

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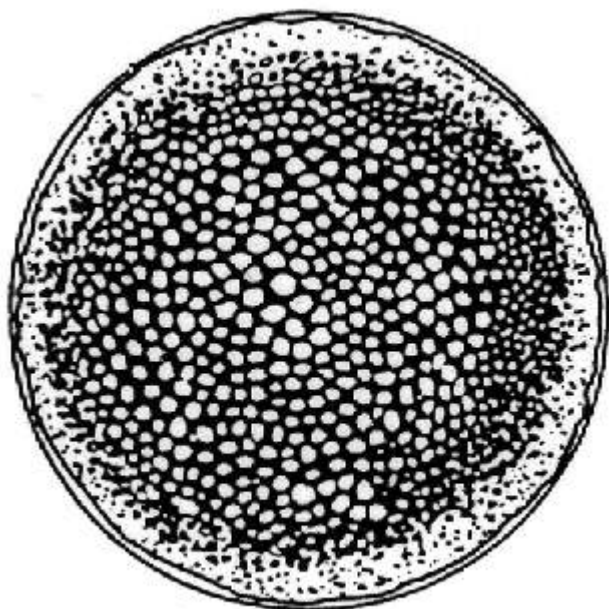


T



U

Coscinodiscus (120 μm)

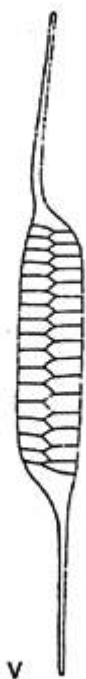


U

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V

Rhizosolenia (5–15 μm)



W

Skeletonema (5–15 μm)

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X

Biddulphia

(100 μm)

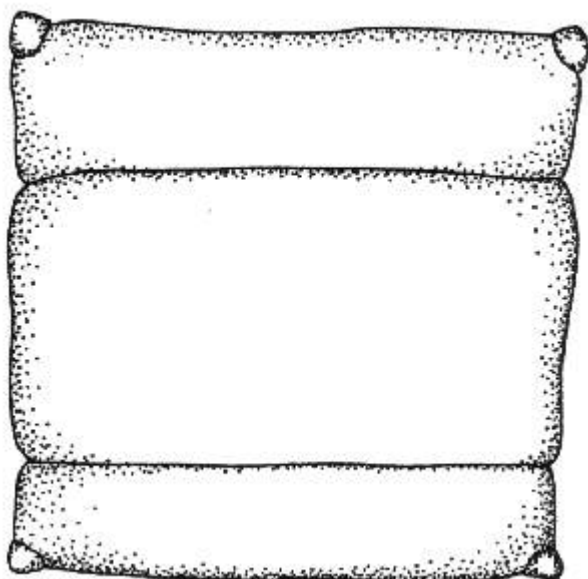


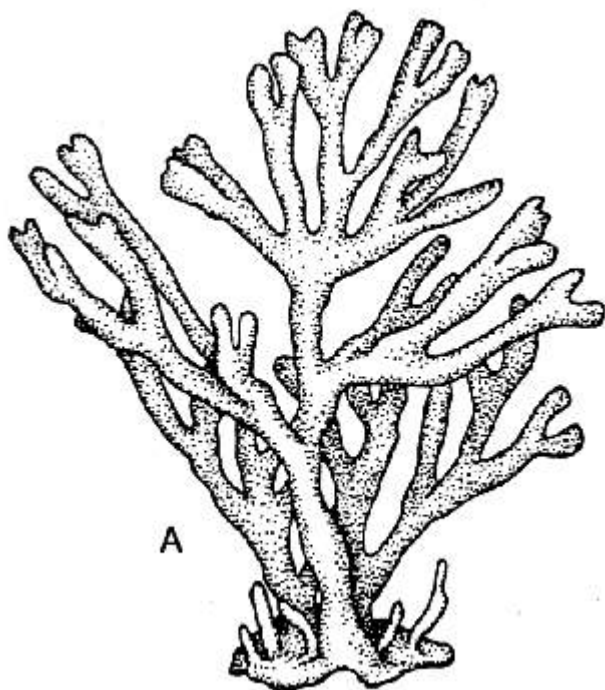
Plate 2A. Types of large marine algae.

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Green algae (Phylum Chlorophyta):

A

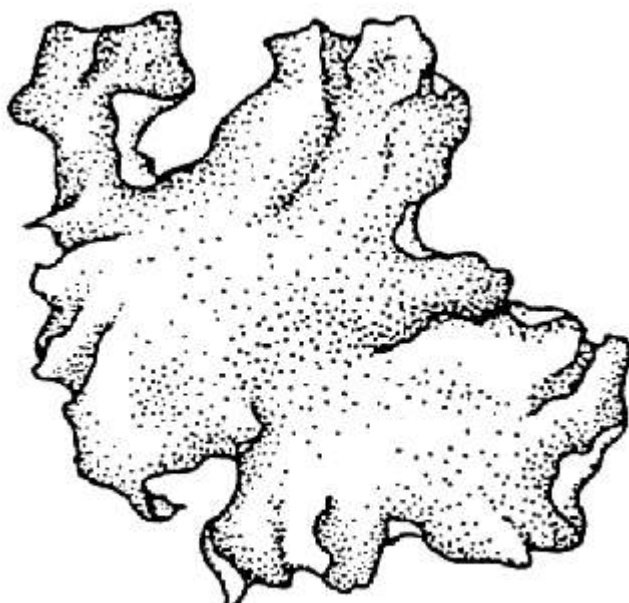
Sponge weed, *Codium* (30–40 cm)



B

Sea lettuce, *Ulva* (20 cm)

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B

C

Sargassum

(20–100
cm)



C

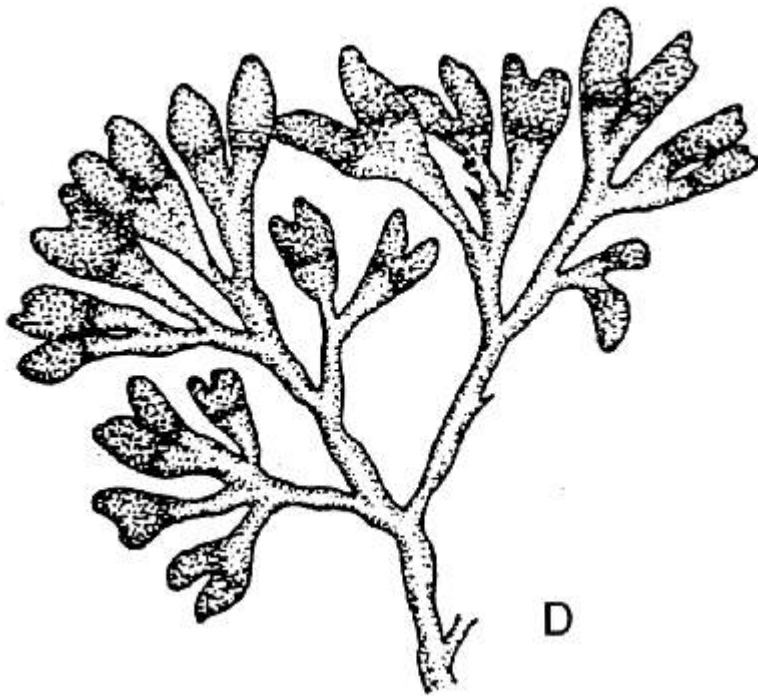
Brown algae (Phylum Phaeophyta):

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D

Rockweed, *Fucus*

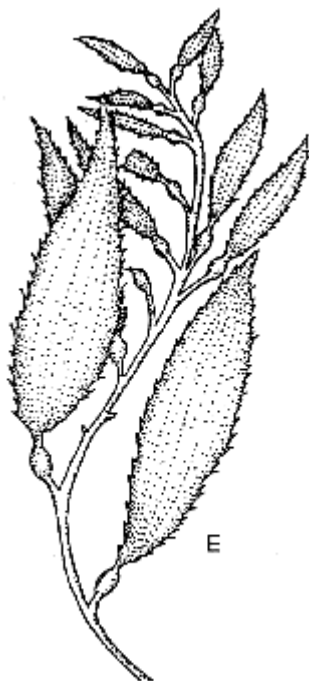
(75 cm)



E

Giant kelp, *Macrocystis* (30 m)

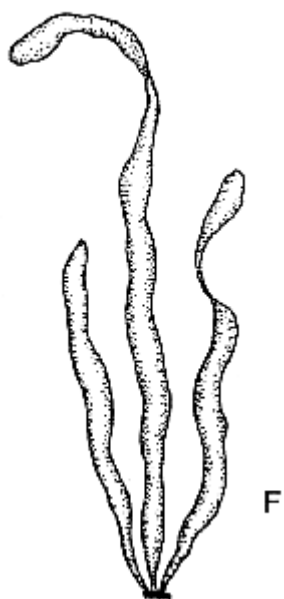
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F

Enteromorpha

(40 cm)



Standard Methods for the Examination of Water and Wastewater

G

Bull whip, *Nereocystis* (20 m)

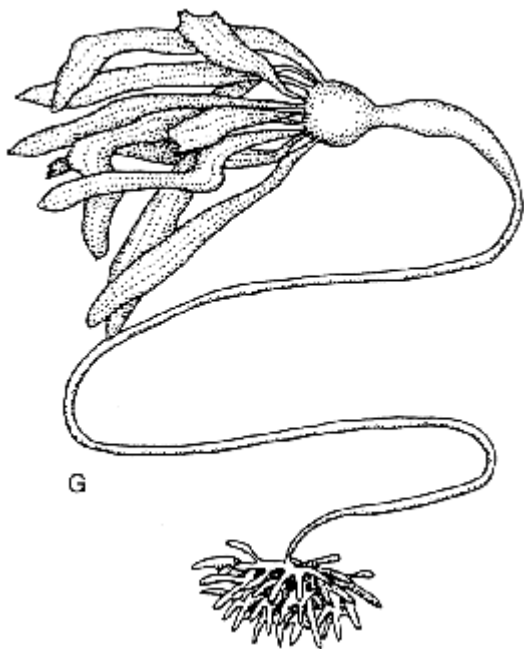


Plate 2B. Types of large marine algae and marine grasses:

Red algae (Phylum Rhodophyta):

H

Corallina

(4 cm)

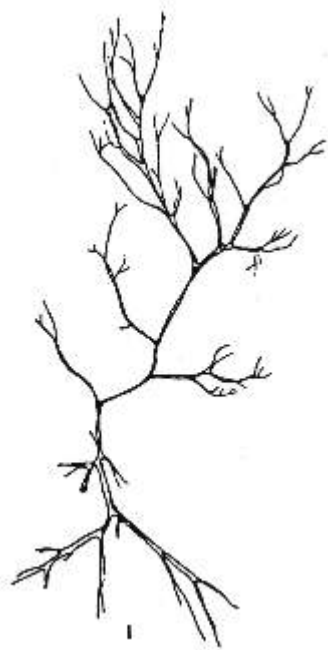
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I

Gracilaria

(50 cm)

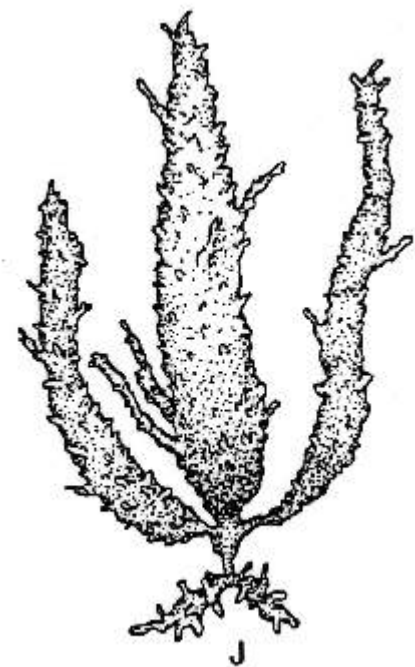


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J

Gigartina

(30–40
cm)



K

Plocamium

(30 cm)

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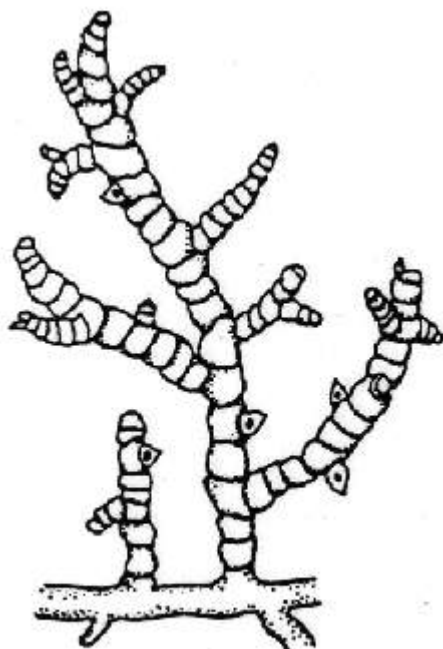


K

L

Champia

(1–6 cm)



L

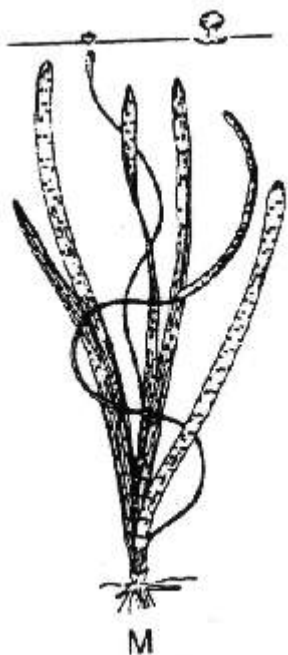
Marine grasses (Phylum Spermatophyta):

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M

Eelgrass, *Vallisneria* (50 cm)



N

Eelgrass, *Zostera* (45 cm)

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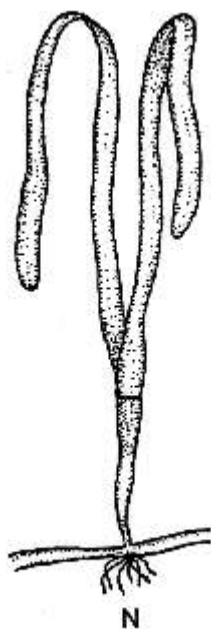


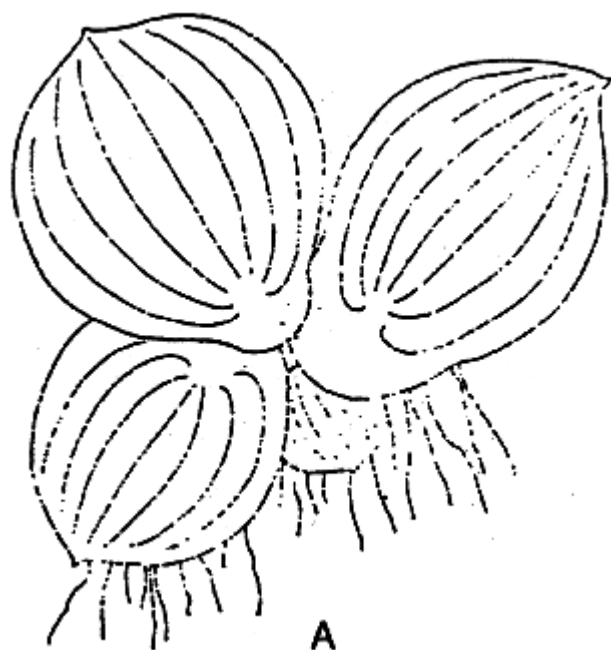
Plate 3A. Higher plants. All floating, freshwater inhabitants.

Phylum Spermatophyta:

A

Great duckweed, *Spirodela* (8 mm)

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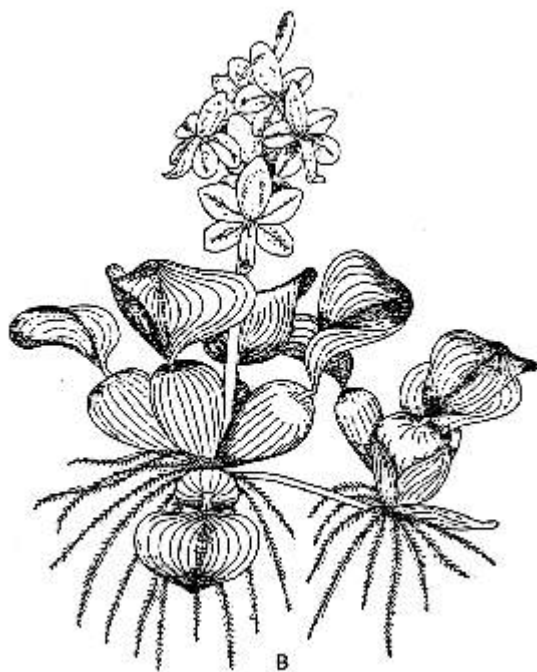


A

B

Water hyacinth, *Eichhornia*

(22 cm)



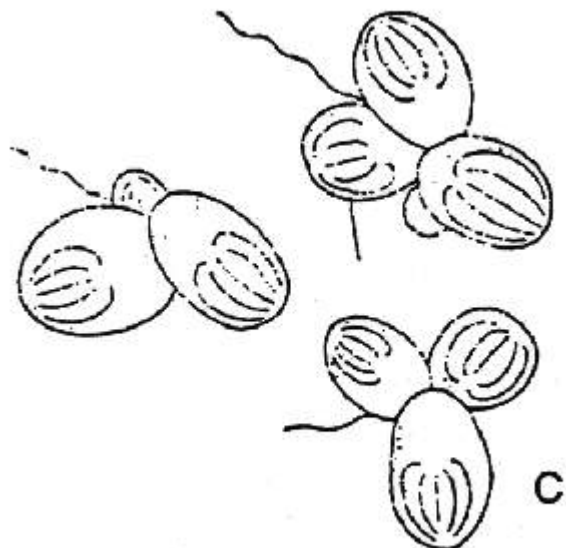
B

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C

Lesser duckweed, *Lemna*

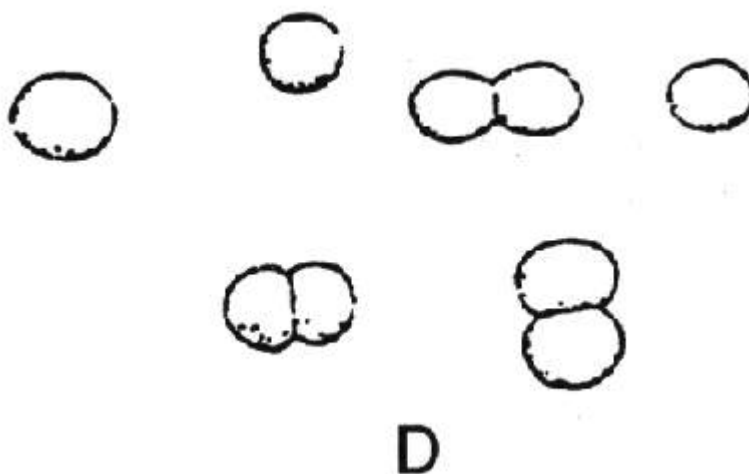
(5 cm)



D

Watermeal, *Wolffia*

(1–1.5 mm)



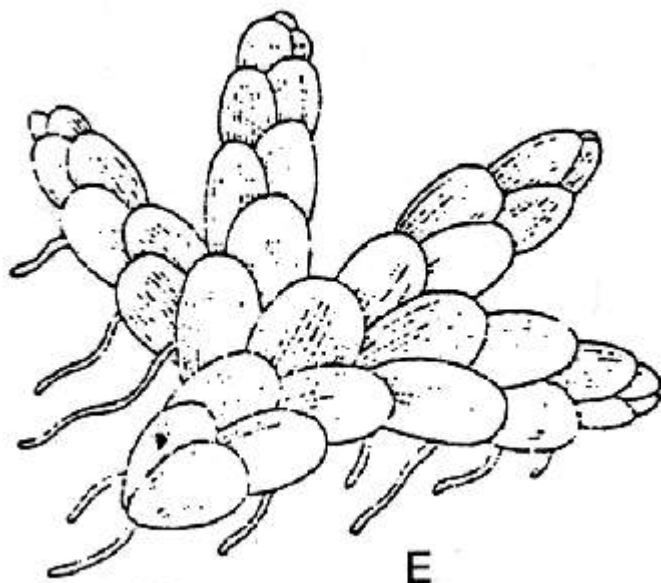
Phylum Pteridophyta:

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E

Water velvet, *Azolla*

(1 cm)



F

Water fern, *Salvinia*

(4 cm)

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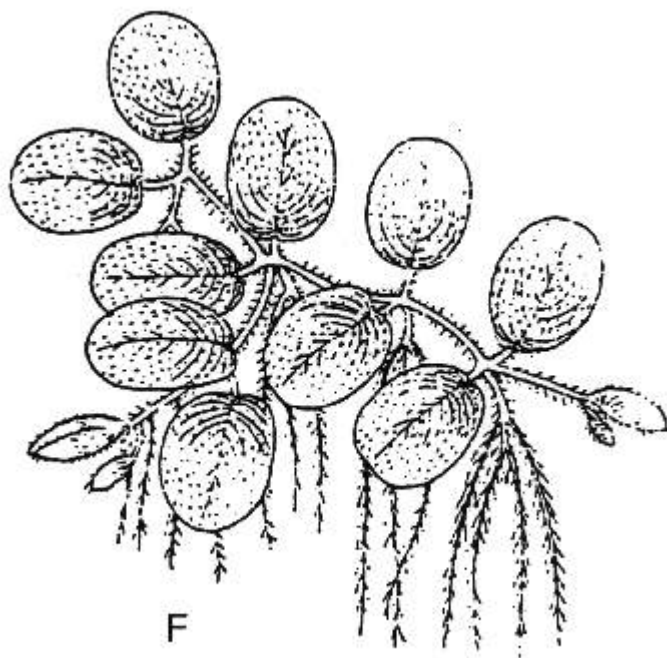


Plate 3B. Higher plants. All freshwater inhabitants (Phylum Spermatophyta).

Submerged:

G

Pondweed, *Potamogeton*

(30–60 cm)

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H

Coontail, *Ceratophyllum*

(30 cm)



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I

Waterweed, *Elodea*

(15 cm)

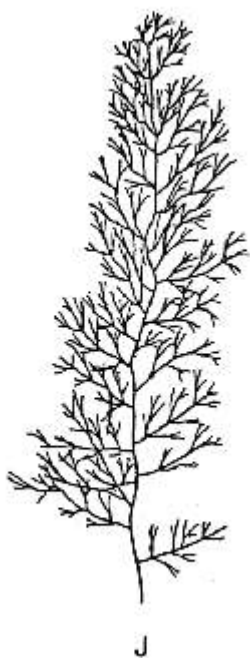


J

Water milfoil, *Myriophyllum*

(30 cm)

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K

Naiad, *Najas*

(60 cm)



Emersed:

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L

Pickereelweed, *Pontederia*

(60 cm)

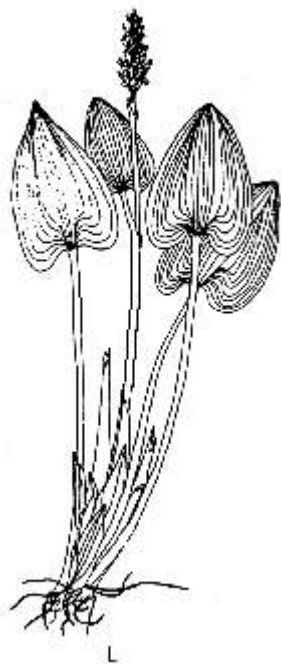


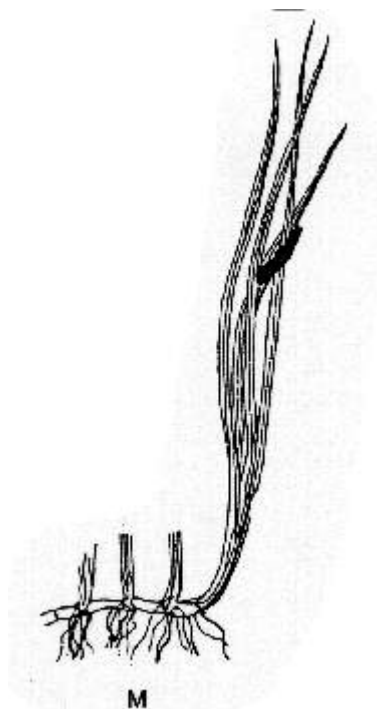
Plate 3C. Higher plants. All freshwater inhabitants (Phylum Spermatophyta), emersed.

M

Sweet flag, *Acorus*

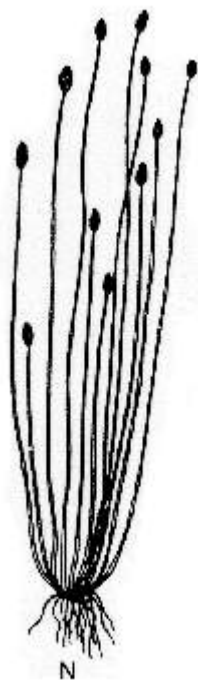
(30 cm)

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N Spike rush, *Eleocharis*

(30 cm)



O Cattail, *Typha*

(1–2 m)

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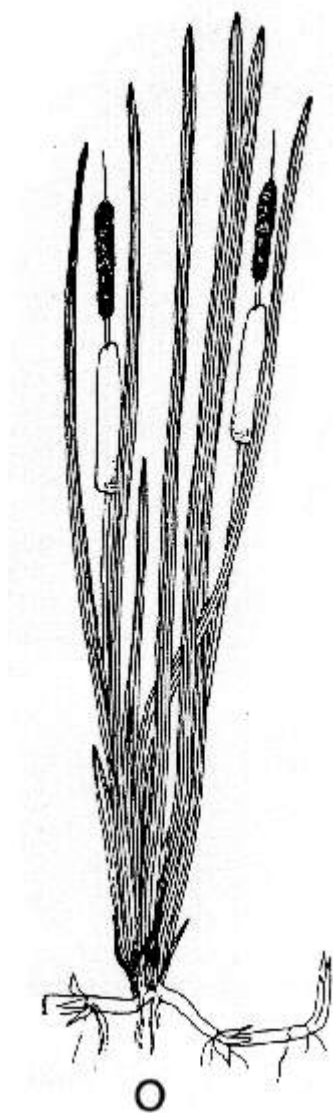


Plate 4A. Flagellates. Pigmented flagellates, various phyla. Freshwater inhabitants.

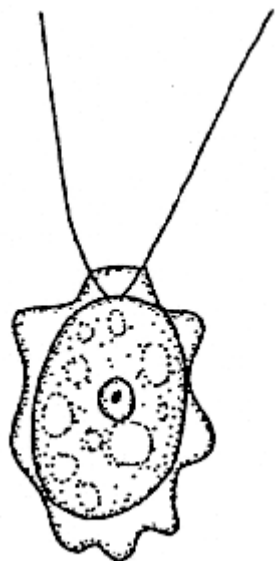
A *Pteromonas* (5–18 μm)

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B *Lobomonas*

(5–14 μm)

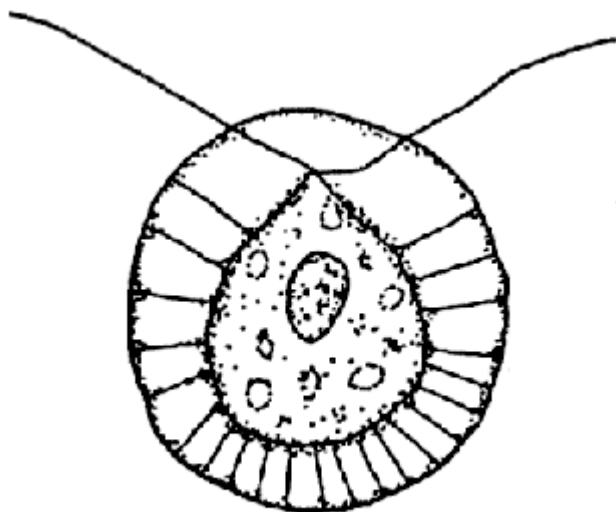


B

C *Haematococcus*

(40–45 μm)

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C

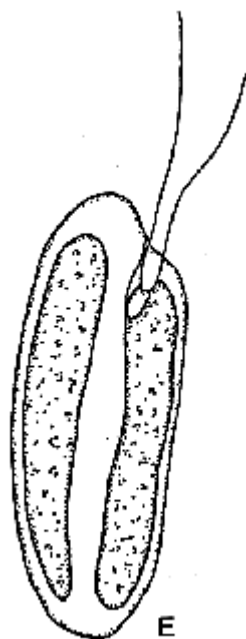
D *Chromulina* (4–10 μm)



D

E *Cryptomonas* (6–12 μm)

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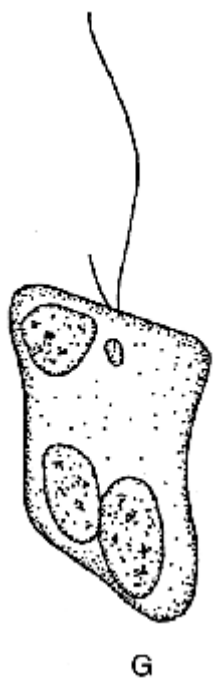


F *Ochromonas* (7–14 μm)

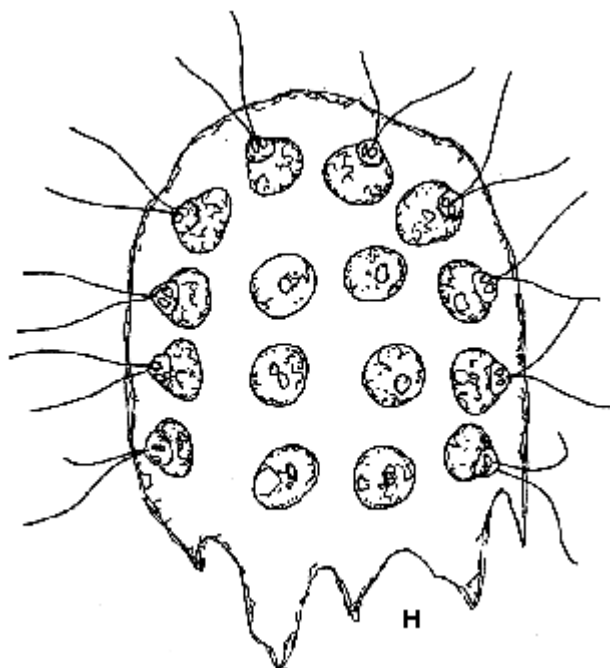


G *Chloramoeba* (10–15 μm)

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H *Platydorina* (66–70 μm , colony)



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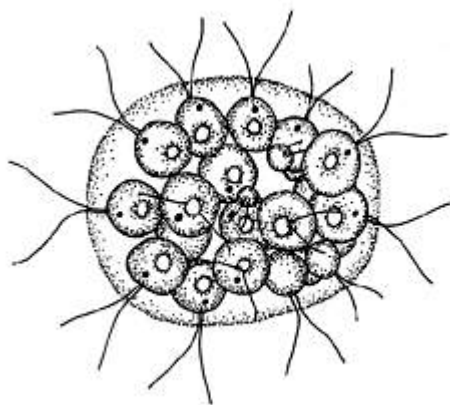
Plate 4B. Flagellates. Freshwater inhabitants except as noted.

Pigmented (various phyla):

I

Pleodorina

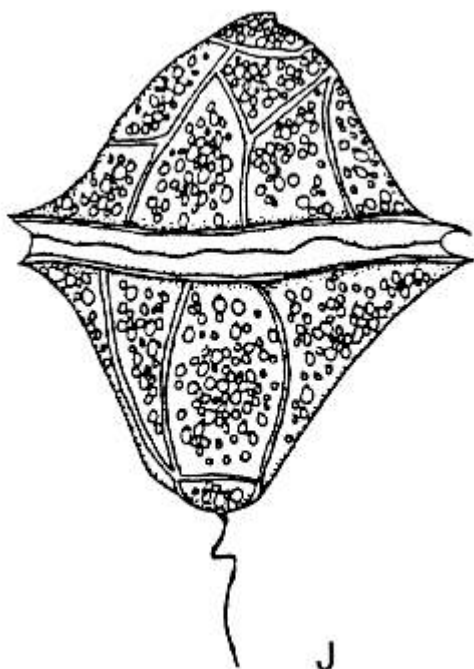
(8–10 μm)



J

Gonyaulax (marine)

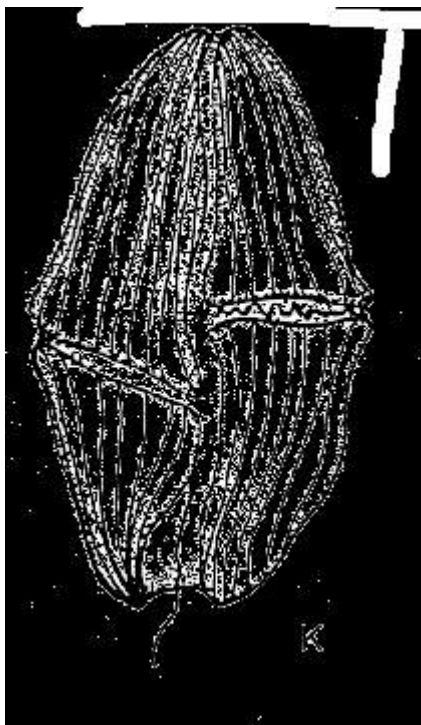
(10 μm)



K

Gymnodinium (marine) (10 μm)

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Nonpigmented (Phylum Protozoa):

L

Astasia

(40–50 μm)



M

Bodo

(11–22 μm)

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N

Dinomonas

(15–16 μm)



O

Oikomonas

(5–20 μm)



P

Anisonema

(14–60 μm)

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Q

Cercomonas

(10–36 μm)



R

Tetramitus

(11–30 μm)



S

Dendromonas

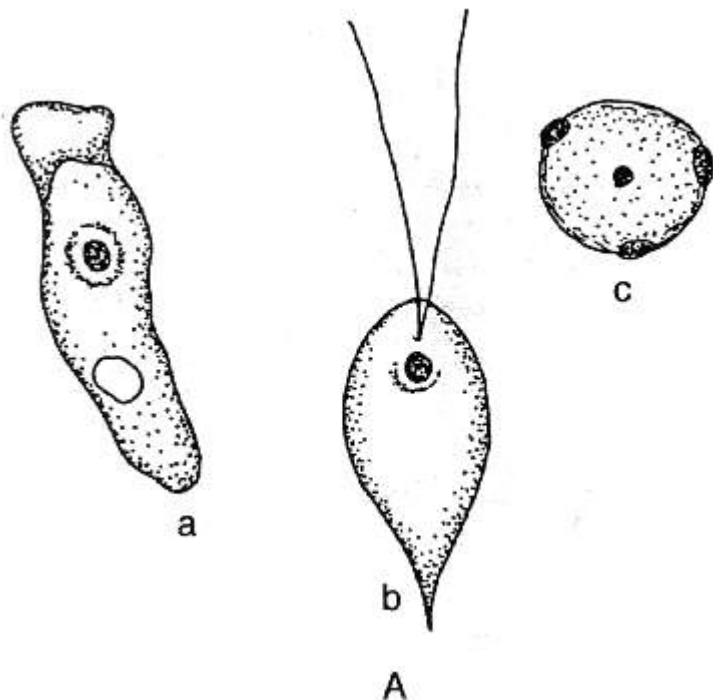
(8 μm)

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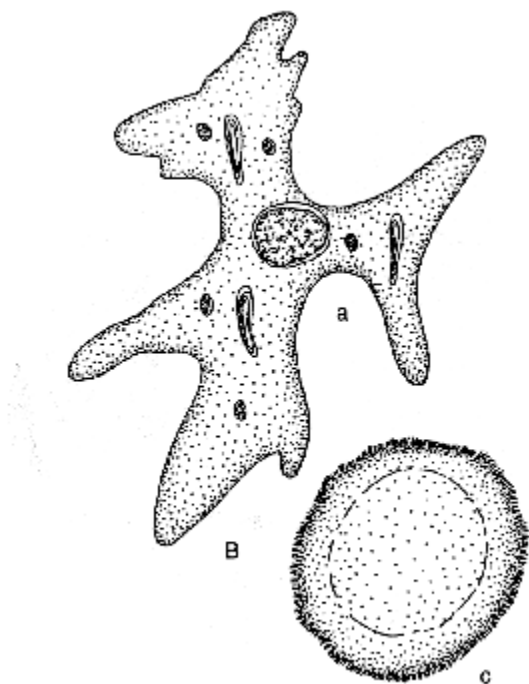
Plate 5A. Amoebas (Phylum Protozoa).

A *Naegleria*, (a) amoeboid stage, (b) flagellated stage, (c) cyst stage (10–36 μm)

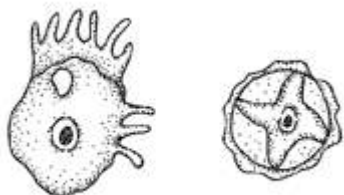


B *Amoeba*, (a) amoeboid state, (c) cyst stage (30–600 μm)

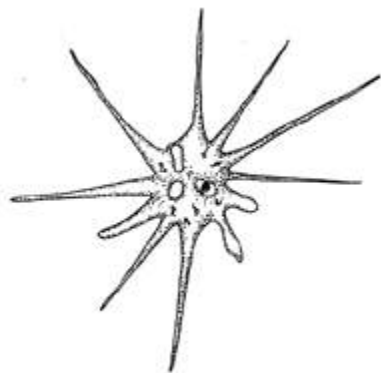
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C *Acanthamoeba* (*Hartmannella*), (a) amoeboid stage, (c) cyst stage (15–25 μm)

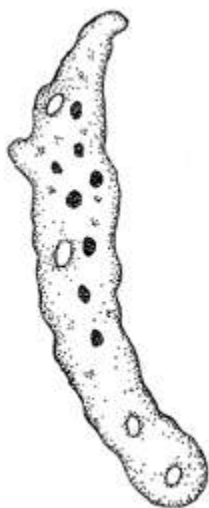


D *Amoeba radiosa* (30–120 μm)



E *Pelomyxa* (0.25–3 mm)

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F *Diffugia*

(40 μm)



G *Actinophrys*

(25–50 μm)

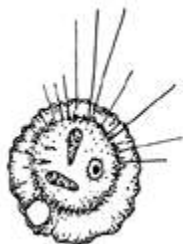


Plate 5B. Amoebas and nonpigmented flagellates (Phylum Protozoa).

Amoebas:

H

Arcella (side view)

(30–260 μm)

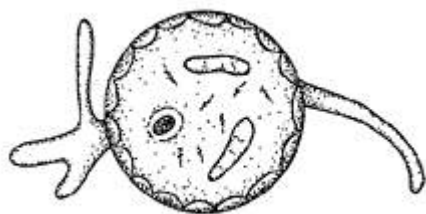
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I

Arcella (top view)

(30–260 μm)



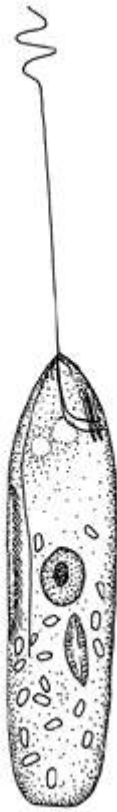
Nonpigmented flagellates:

J

Peranema

(40–70 μm)

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K

Mastigamoeba

(28–200 μm)

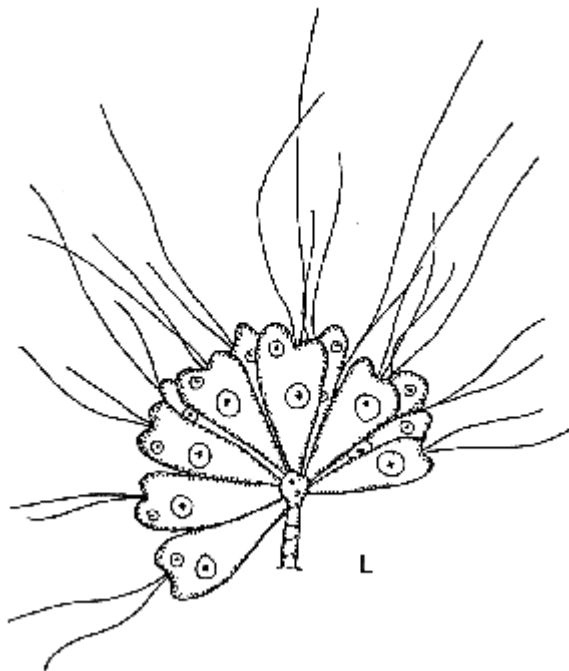
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L

Anthophysa

(5–6 μm)



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M

Monas

(5–16 μm)



Plate 6. Ciliates (Phylum Protozoa).

A

Lionotus

(100 μm)

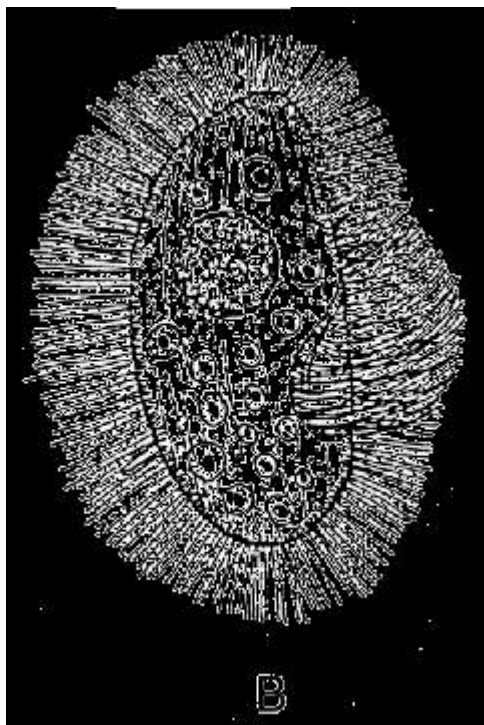


B

Pleuronema

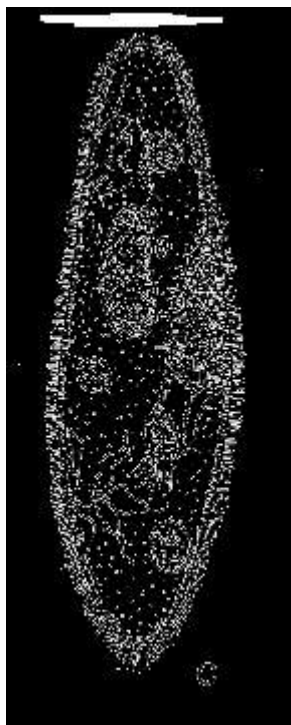
(38–120 μm)

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C *Paramecium*

(50–330 μm)



D *Colpoda*

(12–110 μm)

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E

Stylonychia

(100–300 μm)

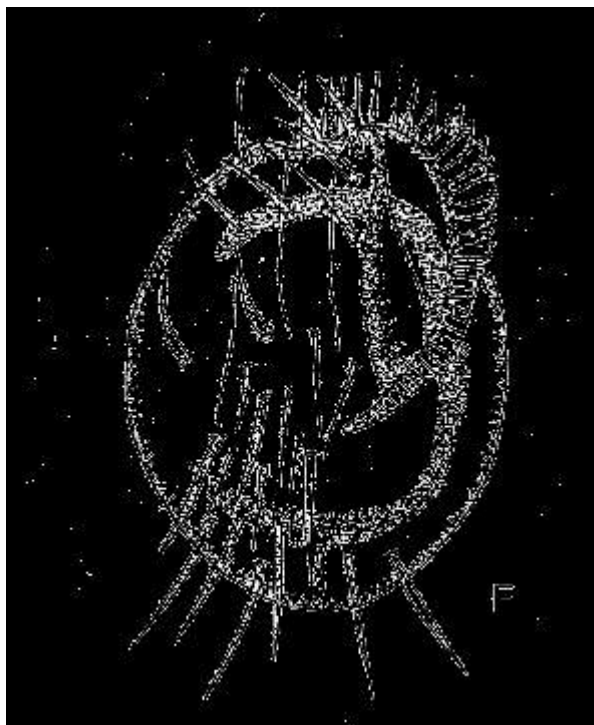


F

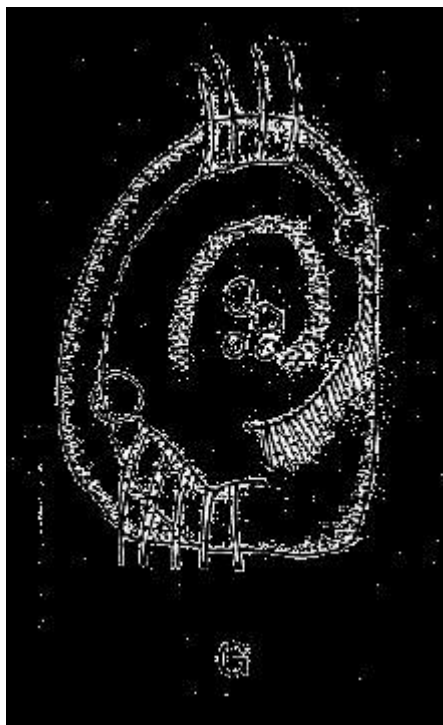
Euplotes

(70–195 μm)

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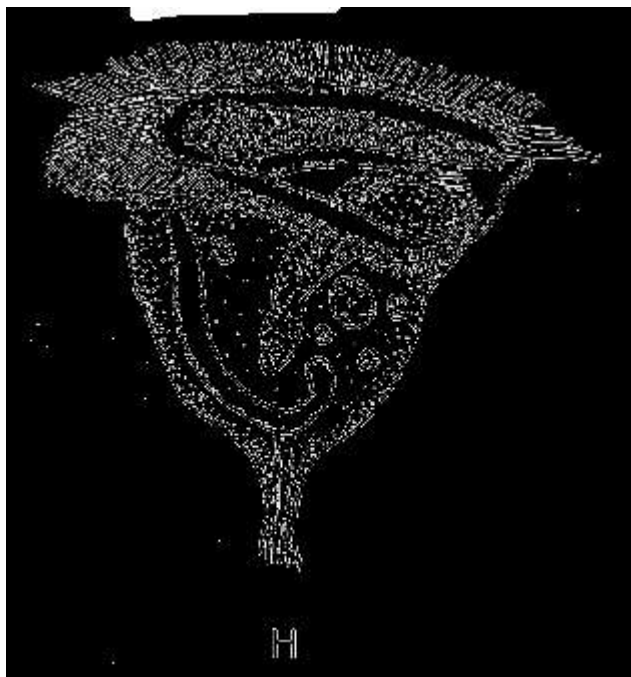


G *Aspidisca* (30–50 μm)



H *Vorticella* (40–175 μm)

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I *Tintinnidium*

(40–200 μm)

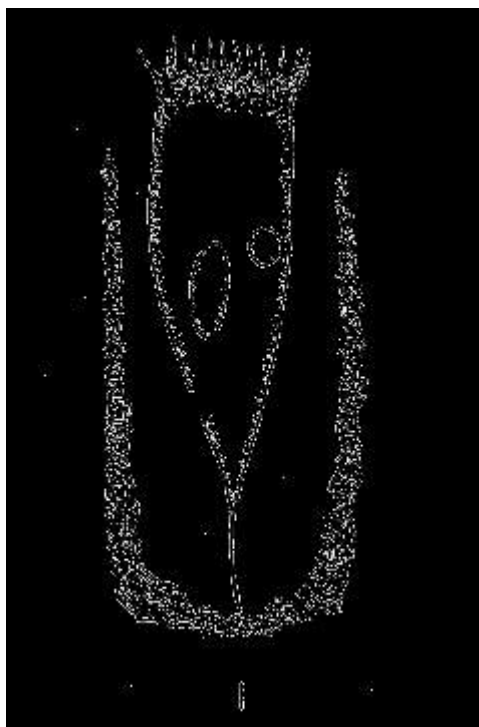


Plate 7. Sponges and coelenterates.

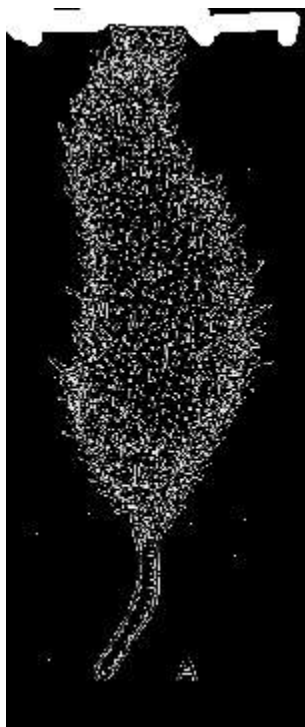
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Sponges (Phylum Porifera):

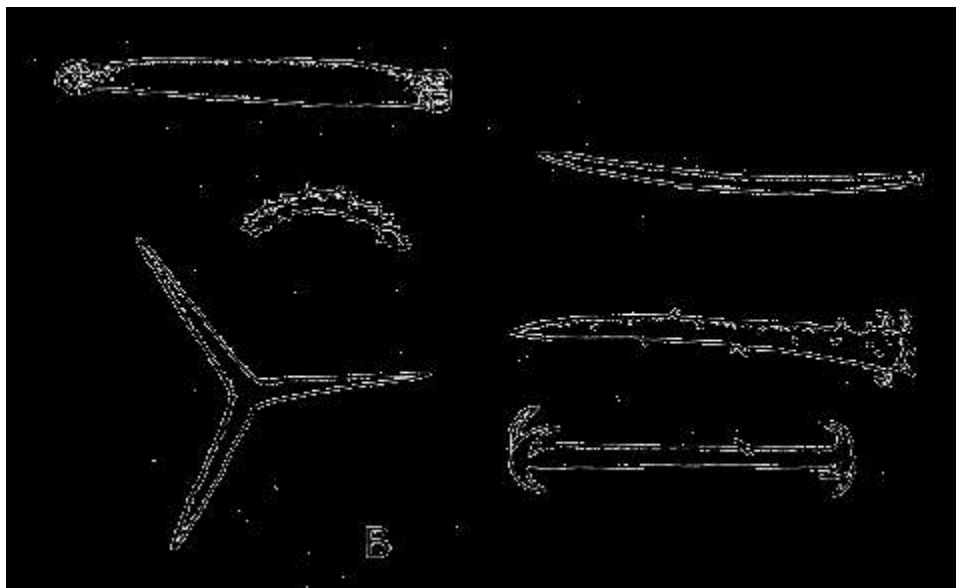
A

Vase sponge, marine



B

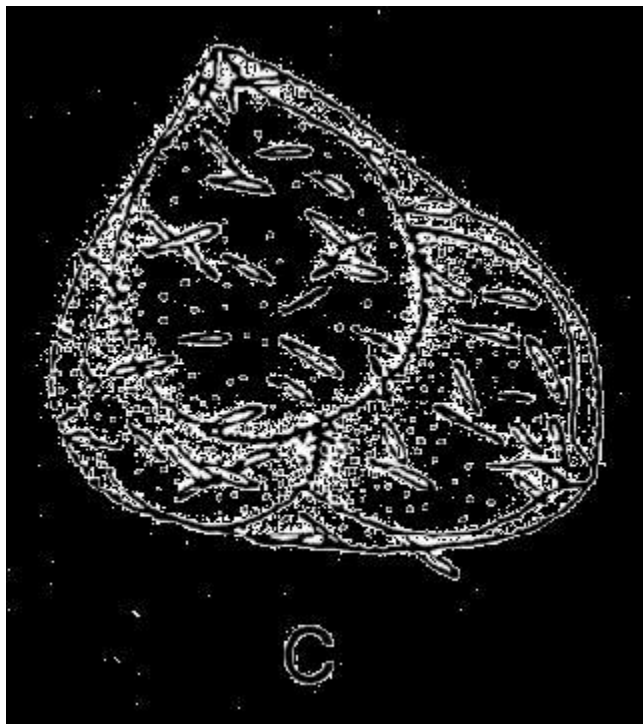
Sponge spicule types



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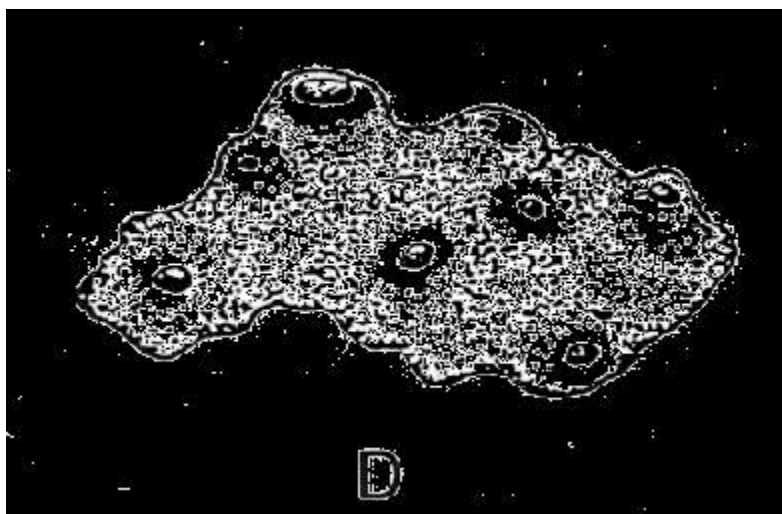
C

Trochospongilla, 3 gemmules, freshwater



D

Purple sponge, *Haliclona*, marine

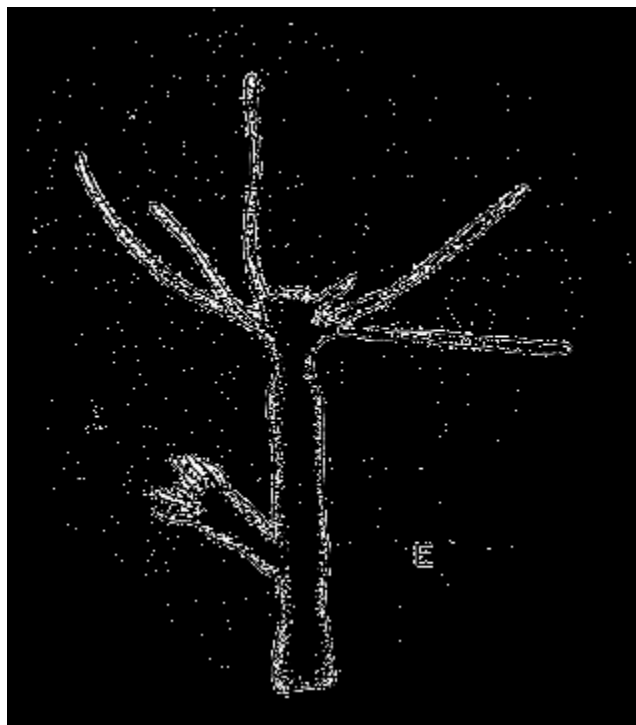


Coelenterates (Phylum Cnidaria):

E

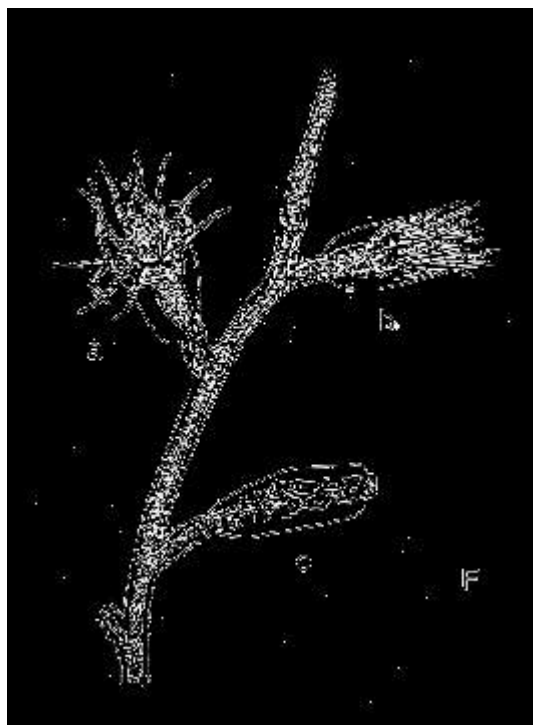
Hydra extended with bud, freshwater

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F

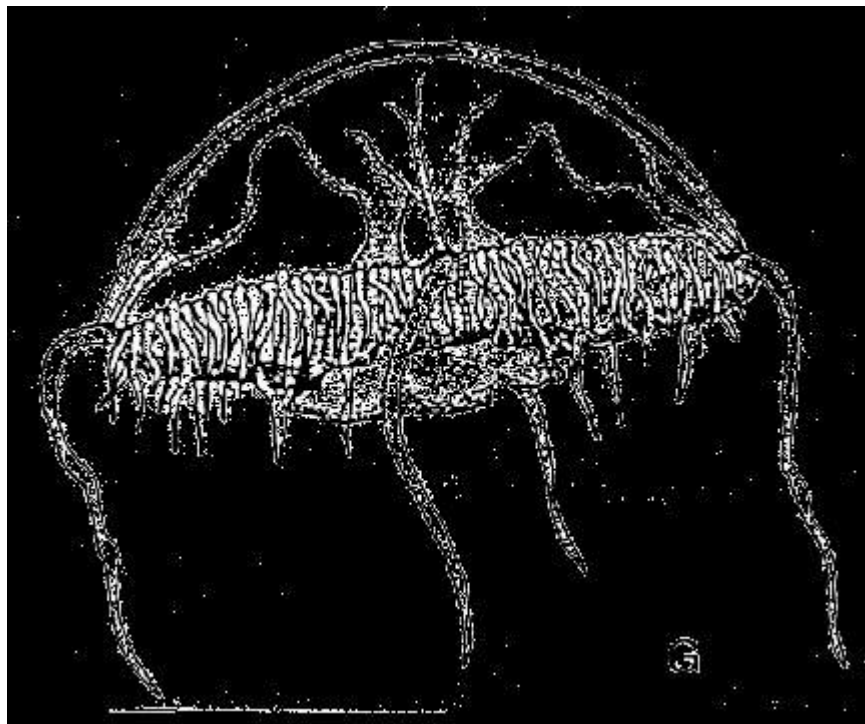
Obelia extended (a) and contracted (b) hydranth and gonotheca (c), marine



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G

Jellyfish (medusoid stage), *Craspedascusta*, freshwater



H

Sea anemone, *Anthopleura*, marine

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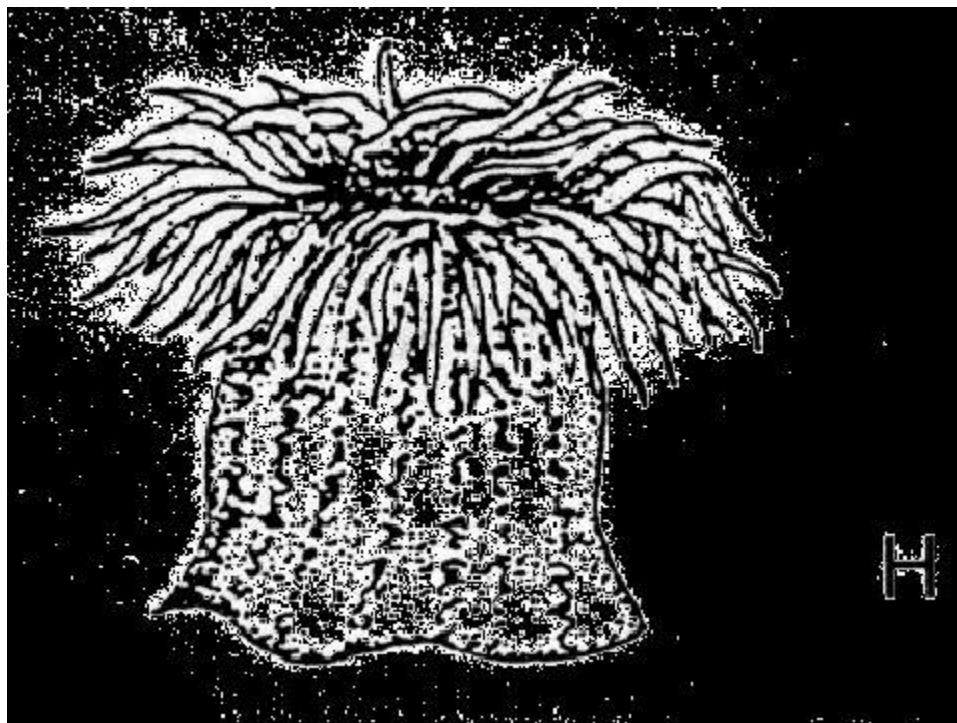


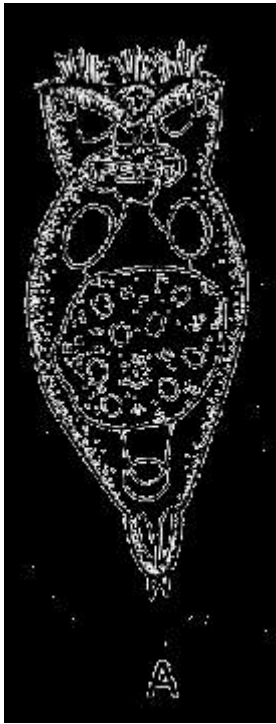
Plate 8. Rotifers (Phylum Rotifera). All freshwater inhabitants. Dimensions include spines.

A

Epiphanes

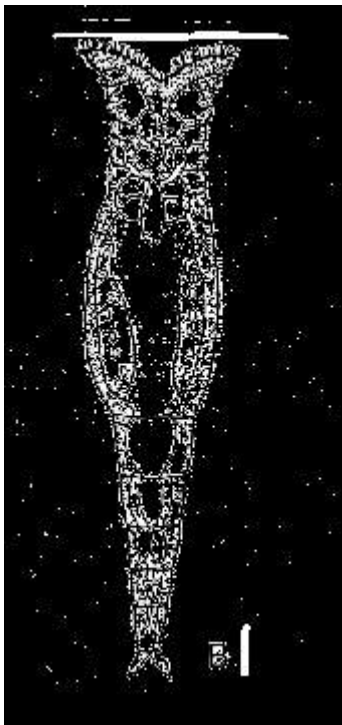
(0.6 mm)

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B *Philodina*

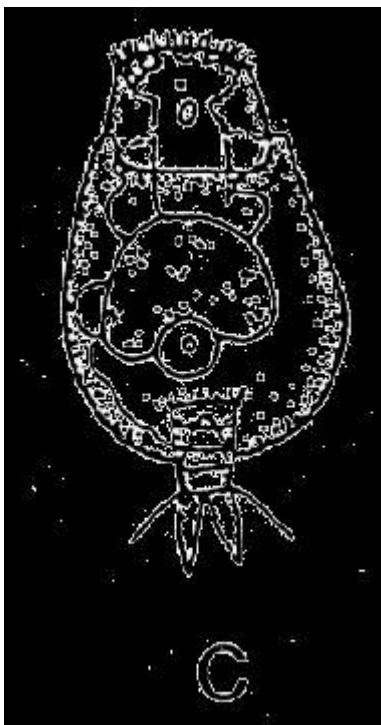
(0.4 mm)



C *Euchlanis*

(0.25 mm)

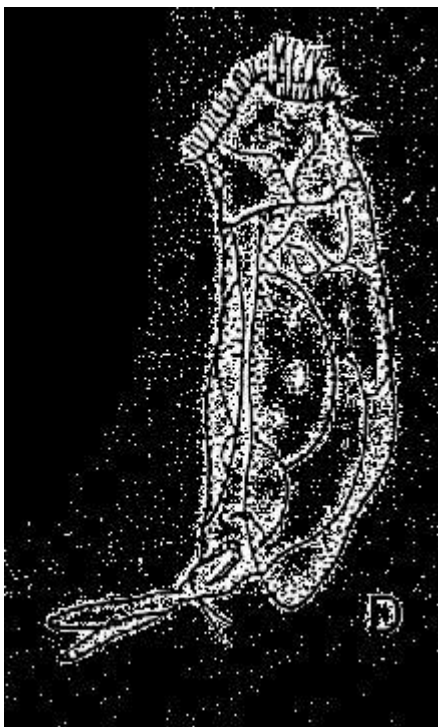
Standard Methods for the Examination of Water and Wastewater



D

Protozoa

(0.45 mm)

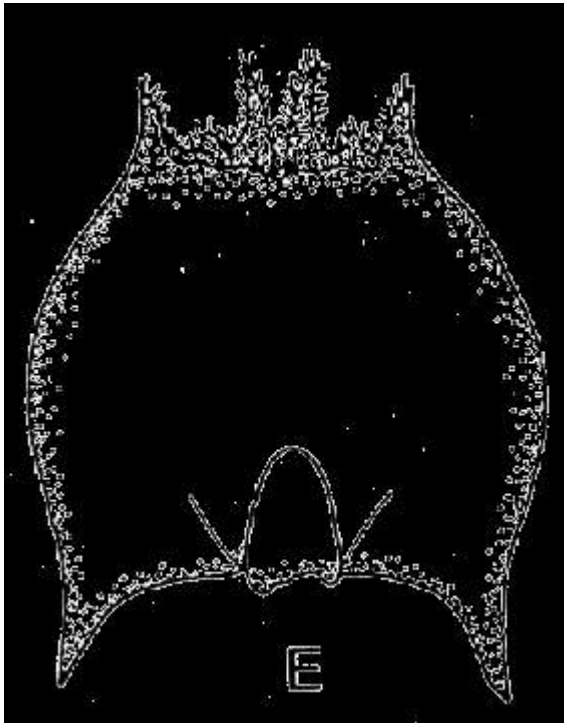


E

Brachionus

(0.2 mm)

Standard Methods for the Examination of Water and Wastewater



F *Monostyla* (0.15 mm)



G *Kellicottia* (1 mm)

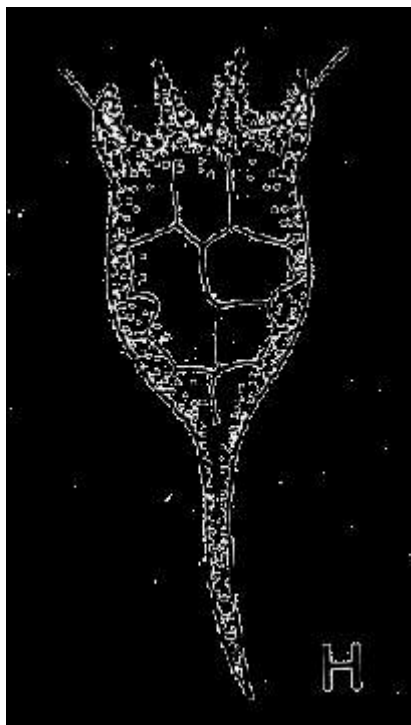
Standard Methods for the Examination of Water and Wastewater



H

Keratella

(0.2 mm)

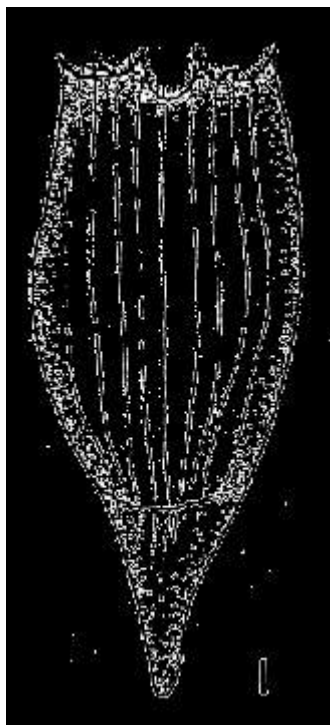


I

Notholca

(0.2 mm)

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J

Trichocerca

(0.6 mm)

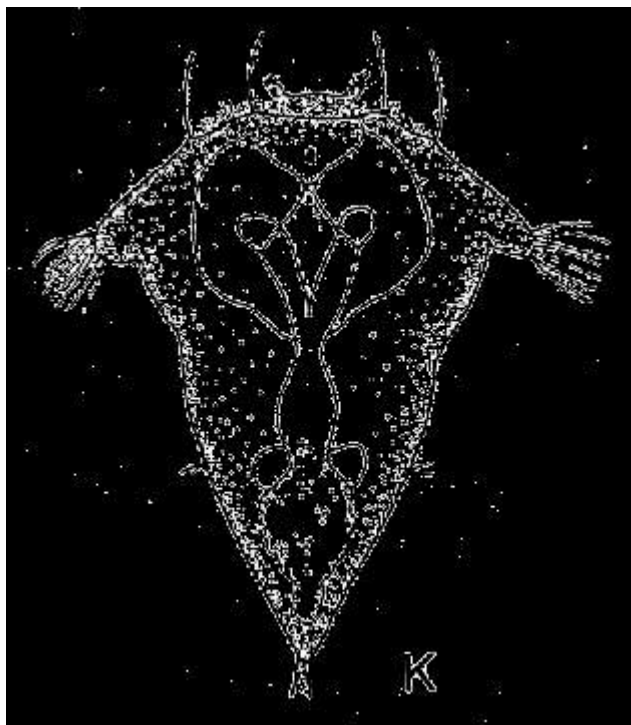


K

Synchaeta

(0.26 mm)

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L

Filinia

(0.15 mm)



M

Polyarthra

(0.175 mm)

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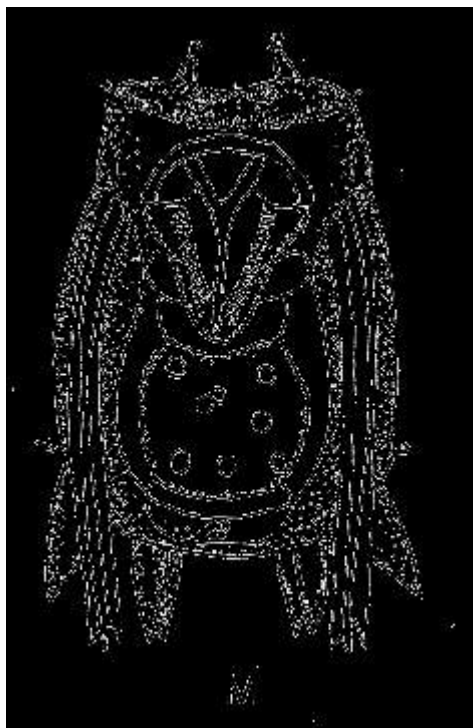


Plate 9. Roundworms, flatworms, and segmented worms. (a) anterior end, (b) posterior end, (c) tubes, (d) mud surface, (e) setae, (f) anterior sucker, (g) posterior sucker. All freshwater inhabitants.

Roundworms (Phylum Nematoda):

A

Plectus

(1.0 mm)

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B

Tripyla

(1.0 mm)



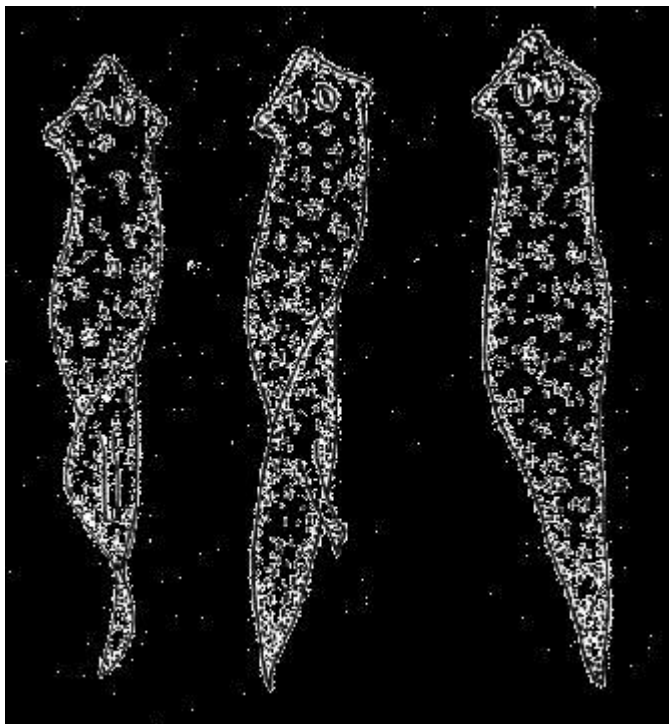
Flatworms (Phylum Platyhelminthes):

Standard Methods for the Examination of Water and Wastewater

C

Planaria

(5–13
mm)



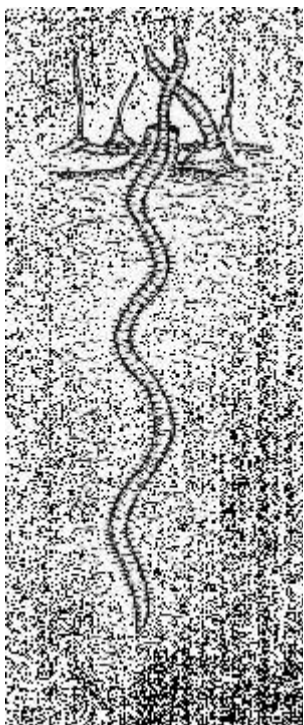
Segmented worms (Phylum Annelida, Class Oligochaeta):

D

Tubifex, sludgeworm

(2.5–5
cm)

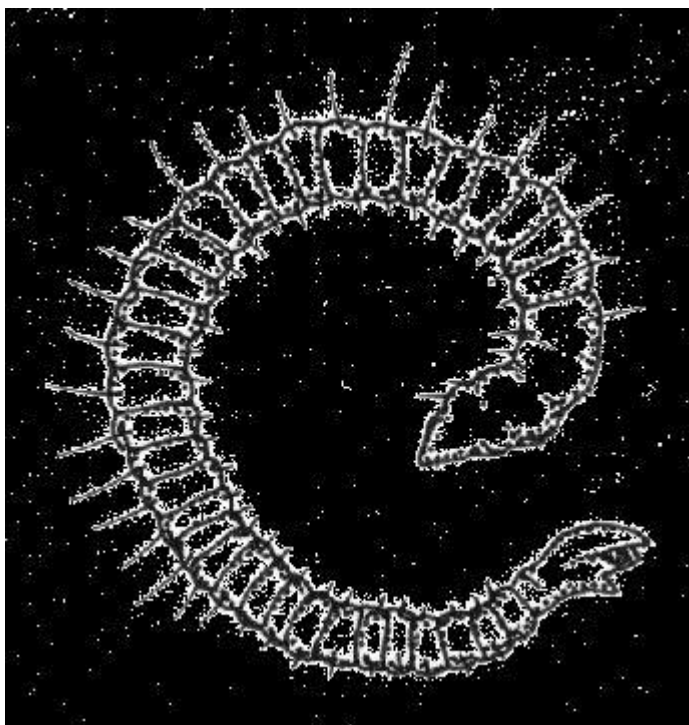
Standard Methods for the Examination of Water and Wastewater



E

Dero

(3–7
mm)



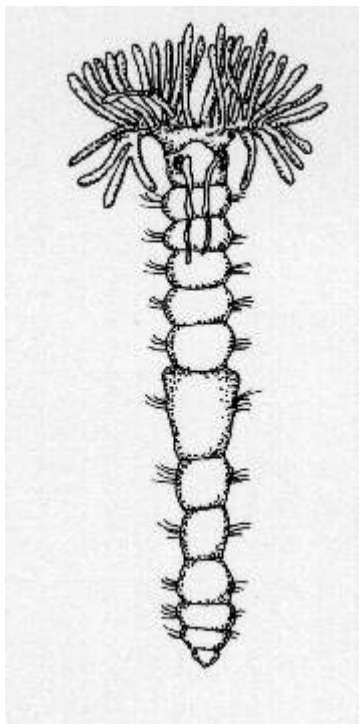
Segmented worms (Phylum Annelida, Class Polychaeta):

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F

Manayunkia, lives in (5 mm)
tube



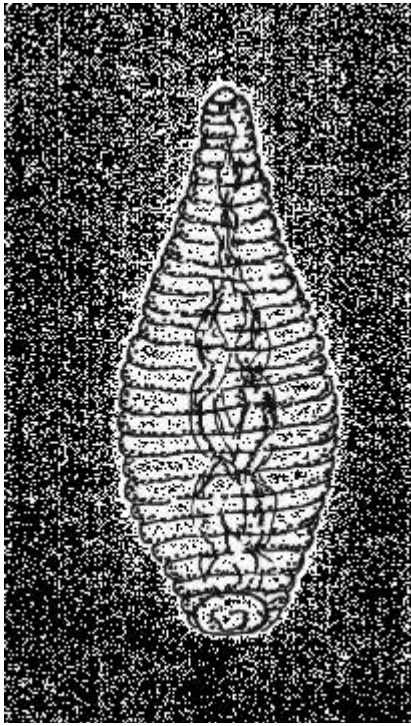
Segmented worms (Phylum Annelida, Class Hirudinea):

G

Leech

(3–6 cm)

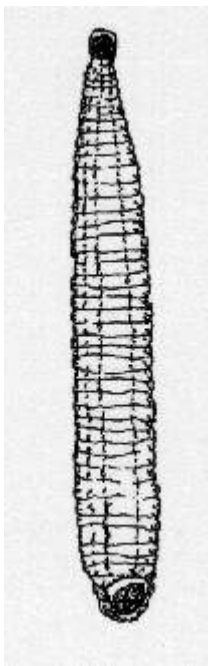
Standard Methods for the Examination of Water and Wastewater



H

Leech

(3–6 cm)

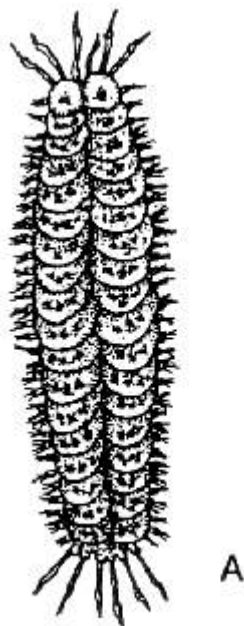


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Plate 10. Segmented marine worms (Phylum Annelida, Class Polychaeta).

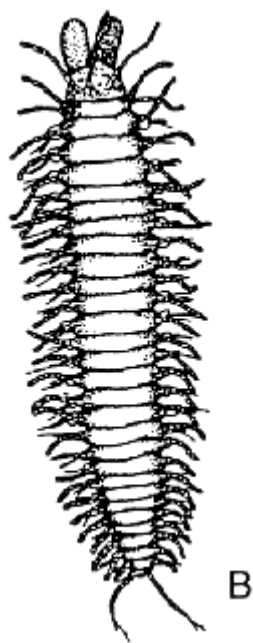
A Scale worm, *Halosydna*

(5 cm)



B Syllids, *Typosyllis*

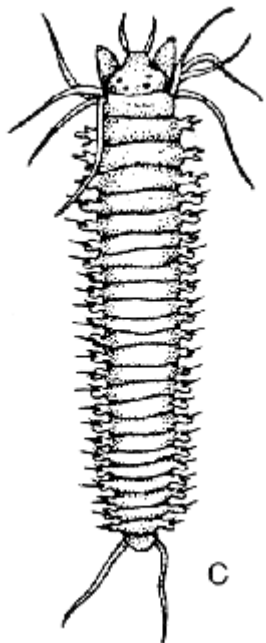
(2–5 cm)



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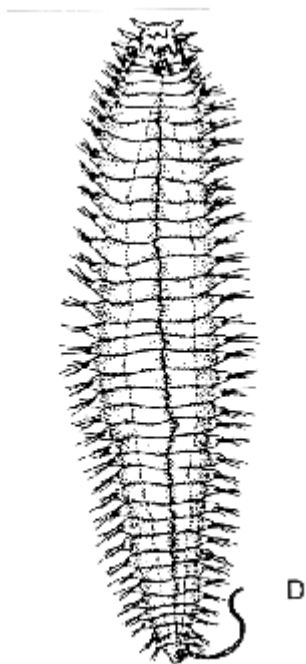
C Nereid, *Nereis*

(4–15 cm)



D Nephtyid, *Nephtys*

(5–25 cm)



E Onuphid, *Diopatra*, in tube covered with shells

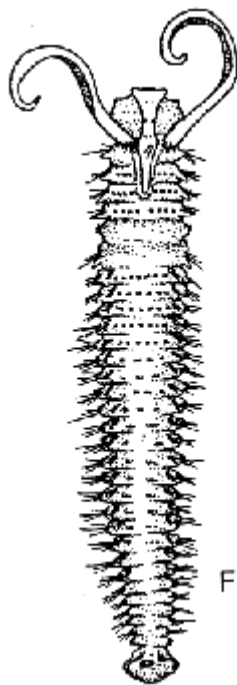
(5–25 cm)

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F Spionid, *Polydora*

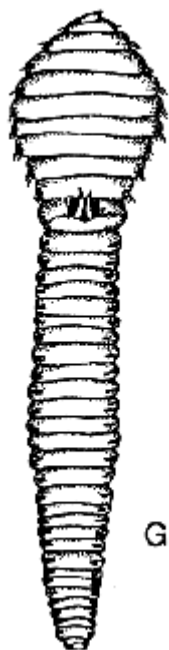
(2–4 cm)



G Capitellid, *Capitella*

(2–4 cm)

Standard Methods for the Examination of Water and Wastewater



H Ice cream cone worm, *Pectinaria*

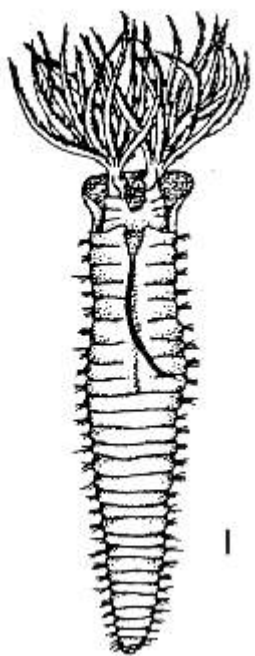
(1–3 cm)



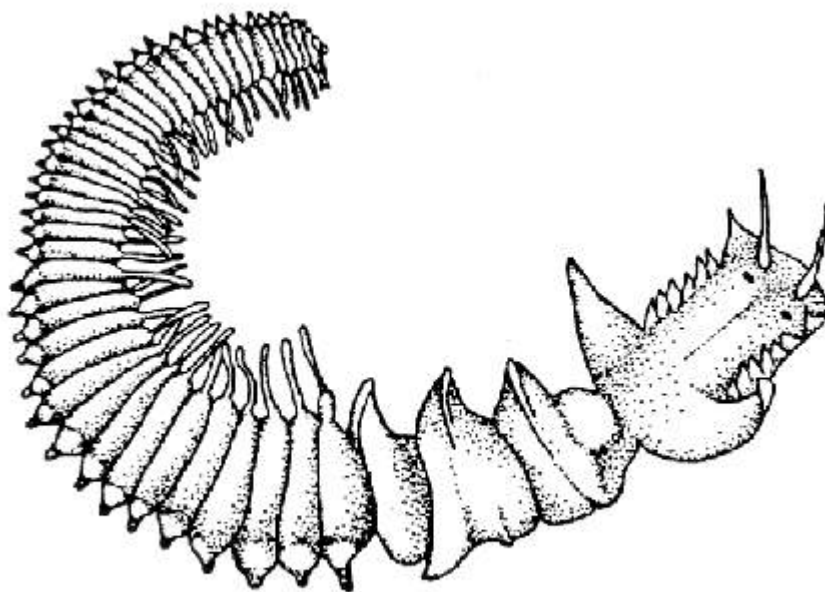
I Feather duster worm, *Sabella*

(10–15 cm)

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J Chaetopterid, *Chaetopterus*, lives in U-shaped tube (10–20 cm)



J

K Terebellid, *Pista* (10–15 cm)

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K

Plate 11. Crustaceans (Phylum Arthropoda)—all freshwater inhabitants, except as noted.

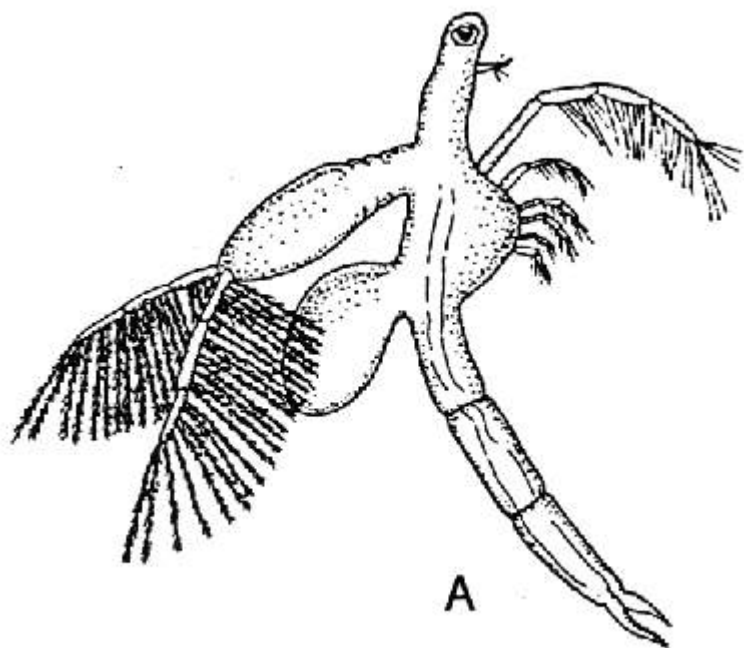
Order Cladocera:

A

Leptodora

(9 mm)

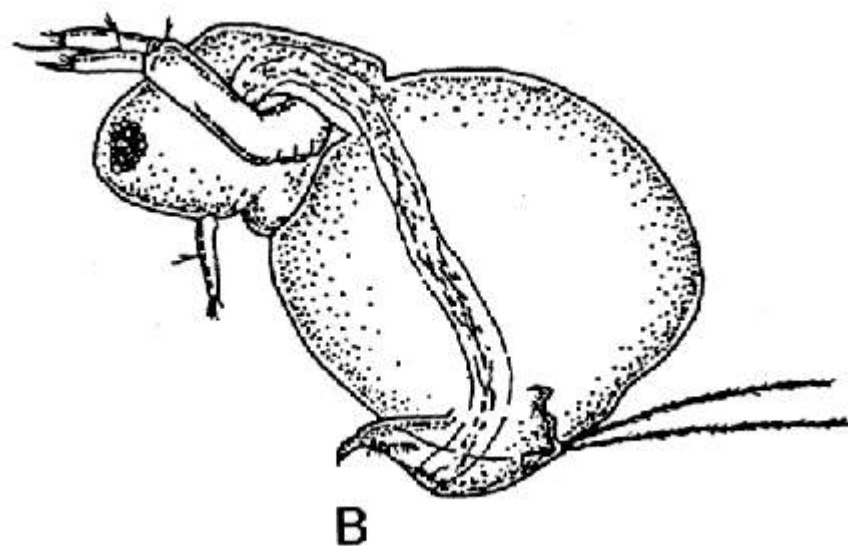
Standard Methods for the Examination of Water and Wastewater



B

Moina

(1.5 mm)

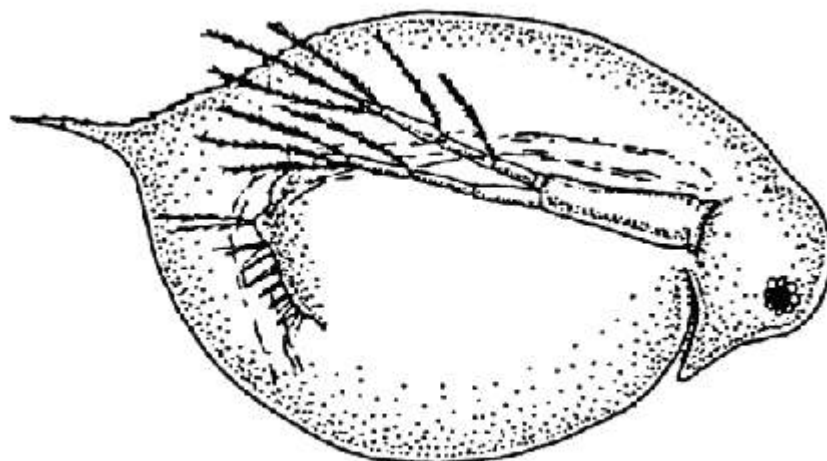


C

Daphnia

(2 mm)

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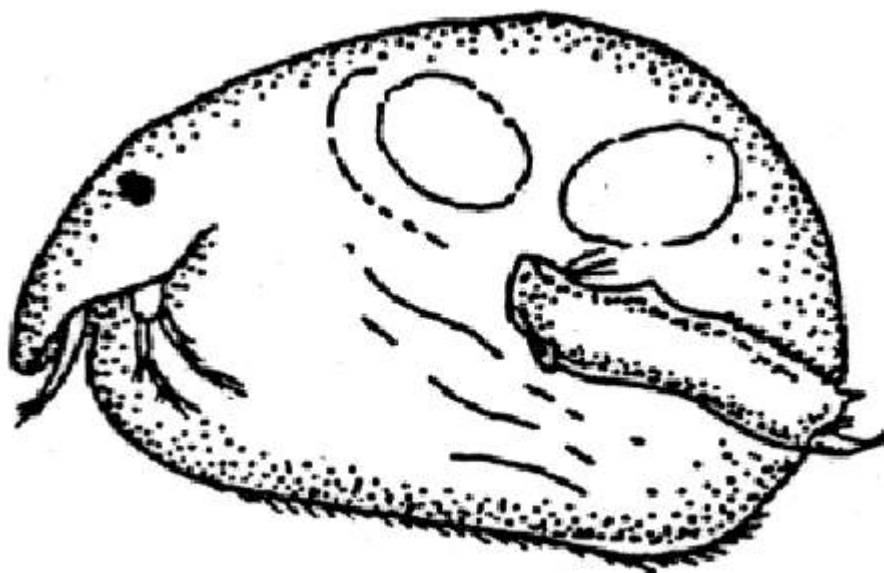


C

D

Alona

(0.4 mm)



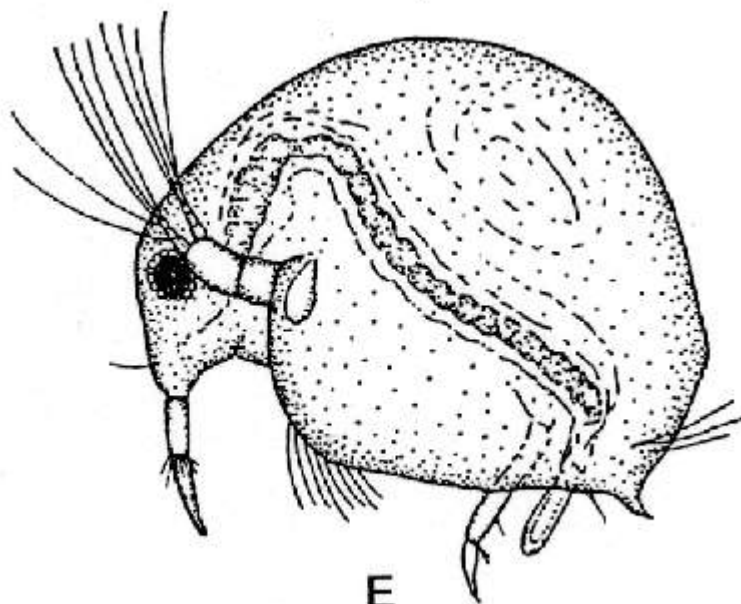
D

Standard Methods for the Examination of Water and Wastewater

E

Bosmina

(0.4 mm)

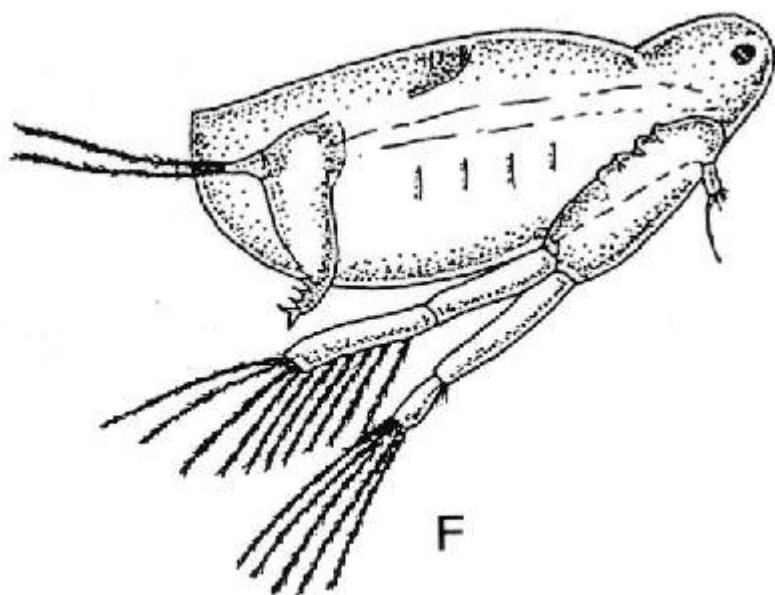


F

Diaphanosoma

(1.5 mm)

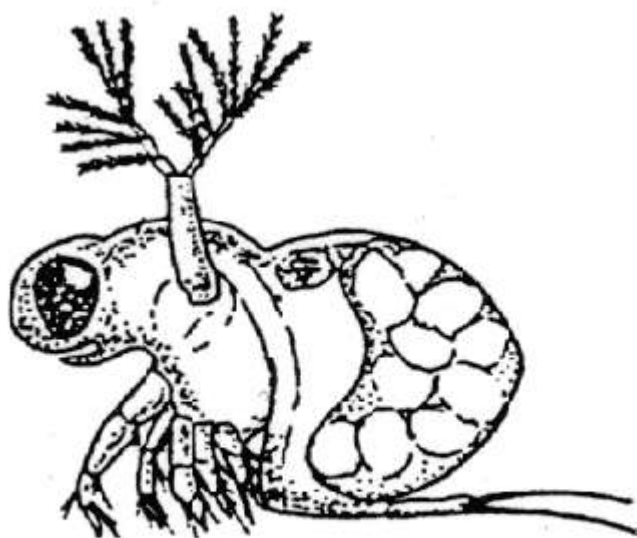
Standard Methods for the Examination of Water and Wastewater



G

Polyphemus

(1.5 mm)



G

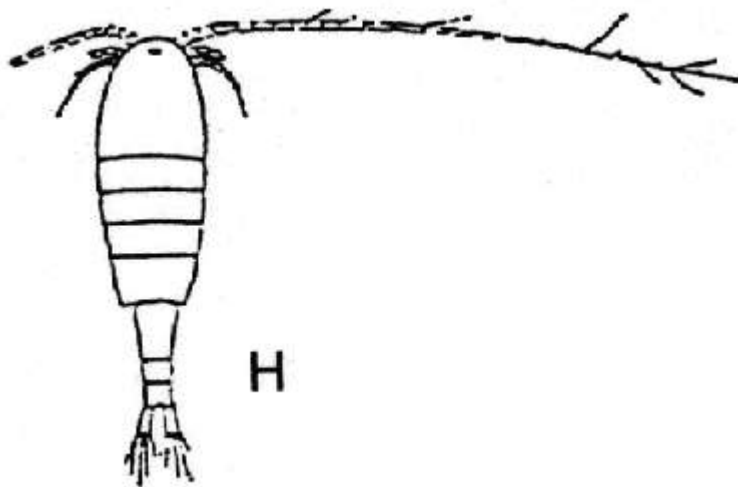
Order Copepoda:

Standard Methods for the Examination of Water and Wastewater

H

Diaptomus, freshwater

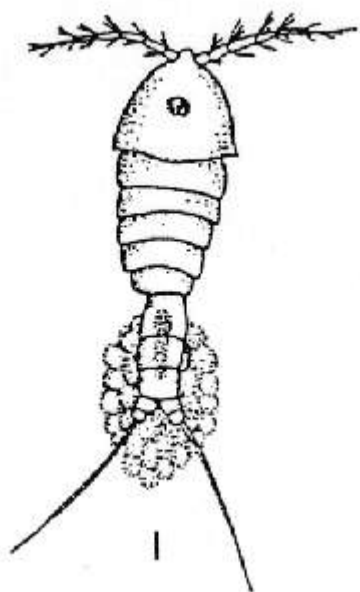
(2 mm)



I

Tigriopus, marine

(2–4 mm)



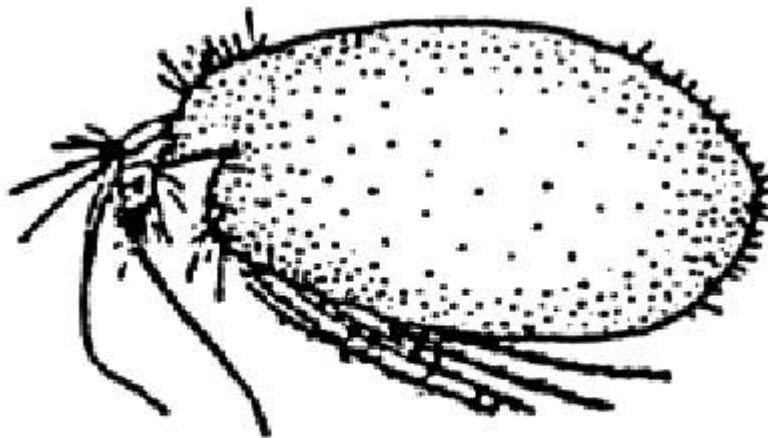
Order Ostracoda:

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J

Ostracod, marine

(2–3 mm)



J

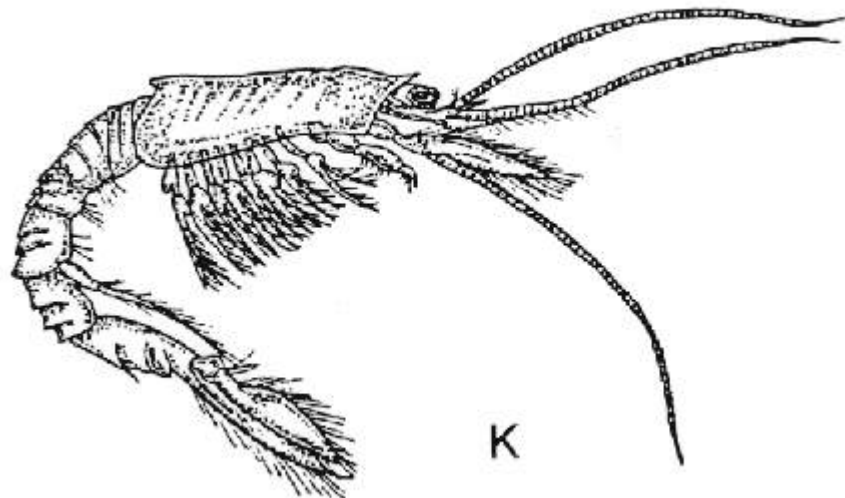
Order Mysidacea:

K

Holmesimysis, marine

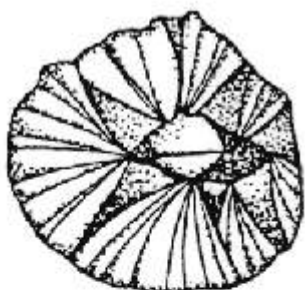
(10–20
mm)

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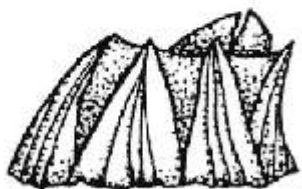


Order Cirripedia:

L Barnacle, *Balanus*, marine, a. top view, b. side (10 mm)
view



a



b

L

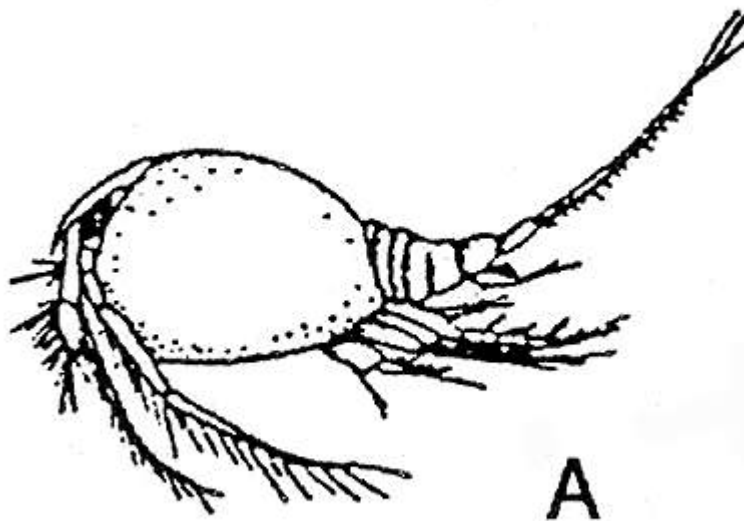
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Plate 12. Crustaceans and Pycnogonid (Phylum Arthropoda).

Crustaceans:

Order Leptostraca:

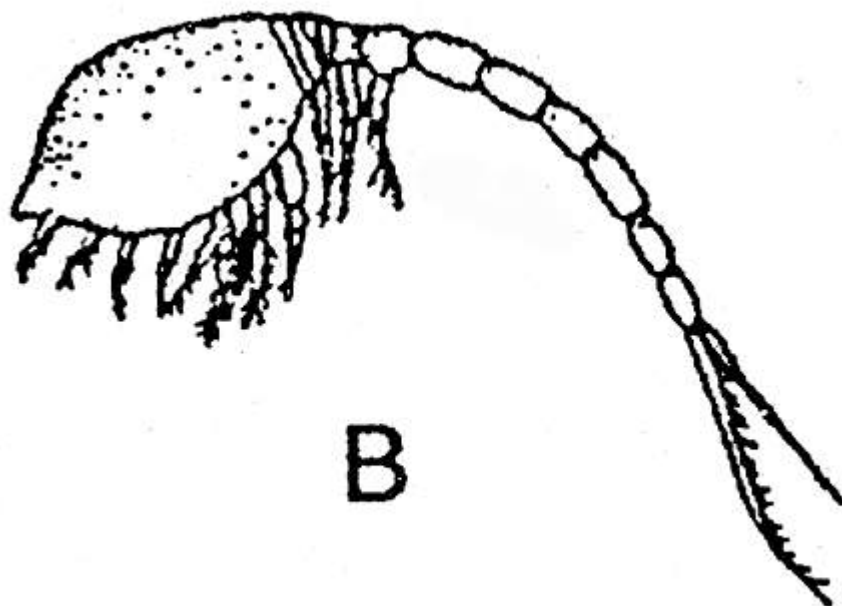
A *Epinebalia*, marine (10–20 mm)



Order Cumacea:

B *Oxyurostylis*, marine (8–12 mm)

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Order Tanaidacea:

C *Anatania*, marine

(8–10 mm)

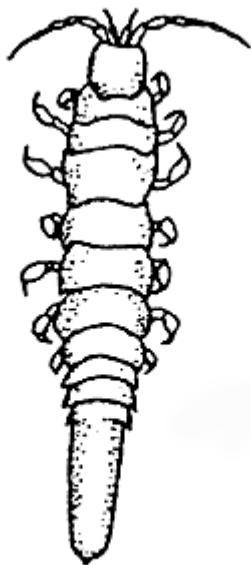


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Order Isopoda:

D *Idotea*, marine

(1–3 cm)

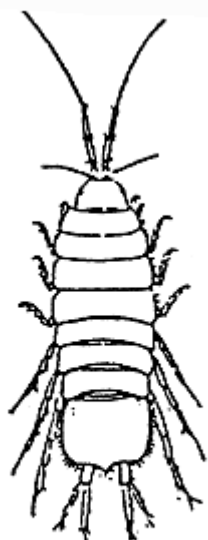


D

E Sowbug, *Asellus*, freshwater

(2 cm)

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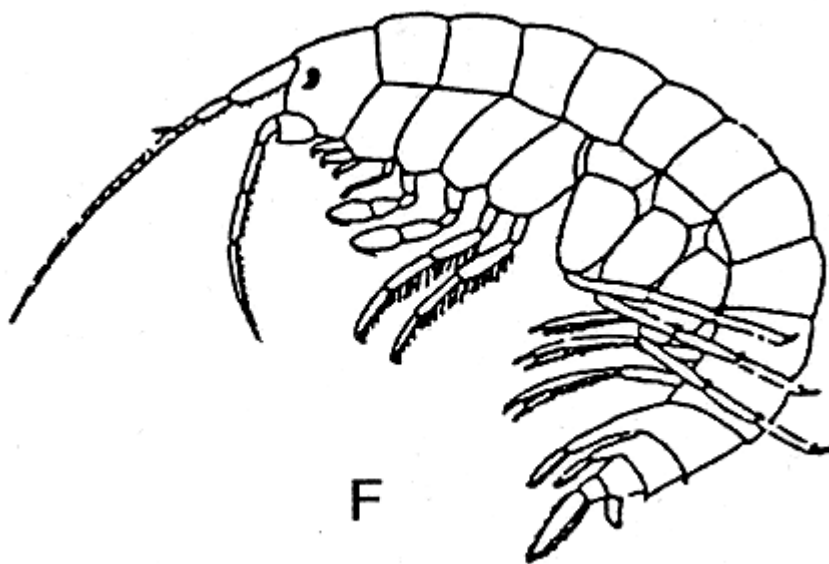


E

Order Amphipoda:

F Scud, *Gammarus*, freshwater

(1.5 cm)

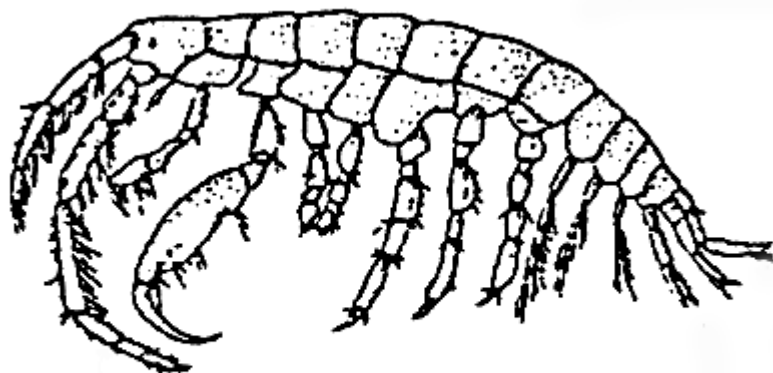


F

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G *Jassa*, marine

(1.5 cm)

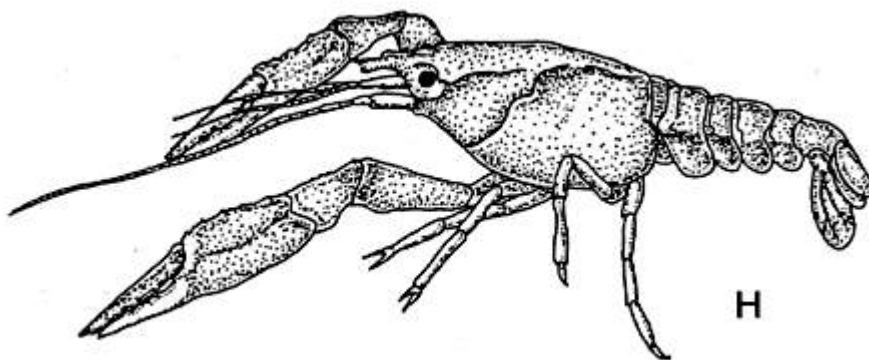


G

Order Decapoda:

H Crayfish or crawdad, *Cambarus*, freshwater

(150 cm)

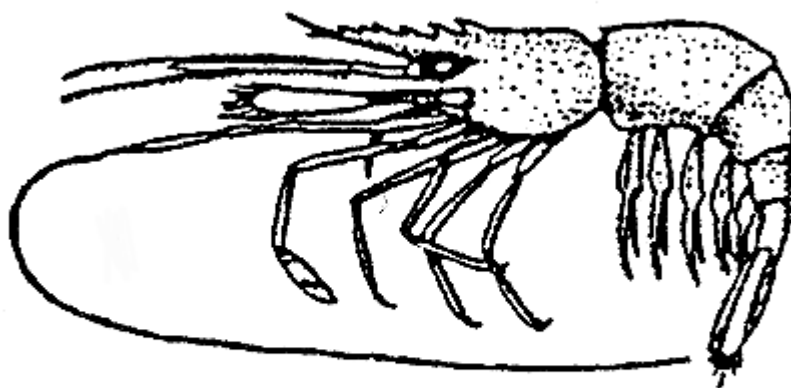


H

I Shrimp, *Spirontocaris*, marine

(3–6 cm)

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I

J

Mud crab, *Hemigrapsus*, marine

(3–6 cm)



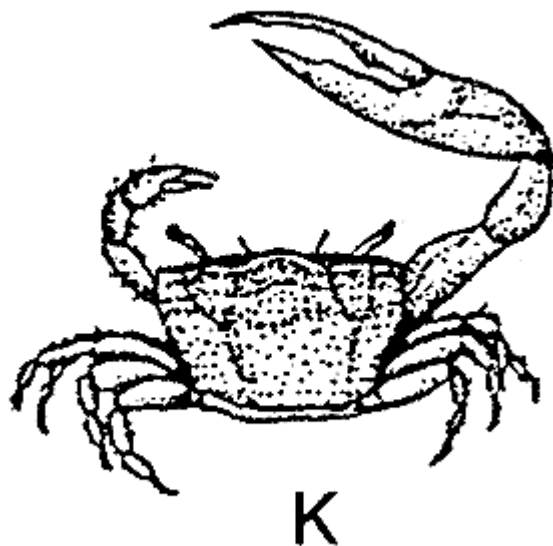
J

K

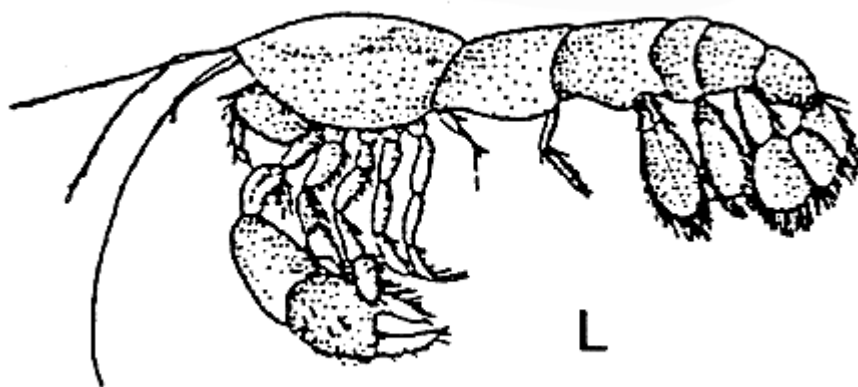
Fiddler crab, *Uca*, marine

(3–6 cm)

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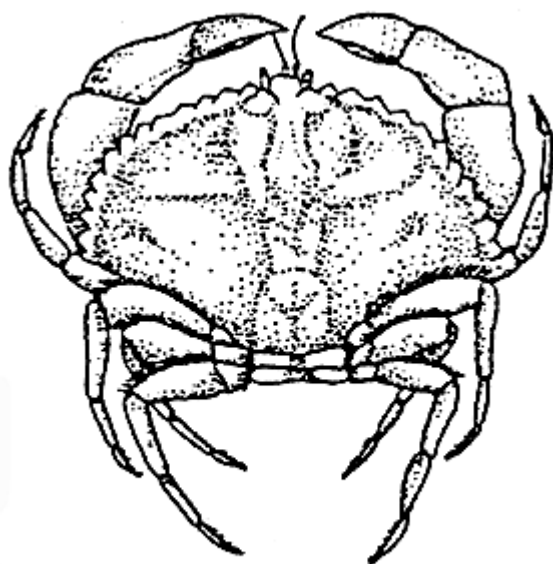


L Ghost shrimp, *Callinassa*, marine (5–10 cm)



M Cancer crab, *Cancer*, marine (3–18 cm)

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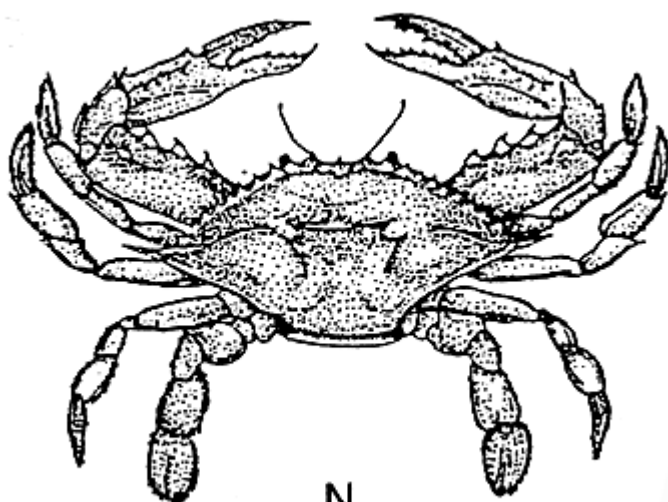


M

N

Blue crab, *Callinectes*, marine

(10–20 cm)

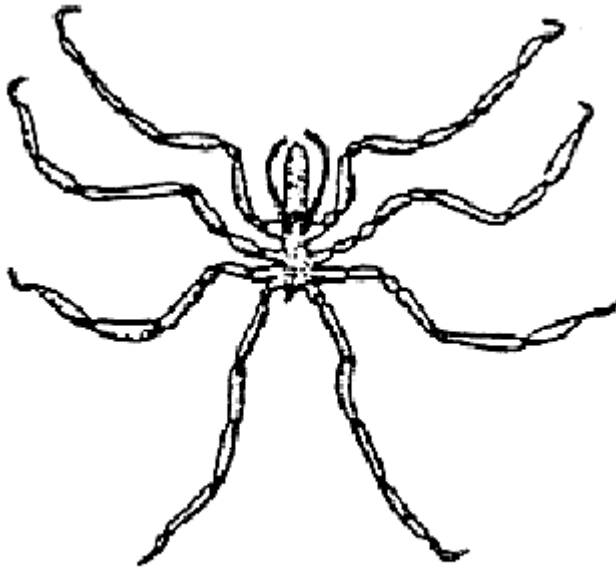


N

Pycnogonid, Class Pycnogonidea:

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- O Sea spider, pycnogonid, marine (10–20 mm)



O

Plate 13. Stoneflies and mayflies. All freshwater inhabitants.

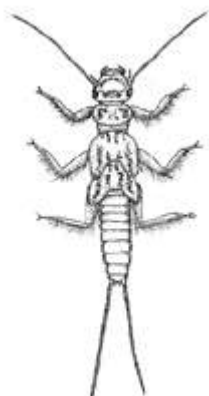
Stoneflies (Order Plecoptera):

- A Adult, *Isoperla*, Family Perlodidae (14–23 mm)

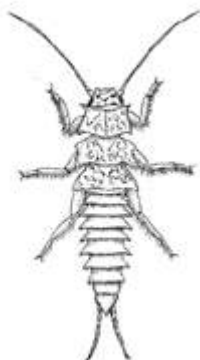


-
- B Nymph, *Isoperla*, Family Perlodidae (10–14 mm)

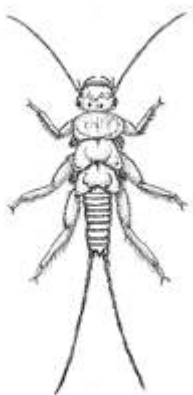
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C	Nymph, <i>Pteronarcys</i> , Family Pteronarcidae	(10–40 mm)



D	Nymph, <i>Acroneuria</i> , Family Perlidae	(20–30 mm)



Mayflies (Order Ephemeroptera):		
E	Adult, mayfly, Family Heptageniidae	(12–18 mm)

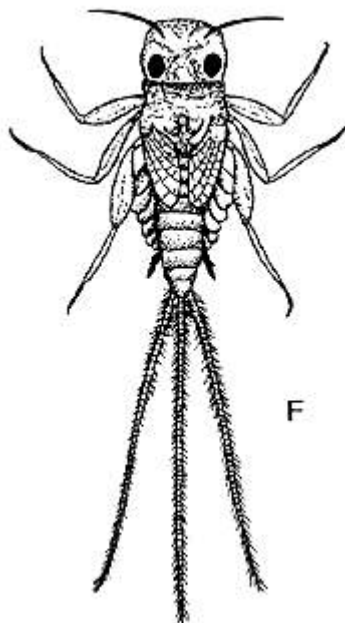
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F

Nymph, *Stenonema*, Family
Heptageniidae

(10–14
mm)

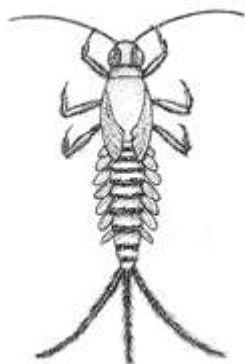


G

Nymph, *Baetis*, Family Baetidae

(7–14
mm)

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H

Nymph, *Hexagenia*, Family Ephemeridae (20–30 mm)



I

Nymph, *Ephemerella*, Family Ephemerellidae (8–15 mm)



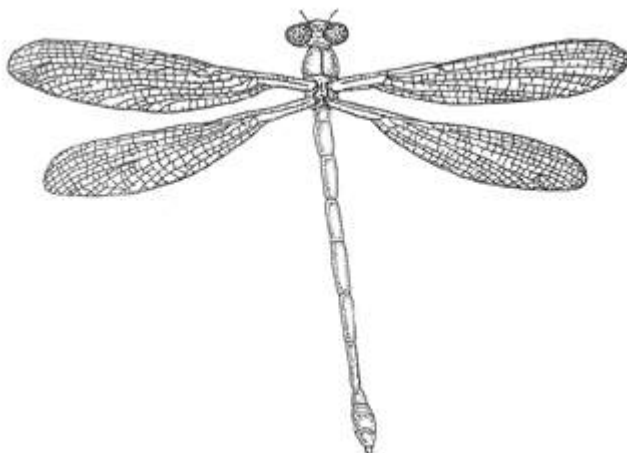
Plate 14. Damselflies, dragonflies (Order Odonata), all freshwater inhabitants.

A

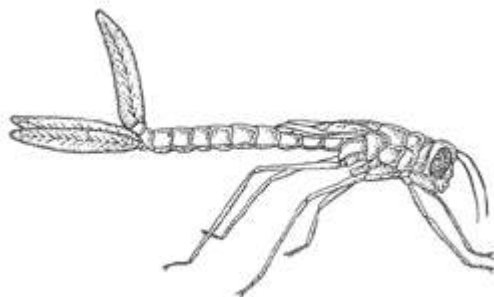
Adult, damselfly

(35–55 mm)

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B Nymph, damselfly, *Lestes*, Family Lestidae (20–30 mm)

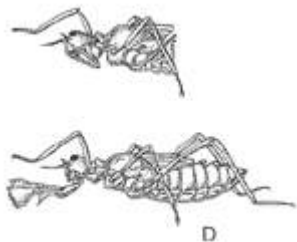


C Adult, dragonfly, *Macromia*, Family Macromiidae (50–70 mm)

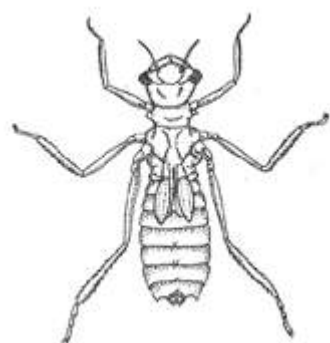


D Nymph, dragonfly, *Macromia*, Family Macromiidae, showing “mask” both extended and contracted

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E Nymph, dragonfly, *Helocordulia*, Family (15–45 mm)
Libellulidae



F Nymph, dragonfly, *Hagenius*, Family (15–20 mm)
Gomphidae



Plate 15. Hellgrammite and relatives, and caddisflies. All freshwater inhabitants.

Hellgrammite and relatives:

A Adult, alderfly, *Sialis*, Family Sialidae (9–15 mm)

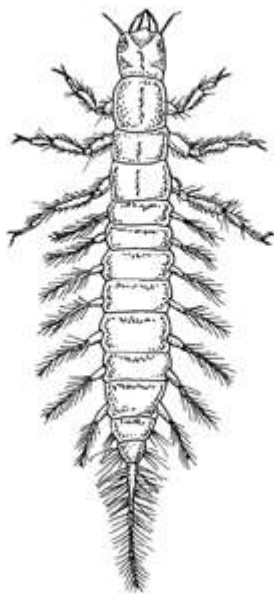
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B

Larva, alderfly, *Sialis*, Family Sialidae

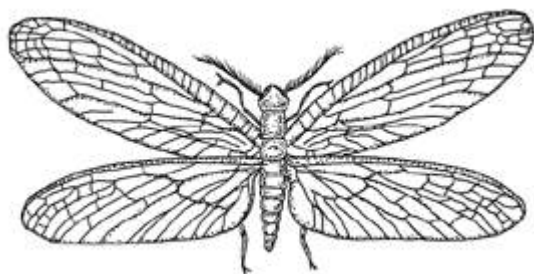
(15–30
mm)



C

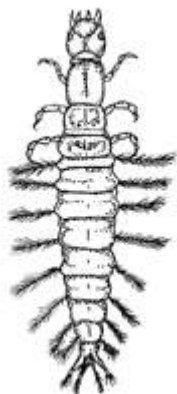
Adult, fishfly, *Chauliodes*, Family Corydalidae (15–30
mm)

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D

Larva, fishfly, *Chauliodes* Family Corydalidae (20–40 mm)



E

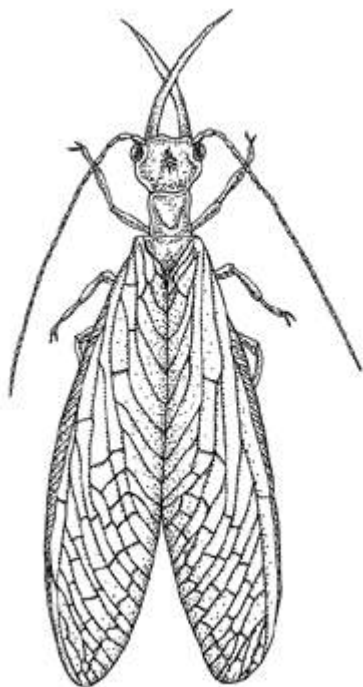
Pupa, hellgrammite, *Corydalus*, Family Corydalidae



F

Adult, dobsonfly, *Corydalus*, Family Corydalidae (25–70 mm)

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G	Larva, dobsonfly, <i>Corydalus</i> , Family Corydalidae	(25–90 mm)



Caddisflies:

H	Larva and case, <i>Triaenodes</i> , Family Leptoceridae	(10–14 mm)

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I

Adult, *Hydropsyche*, Family Hydropsychidae (20–30 mm)

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J

Larva and case, *Ochrotricha*, Family
Hydroptilidae

(4–6 mm)



K

Larva and case, *Leptocella*, Family
Leptoceridae

(14–18
mm)

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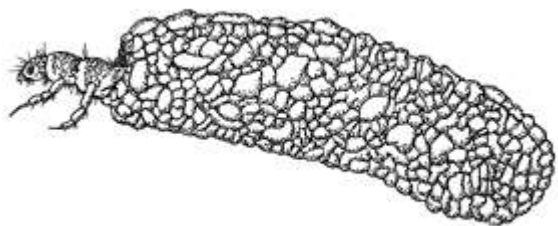
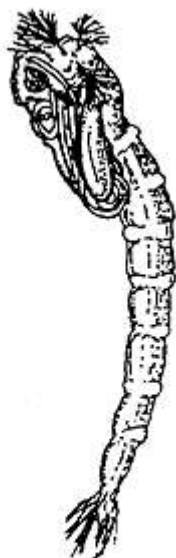


Plate 16. Two-winged flies (Order Diptera). All freshwater inhabitants.

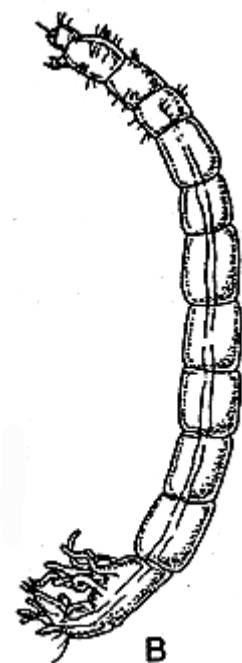
A Pupa, midge, *Chironomus*, Family Chironomidae



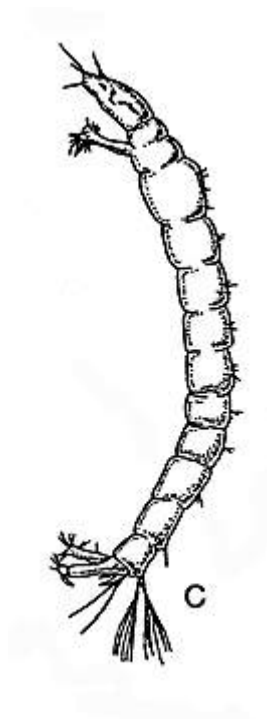
A

B Larva, midge, *Chironomus*, Family Chironomidae (5–30 mm)

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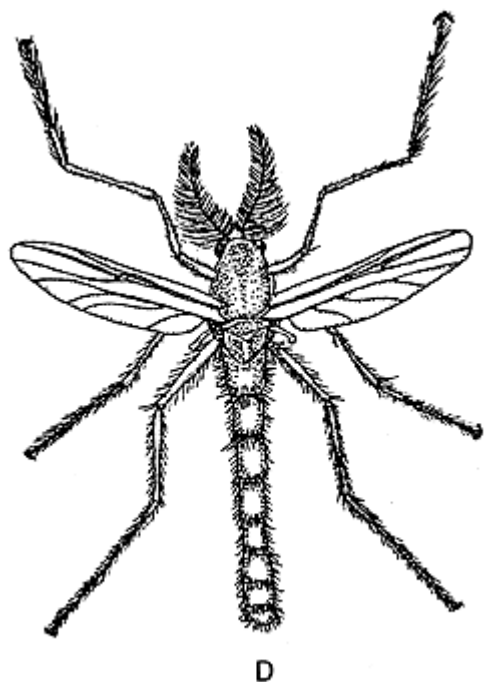


C Larva, midge, *Ablabesmyia*, Family Chironomidae (5–10 mm)

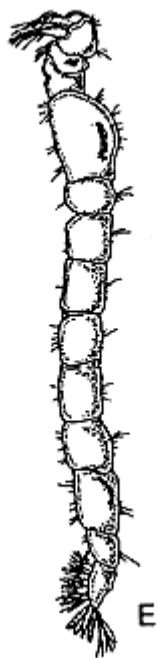


D Adult, midge, Chironomidae
(4–12 mm)

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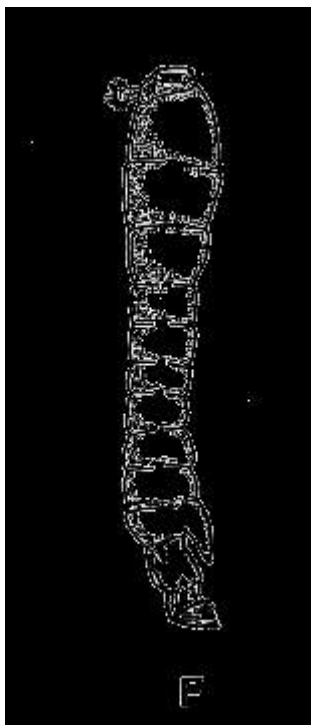


E Larva, phantom midge, *Chaoborus*, Family Culicidae (8–12 mm)



F Larva, housefly, *Musca*, Family Muscidae (3–8 mm)

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G Adult, sewage fly, *Psychoda*, Family Psychodidae (2–5 mm)



H Larva, sewage fly, *Psychoda*, Family Psychodidae (4–6 mm)

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H

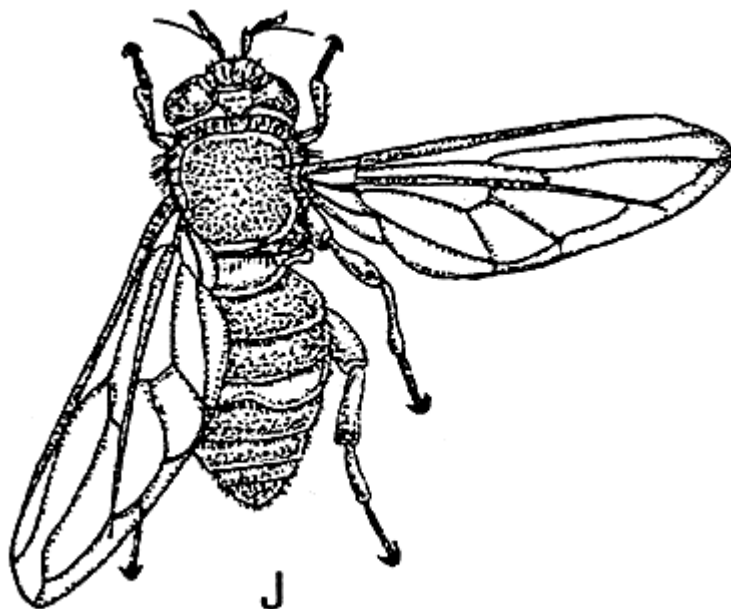
I Adult, blackfly, *Simulium*, Family Simuliidae (2–6 mm)



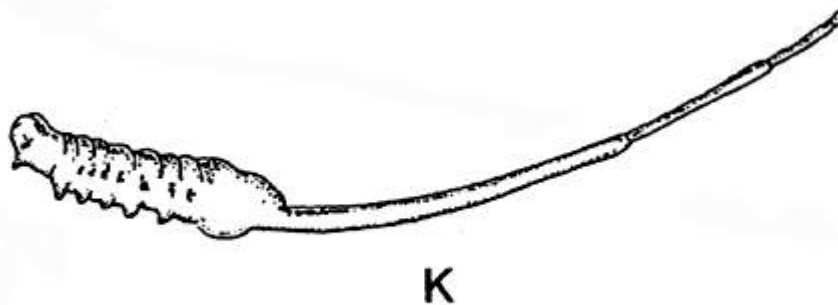
I

J Adult, drone fly, Family Syrphidae (10–15 mm)

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K Rat-tailed maggot, *Eristalis*, Family Syrphidae (15–30 mm)



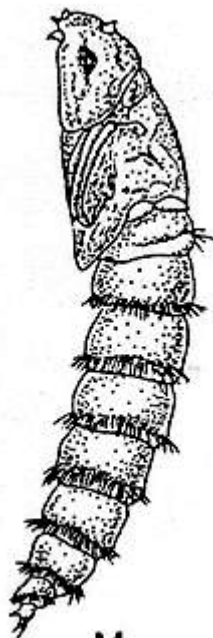
L Larva, *Tabanus*, Family Tabanidae (30–40 mm)

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L

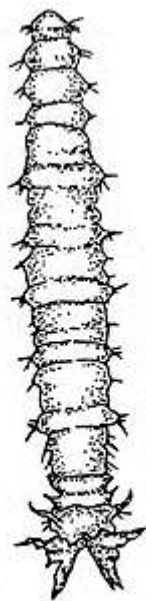
M Blowfly, *Tabanus*, Family Tabanidae



M

N Larva, crane fly, *Tipula*, Family Tipulidae (30–40 mm)

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N

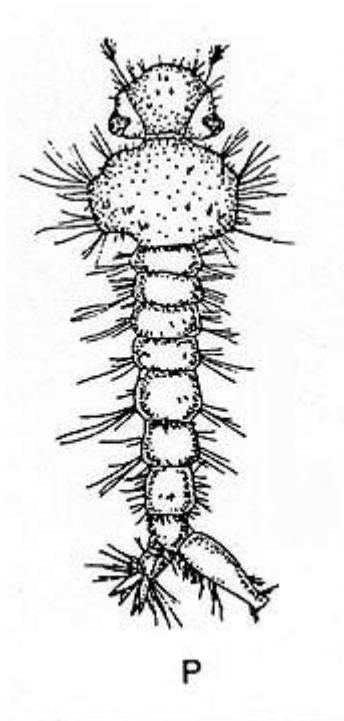
O Pupa, cranefly, *Antocha*, Family Tipulidae



O

P Larva, mosquito, *Aedes*, Family Culicidae (10–15 mm)

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Q Pupa, mosquito, *Culex*, Family Culicidae

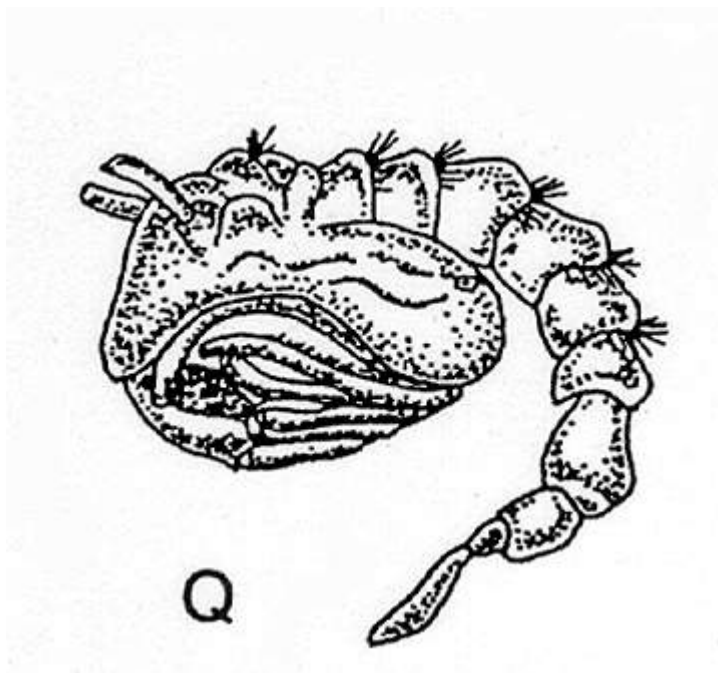
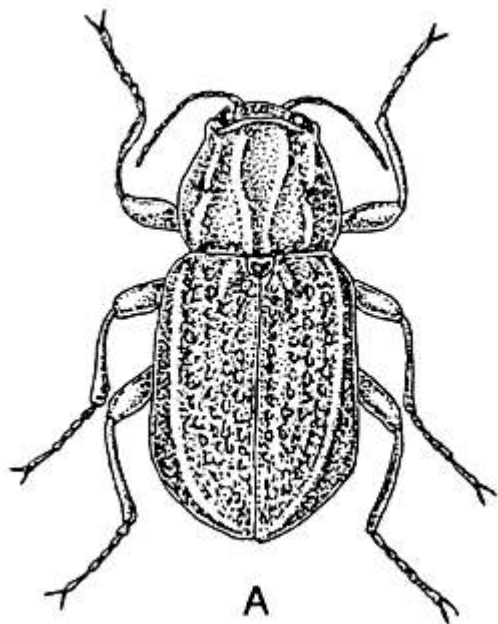


Plate 17. Beetles (Order Coleoptera). All freshwater inhabitants.

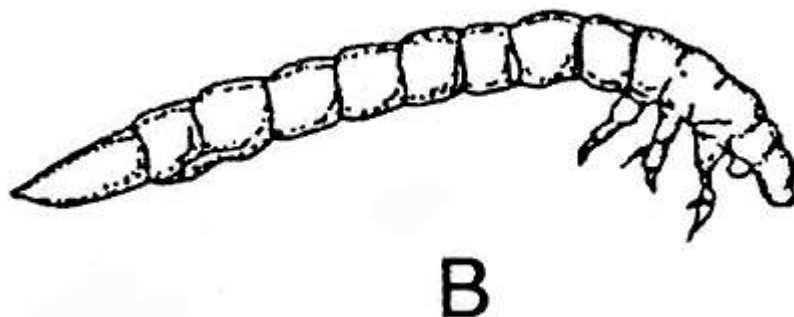
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A Adult, riffle beetle, *Stenelmis*, Family Elmidae (2–5 mm)

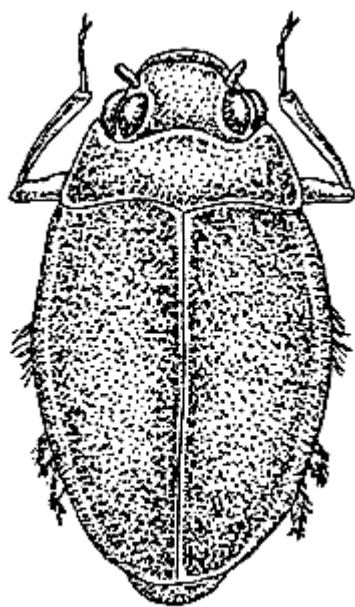


B Larva, *Narpus*, Family Elmidae (4–10 mm)



C Adult, whirligig beetle, *Dineutus*, Family Gyrinidae (7–15 mm)

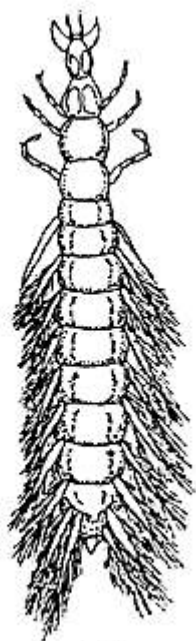
Standard Methods for the Examination of Water and Wastewater



C

D Larva, *Dineutus*, Family Gyrinidae

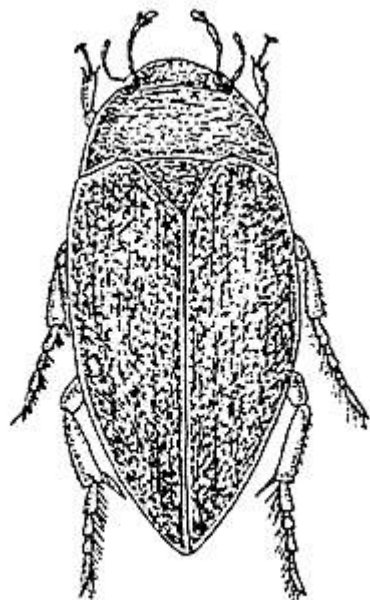
(10–30
mm)



D

Standard Methods for the Examination of Water and Wastewater

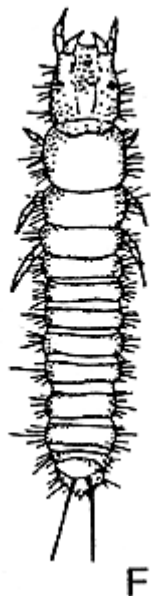
-
E	Adult, water scavenger beetle, <i>Hydrophilus</i> , Family Hydrophilidae	(2–40 mm)



E

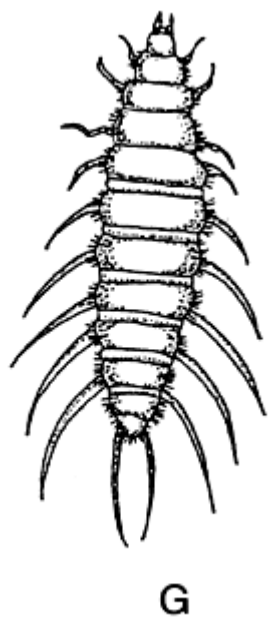
-
F	Larva, <i>Berosus</i> , Family Hydrophilidae	(5–20 mm)

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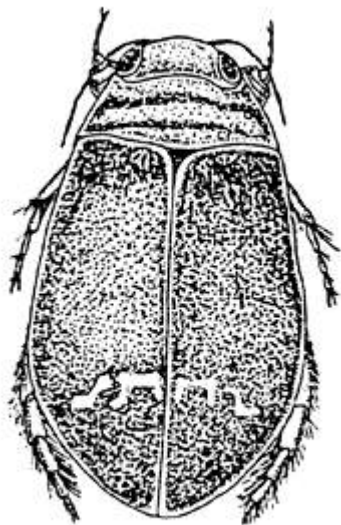
G Larva, *Enochrus*, Family Hydrophilidae

(10–25
mm)



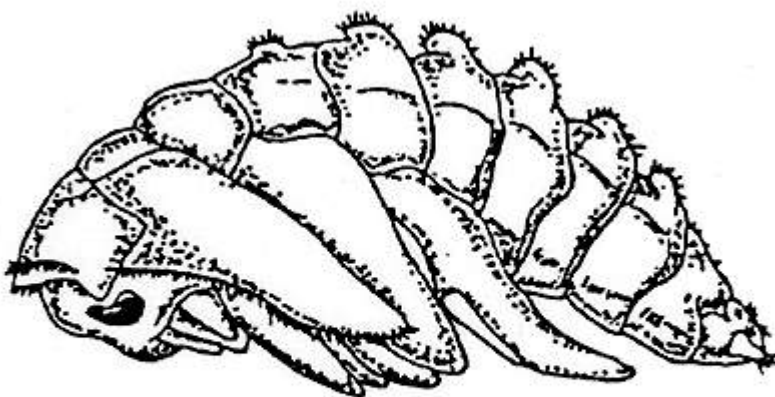
Standard Methods for the Examination of Water and Wastewater

-
H	Adult, predaceous diving beetle, <i>Dytiscus</i> , Family Dytiscidae	(2–40 mm)
-



H

-
I	Pupa, <i>Cybister</i> , Family Dytiscidae	
-



I

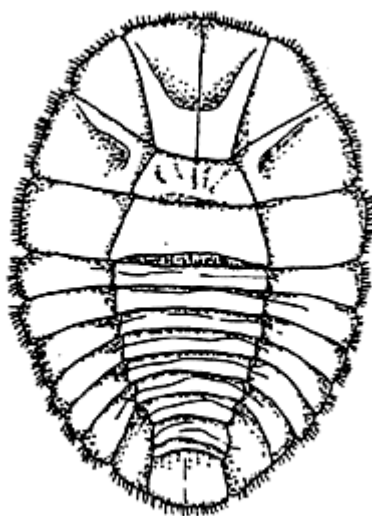
-
J	Larva, <i>Cybister</i> , Family Dytiscidae	(10–25 mm)
-

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J

K Larva, water penny, *Psephenus*, Family Psephenidae (3–10 mm)

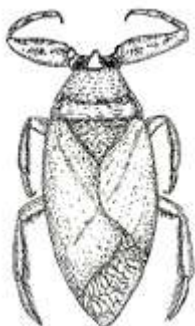


K

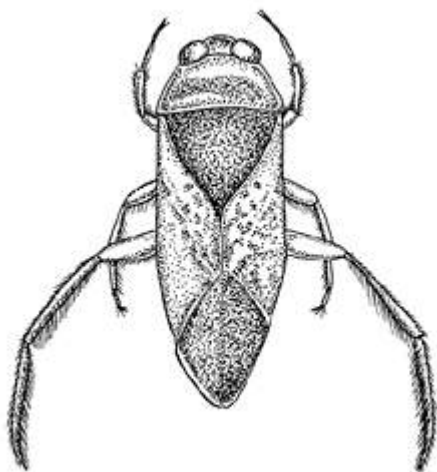
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Plate 18. True bugs (Order Hemiptera, all adults). All freshwater inhabitants.

- A Electric light bug, *Lethocerus*, Family Belostomidae (20–70 mm)

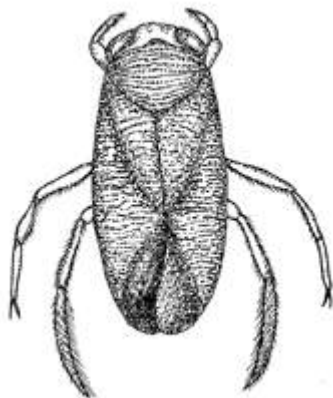


-
- B Backswimmer, *Notonecta*, Family Notonectidae (5–17 mm)

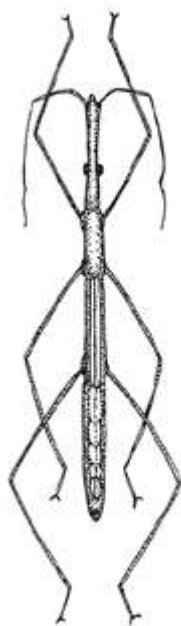


-
- C Water boatman, *Sigara*, Family Corixidae (3–12 mm)

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D Marsh treader, *Hydrometra*, Family Hydrometridae (8–11 mm)



E Water strider, *Gerris*, Family Gerridae (2–15 mm)

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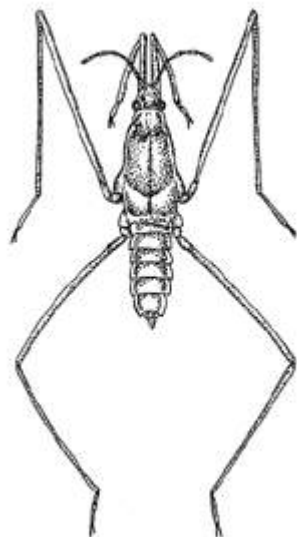
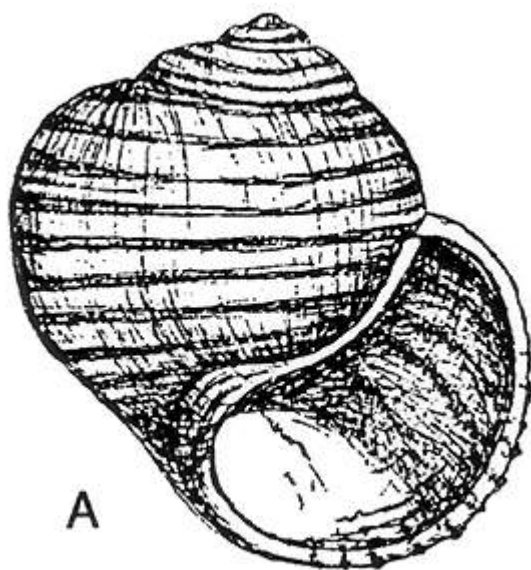


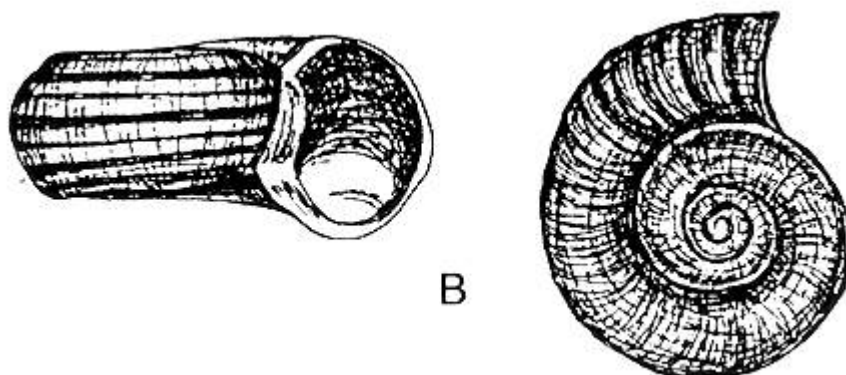
Plate 19. Snails (Phylum Mollusca, Class Gastropoda). All freshwater inhabitants.

A Apple snail, *Pomacea*, Family *Pilidae* (5 cm)



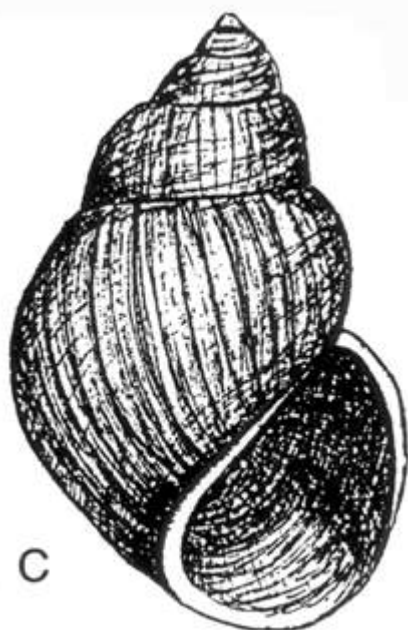
B *Marisa*, side and top views, Family *Pilidae* (15 mm)

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C *Campeloma*, Family Viviparidae

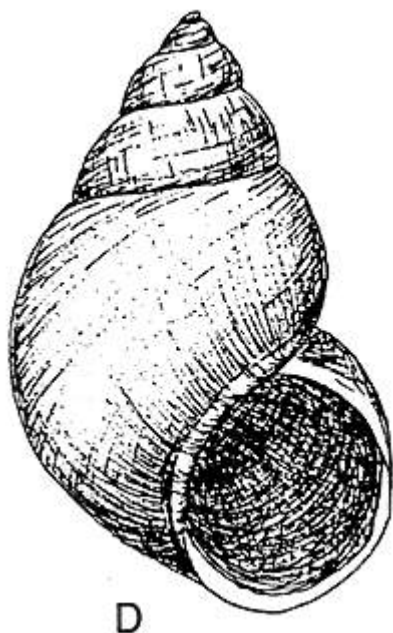
(4 cm)



D Faucet snail, *Bithynia*, Family Ammicolidae

(2 cm)

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E *Tarebia*, Family Thiaidae

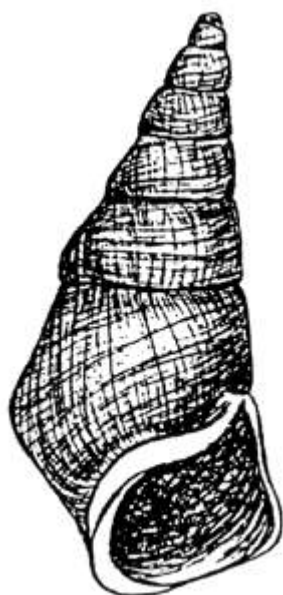
(15 mm)



F River snail, *Pleurocera*, Family Pleuroceridae

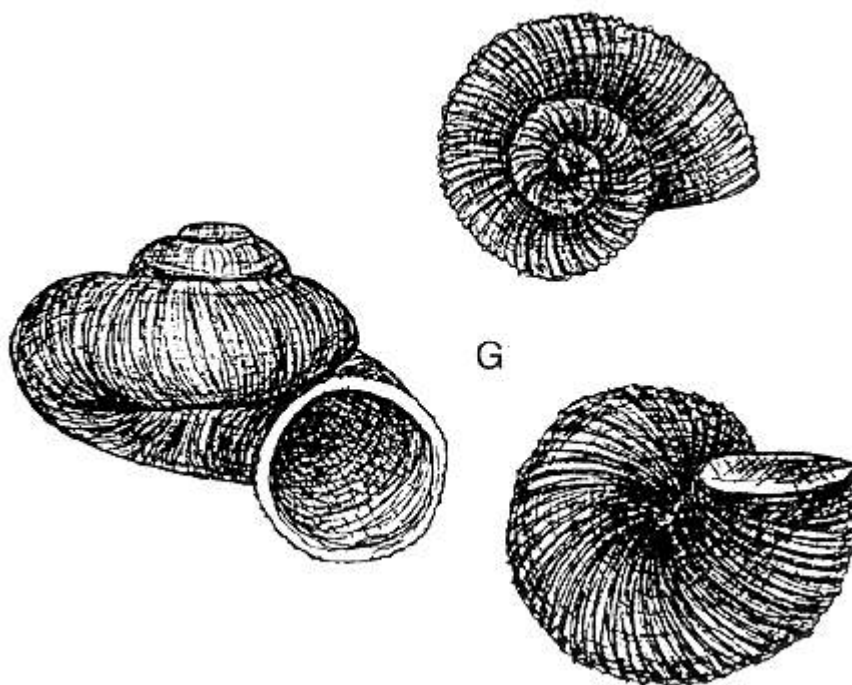
(3 cm)

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F

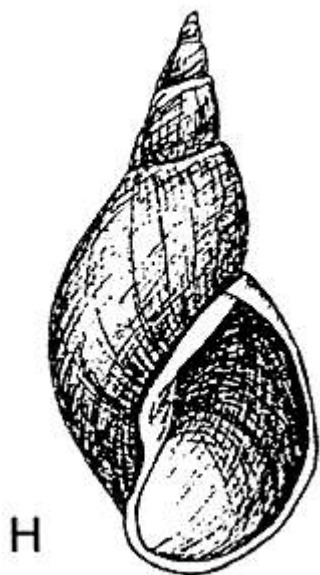
G *Valvata*, side, top and bottom views, Family Valvatidae (1 cm)



G

H Pond snail, *Lymnaea*, Family Lymnaeidae (15 mm)

Standard Methods for the Examination of Water and Wastewater

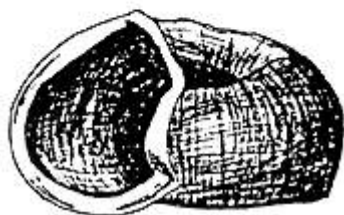


I Orb snail, top and side views, *Helisoma*, Family Planorbidae

(1 cm)



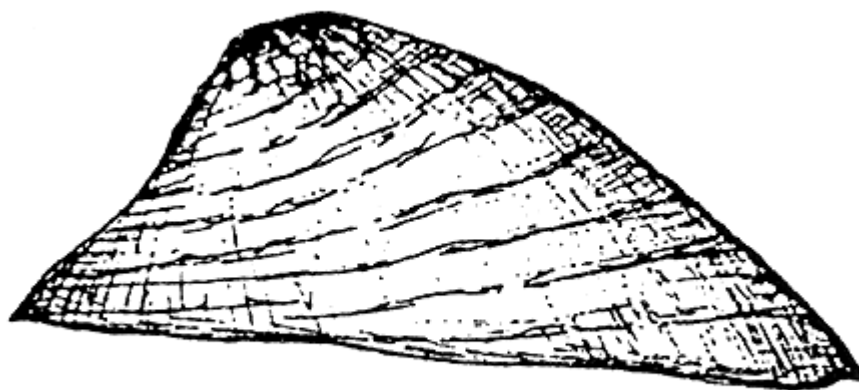
I



J Limpet, *Ferrissia*, Family Ancyliidae

(2 mm)

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J

K Limpet, *Lanx*, Family Lanicidae

(10 mm)

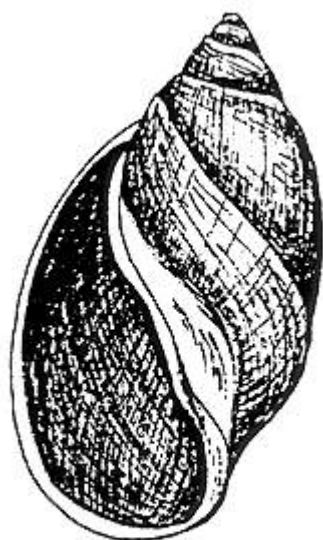


K

L Pouch snail, *Physa*, Family Physidae

(3–5
mm)

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L

Plate 20. Some marine mollusks.

Snails (Class Gastropoda):

A

Basket snail, *Nassarius*, Family
Nassidae

(2–5 cm)

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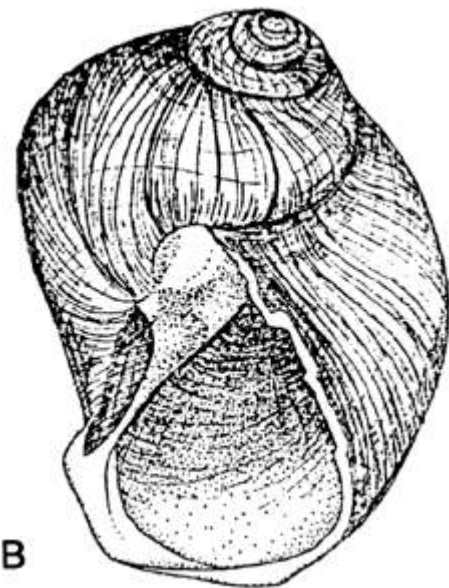


A

B

Moon snail, *Polinices*, Family
Naticidae

(5–8 cm)



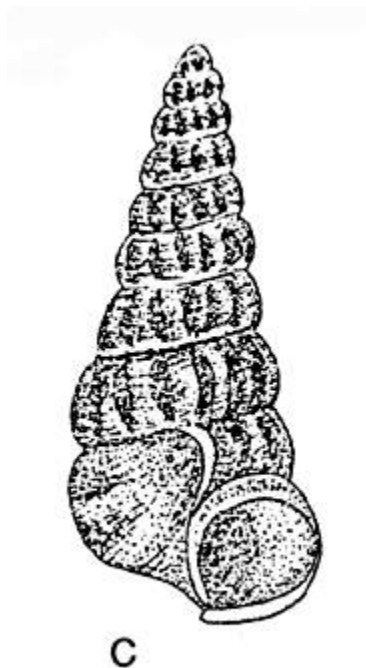
B

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C

Horn snail, *Cerithidea*, Family
Cerithiidae

(1–3 cm)



D

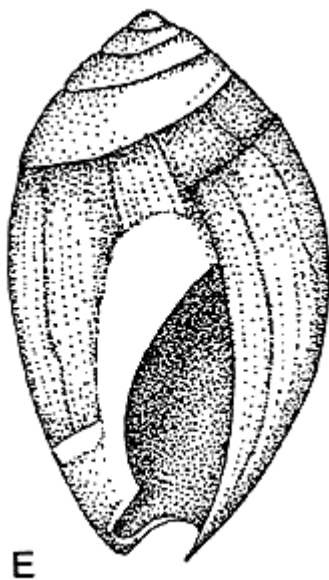
Bubble snail, *Bulla*, Family Bullidae (3–5 cm)

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E

Olive snail, *Olivella*, Family Olividae (2–3 cm)

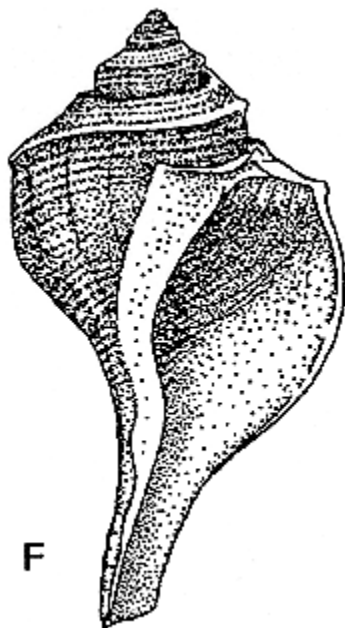


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F

Busycon, Family Melongenidae

(5–8 cm)



G

Periwinkle, *Littorina*, Family
Littorinidae

(1 cm)

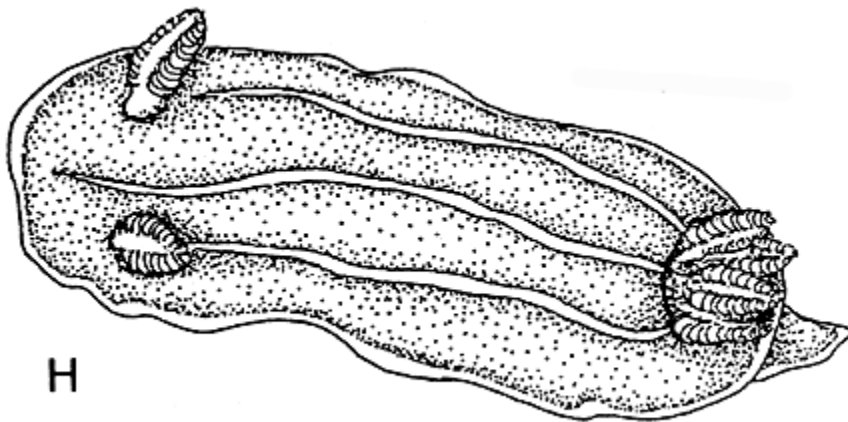
Standard Methods for the Examination of Water and Wastewater



G

H

Sea slug, *Chromodoris*



H

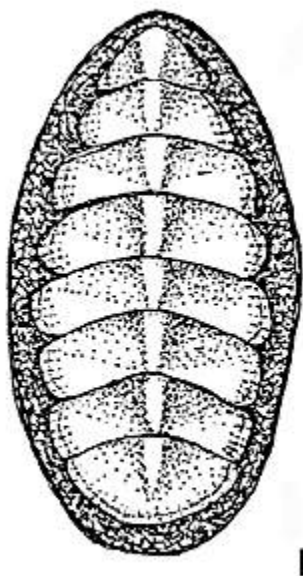
Chitons (Class Polyplacophora):

I

Conspicuous chiton, *Stenoplax*

(3–6 cm)

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Tusk shell (Class Scaphopoda):

J

Dentalium

(2–4 cm)



J

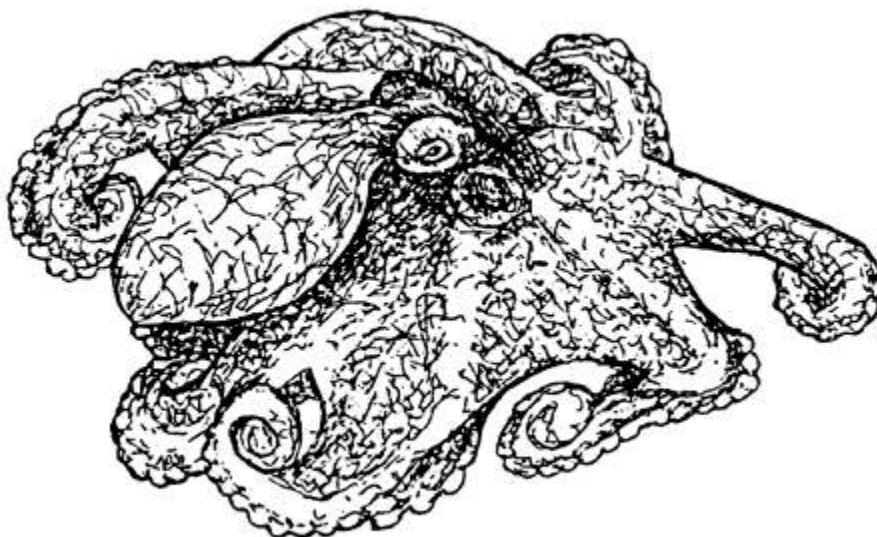
Standard Methods for the Examination of Water and Wastewater

Octopus (Class Cephalopoda):

K

Octopus

(3–10 cm up to 1 m or more)



K

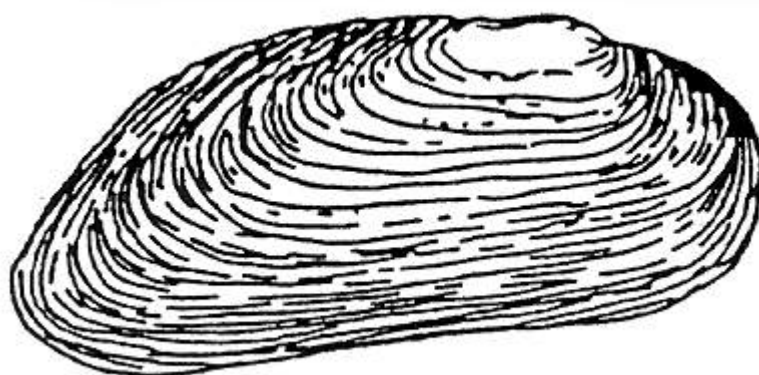
Plate 21. Bivalves (Phylum Mollusca, Class Pelecypoda).

Freshwater species:

A

Spectacle case, *Margaritifera*, Family
Margaritiferidae

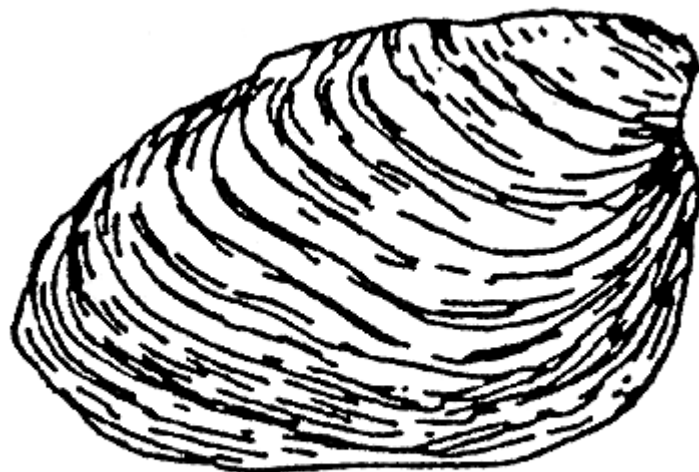
(10 cm)



A

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B Pearly mussel, *Pleurobema*, Family Unionidae (10 cm)



B

C Pearly mussel, *Gonidea*, Family Unionidae (10 cm)

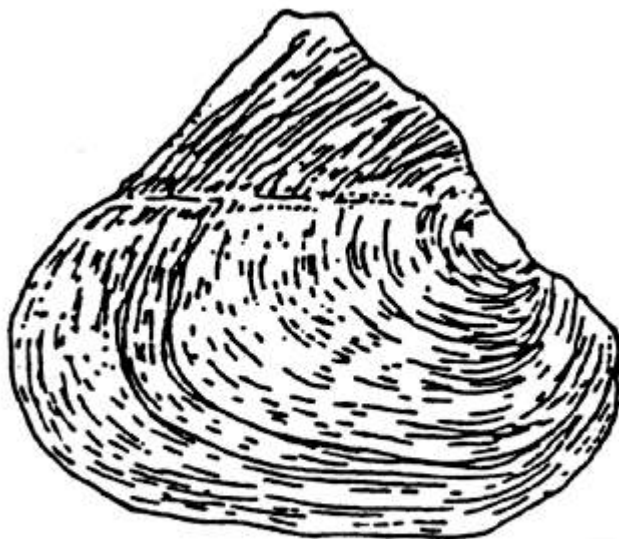
Standard Methods for the Examination of Water and Wastewater



C

D

Winged lampshell, *Proptera*, Family Lampsilinae (13 cm)



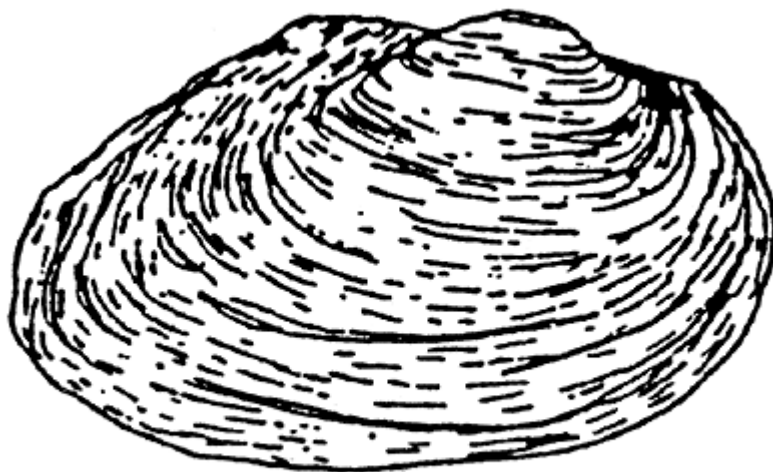
D

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E

Papershell, *Anodonta*, Family Anodontidae

(14 cm)



E

F

Zebra mussel (examples of color patterns),
Dreissena

(5 cm)



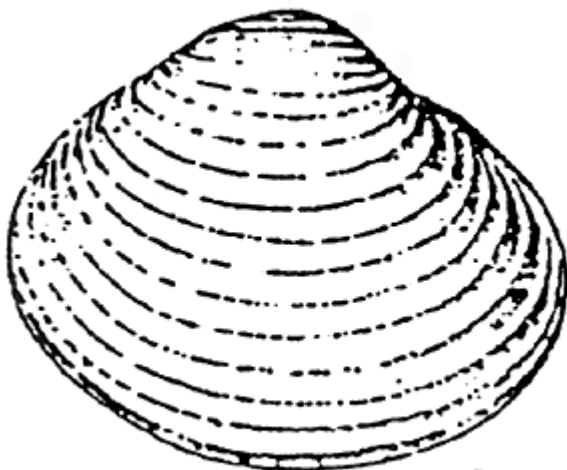
F

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G

Asiatic clam, *Corbicula*, Family Corbiculidae

(4 cm)



G

H

Fingernail clam, *Sphaerium*, Family Sphaeriidae (1 cm)

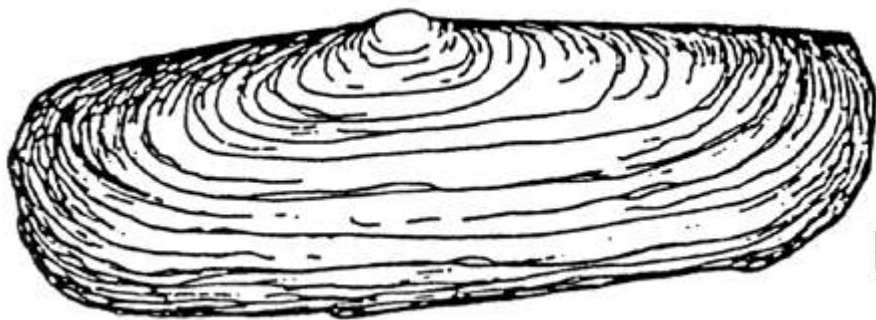


H

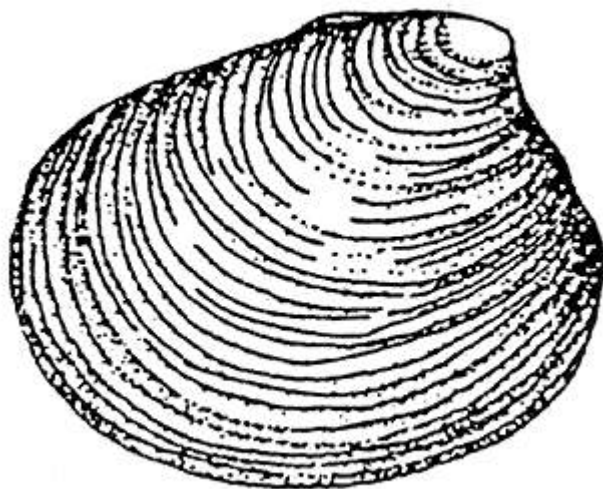
Marine species:

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- I Jackknife clam, *Tagelus*, Family Psammobiidae (6–10 cm)



-
- J Quahog clam, *Mercenaria*, Family Veneridae (6–10 cm)



-
- K Soft-shelled clam, *Mya*, Family Myidae (5–10 cm)

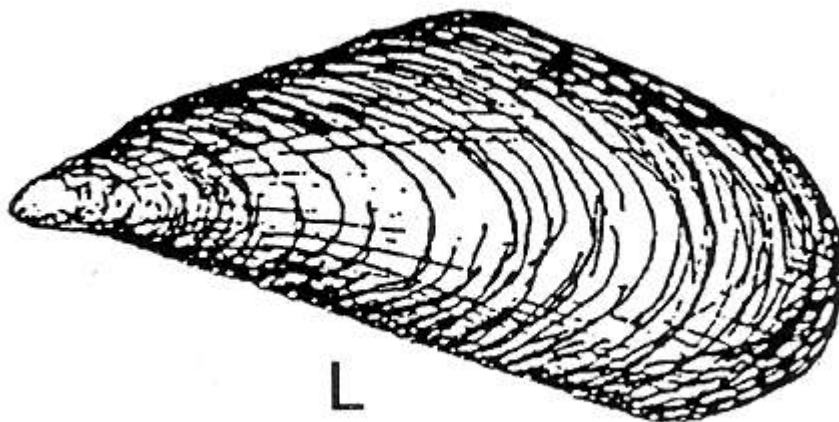
Standard Methods for the Examination of Water and Wastewater



L

Blue mussel, *Mytilus*, Family Mytilidae

(6 cm)

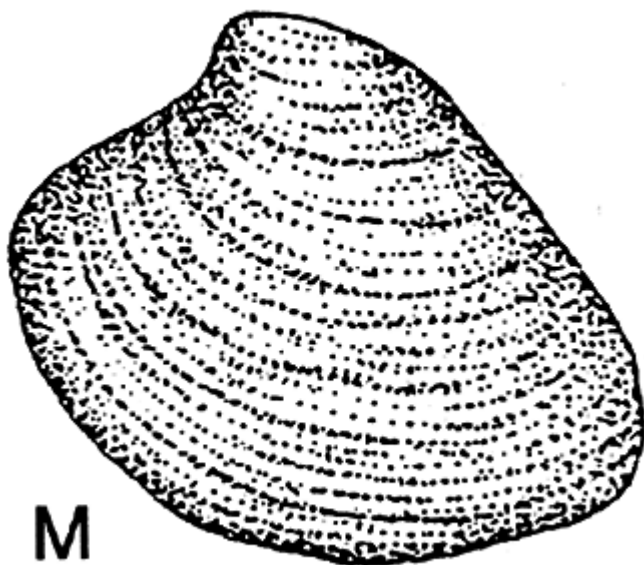


M

Rangia clam, *Rangia*, Family Matricidae

(5 cm)

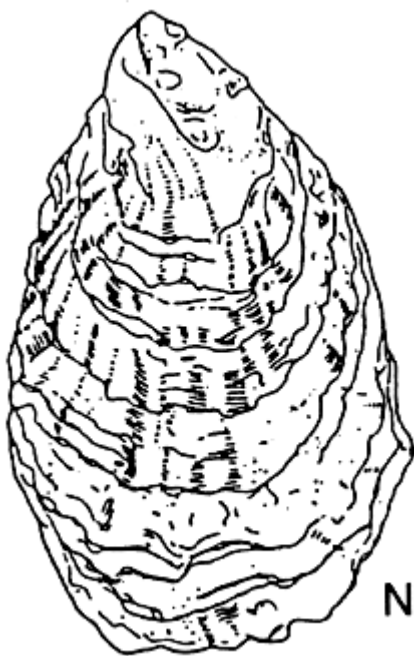
Standard Methods for the Examination of Water and Wastewater



N

Oyster, *Crassostrea*, Family Ostreidae

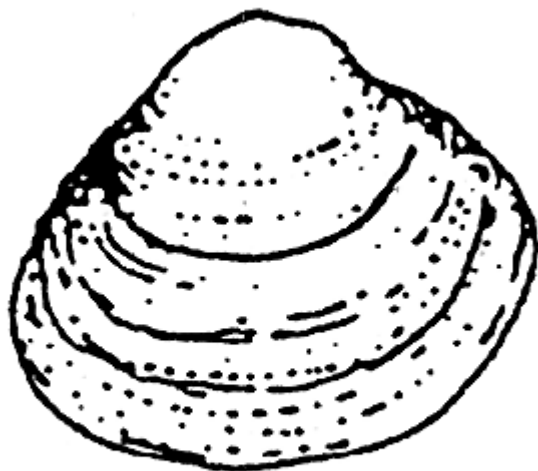
(9 cm)



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O

Marsh clam, *Polymesoda*, Family Corbiculidae (4 cm)



O

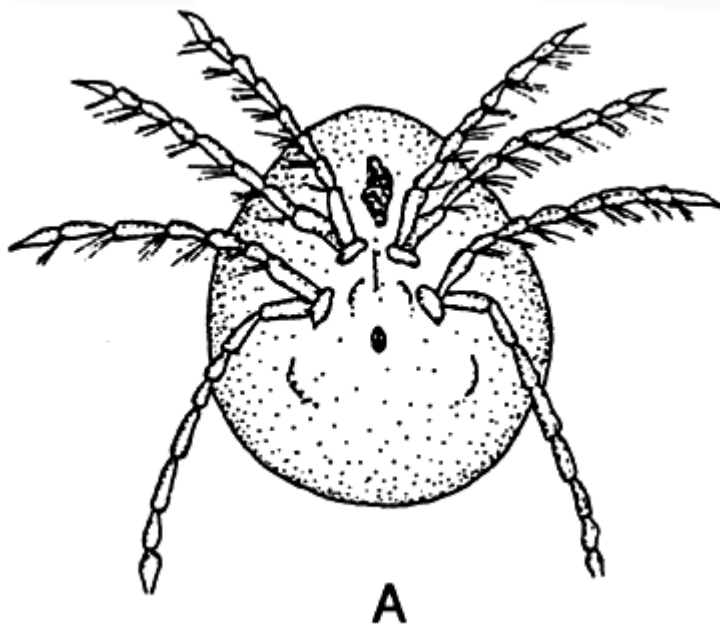
Plate 22. Miscellaneous invertebrates.

Arthropods (Phylum Arthropoda):

A

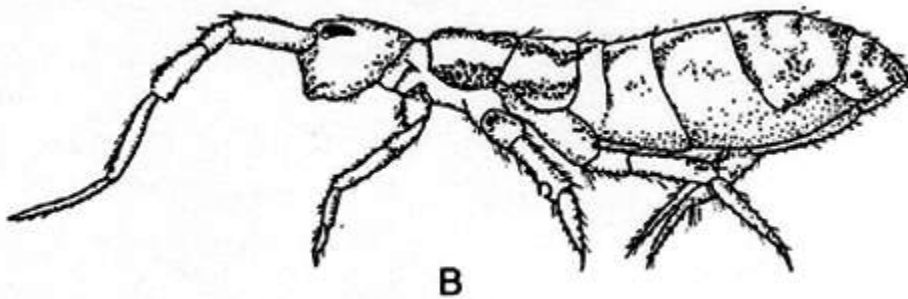
Water mite, *Limnochares*, Class Arachnoidea, freshwater

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B

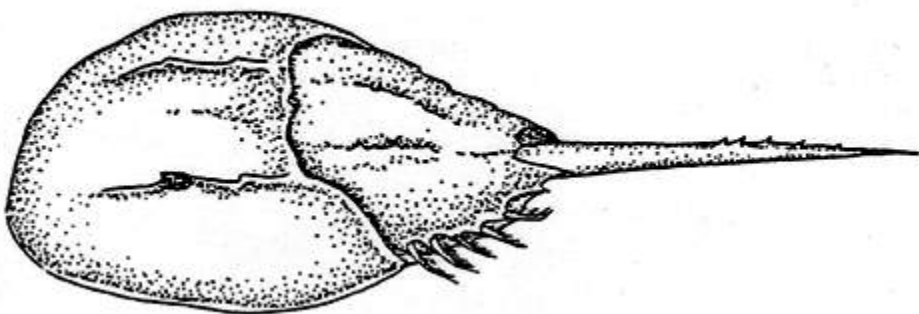
Springtail, *Orchesella*, Class Insecta, Order Collembola, freshwater



C

Horseshoe crab, *Limulus*, Class Arachnoidea, marine

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C

D

Phoronis, Phylum Phoronidea, marine

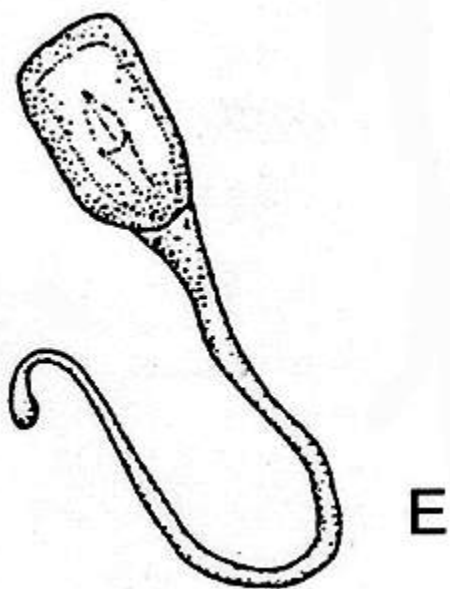


D

E

Glottidia, Phylum Brachiopoda, marine

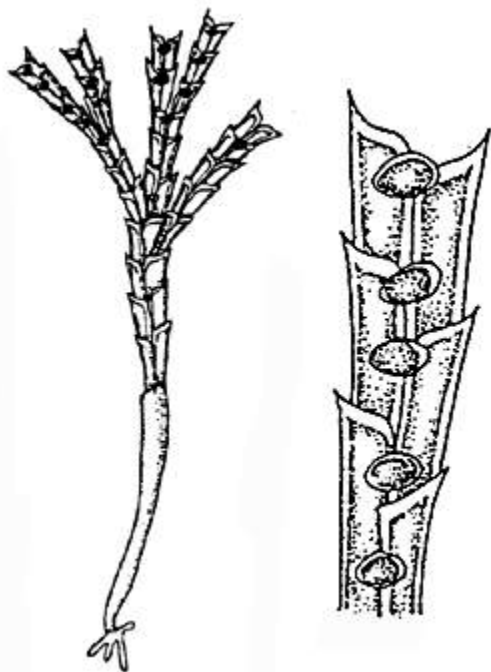
Standard Methods for the Examination of Water and Wastewater



Phylum Ectoprocta (Bryozoa):

F

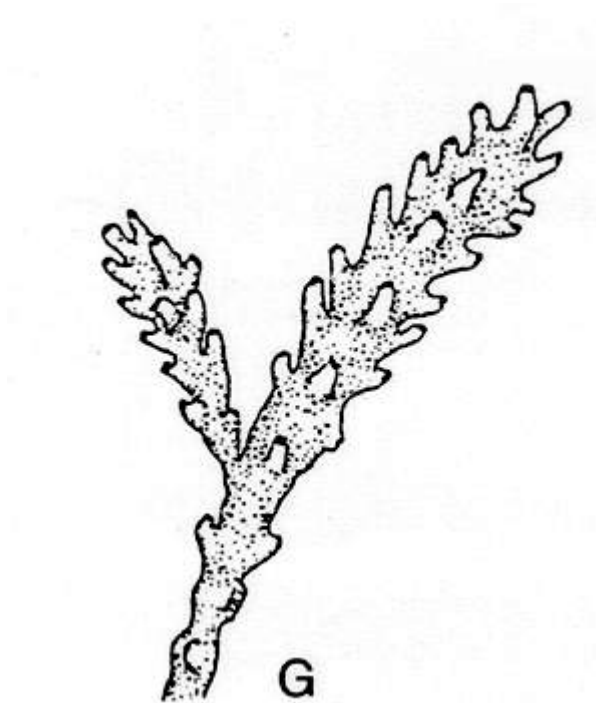
Bugula, a. colony, b. enlarged view, marine



G

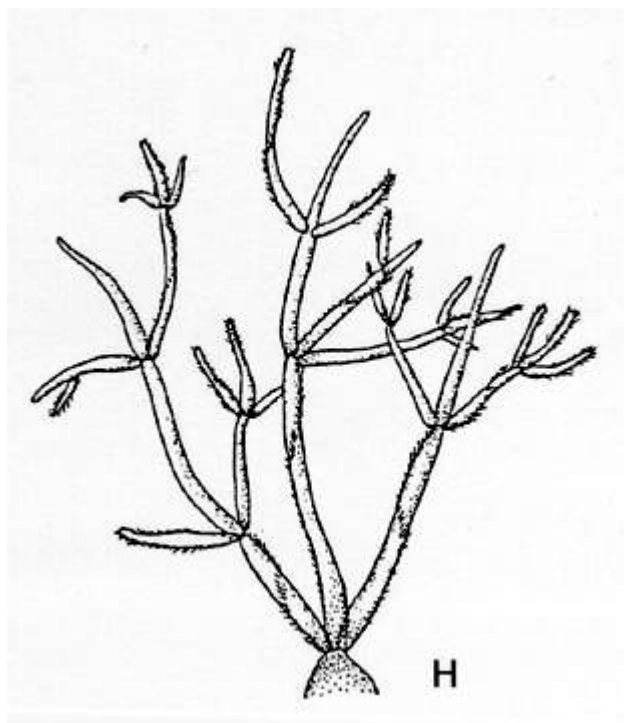
Crisulipora, calcareous colony, marine

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H

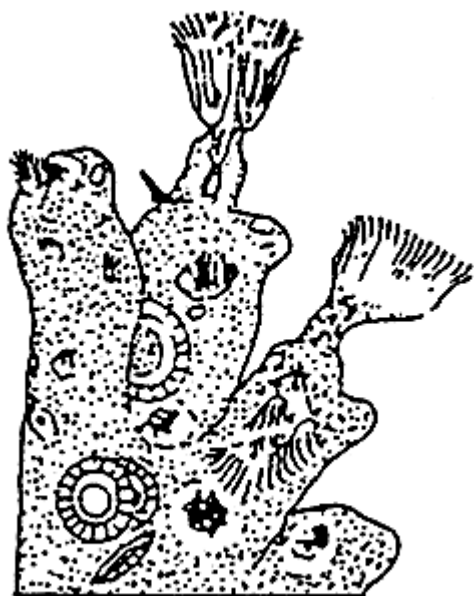
Zoobotryon, gelatinous colony, marine



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I

Jellyball, *Pectinatella*, young colony, freshwater



J

Pectinatella, statoblast, freshwater

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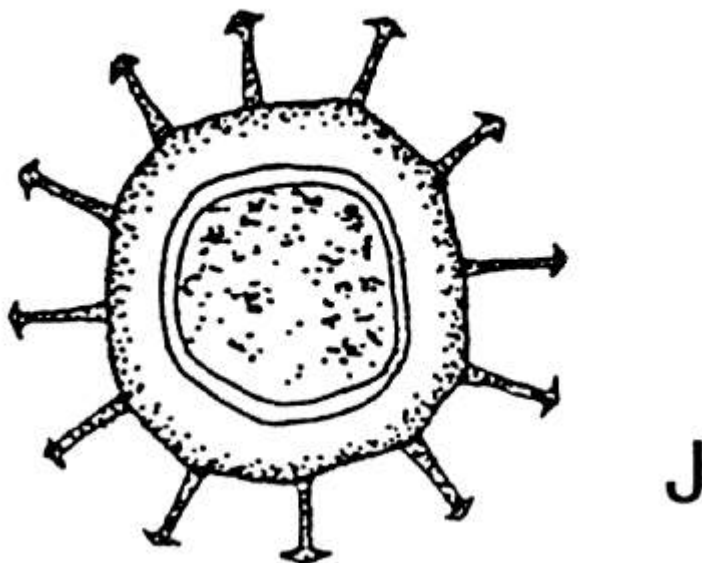
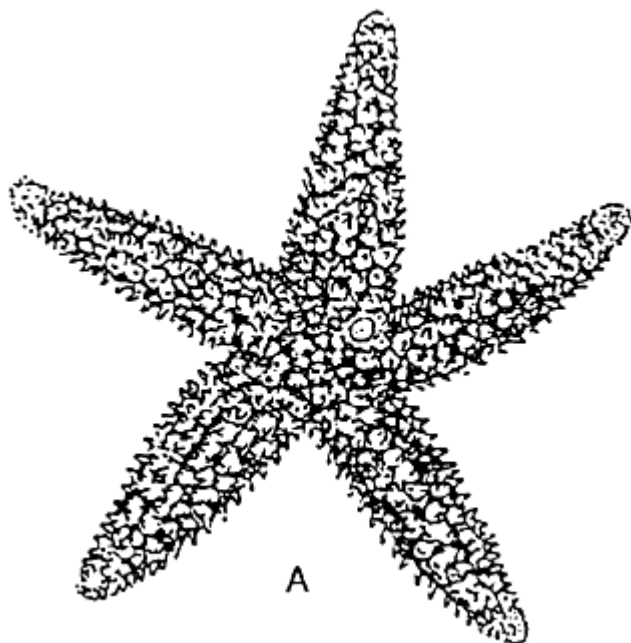


Plate 23. Echinoderm types (Phylum Echinodermata). All marine.

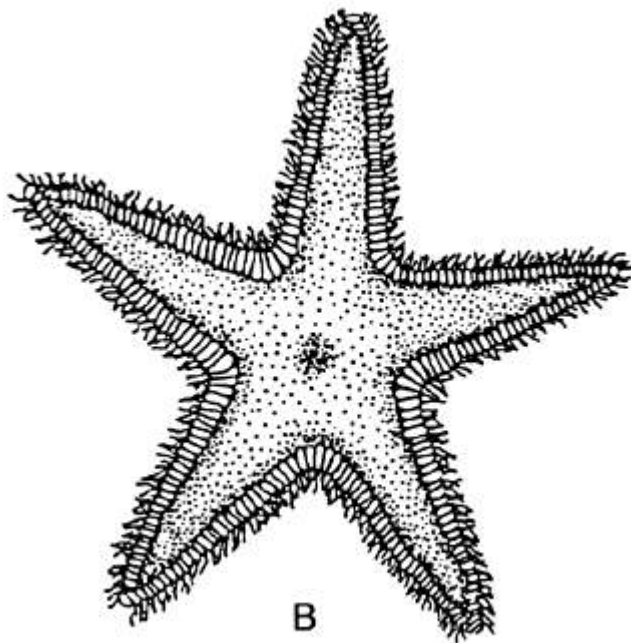
A Starfish, *Asterias*, Class Asteroidea

(15 cm)

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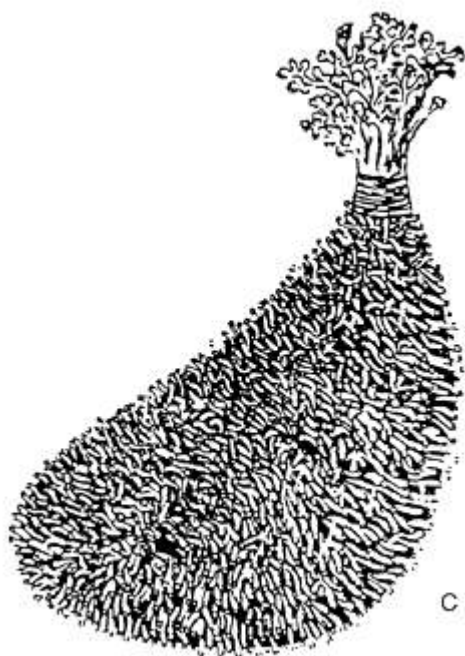


B Sand starfish, *Astropecten*, Class Asteroidea (10–20 cm)

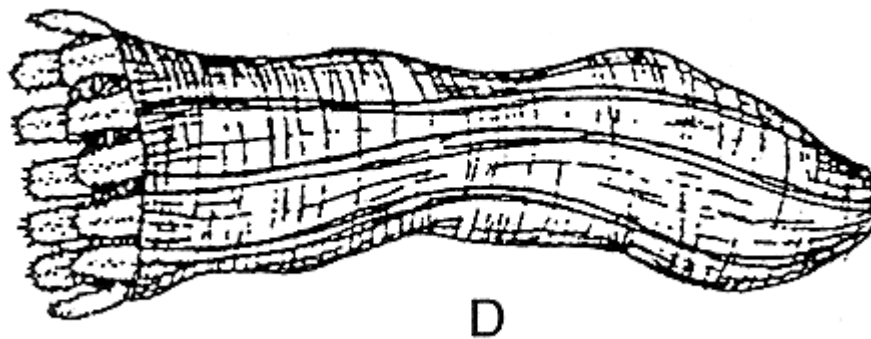


C Sea cucumber, *Thyrone*, Class Holothuroidea (10 cm)

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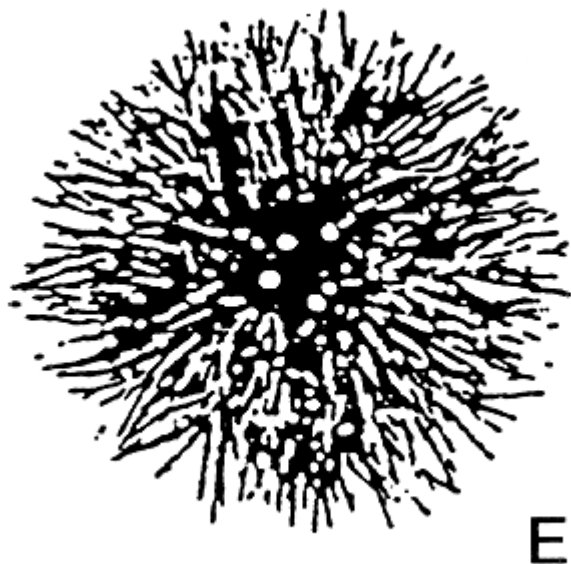


D Sea cucumber, *Leptosynapta*, Class Holothuroidea (5–8 cm)



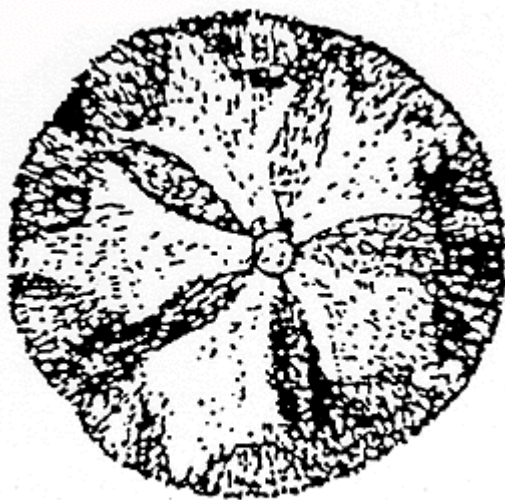
E Sea urchin, *Strongylocentrotus*, Class Echinoidea (6 cm)

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E

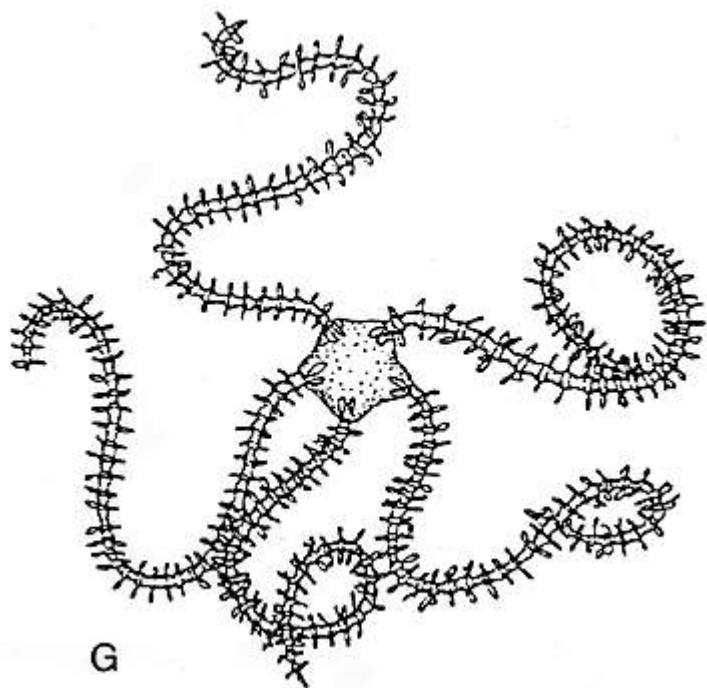
F Sand dollar, *Echinarachnius*, Class Echinoidea (7 cm)



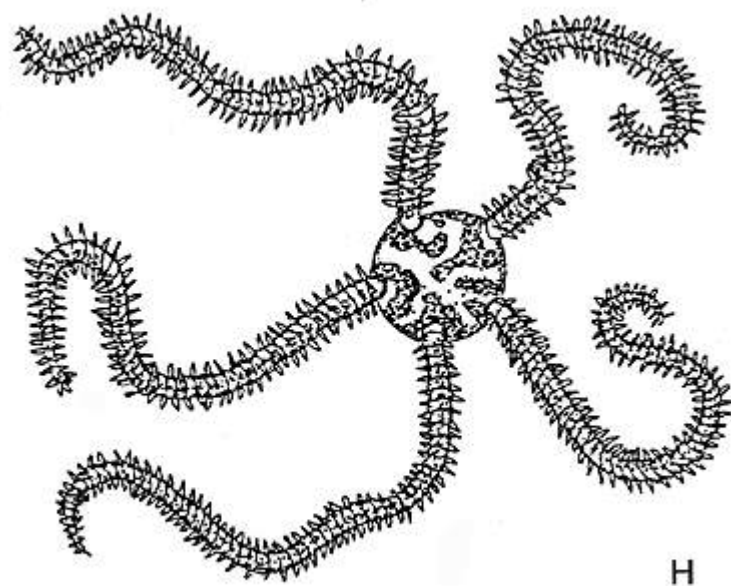
F

G Brittle star, *Amphiodia*, Class Ophiuroidea (disk 15 mm)

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H Brittle star, *Ophiopholis*, Class Ophiuroidea (disk 15 mm)



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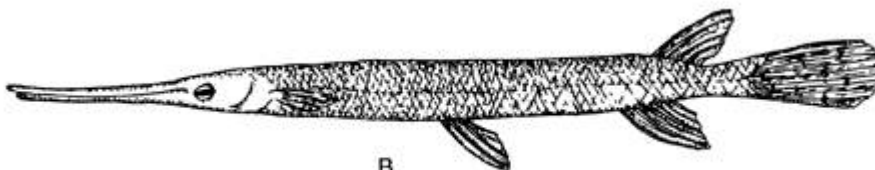
Plate 24. Some types of fishes (Phylum Chordata).

-
- A Jawless fish, lamprey, *Petromyzon*, Class Agnatha, freshwater (30–45 cm)



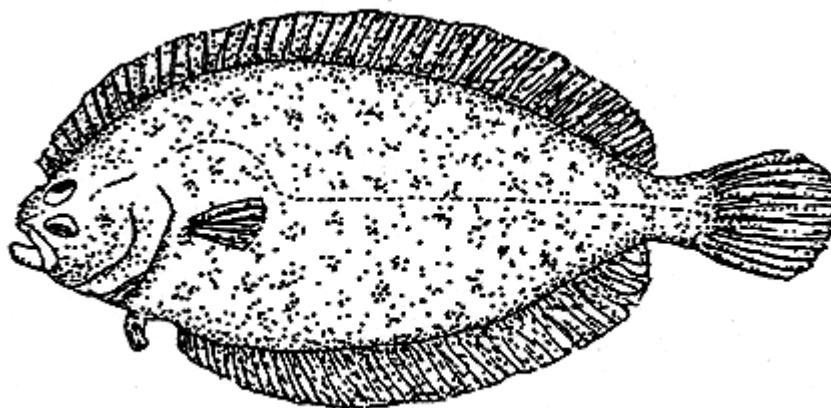
A

-
- B Ganoid fish, long-nosed gar, *Lepisosteus*, Class Osteichthys, (2.4 m) freshwater



B

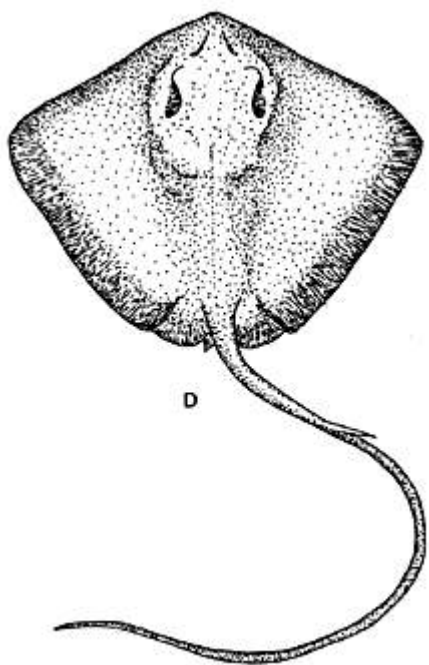
-
- C Flatfish, flounder, *Paralichthys*, Class Osteichthys, marine (30–60 cm)



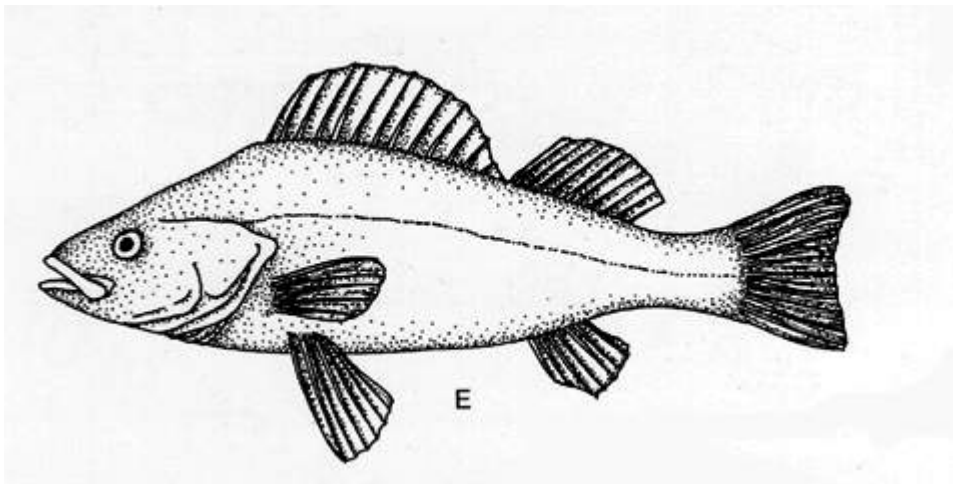
C

-
- D Cartilage fish, stingray, *Dasyatis*, Class Chondrichthys, marine (2 m)

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D Spiny-rayed fish, perch, *Perca*, Class Osteichthys, freshwater (30 cm)



E Soft-rayed fish, rainbow trout, *Salmo*, Class Osteichthys, freshwater (30 cm)

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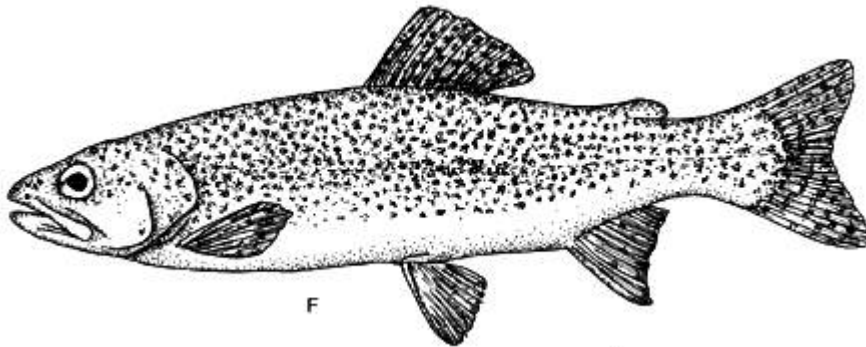
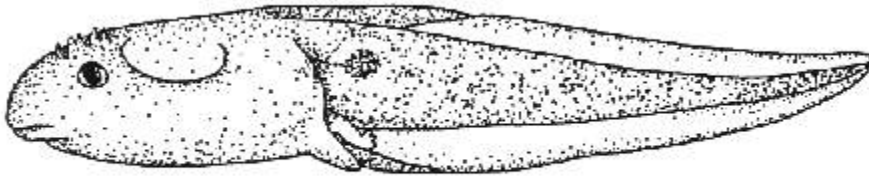


Plate 25. Types of amphibians (Phylum Chordata, Class Amphibia). All freshwater.

Frogs and toads (Order Salientia):

A

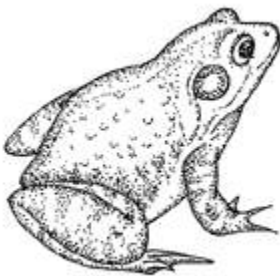
Tadpole larva with hind legs beginning to develop from the t



A

B

Frog, *Rana*

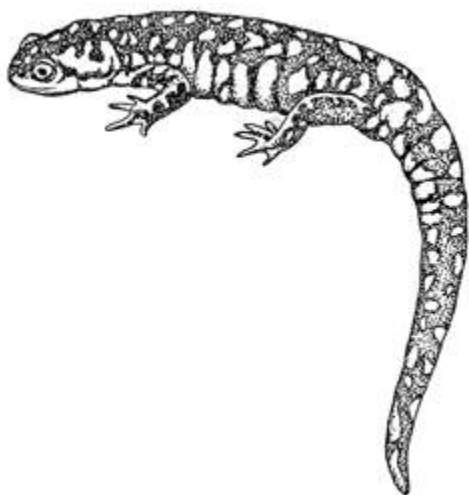


Salamanders (Order Caudata):

C

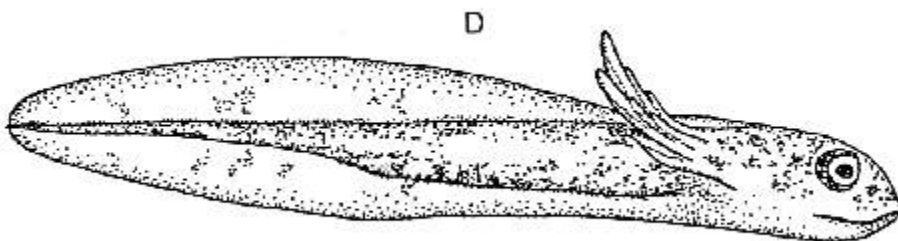
Ambystoma, adult, typically terrestrial

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D

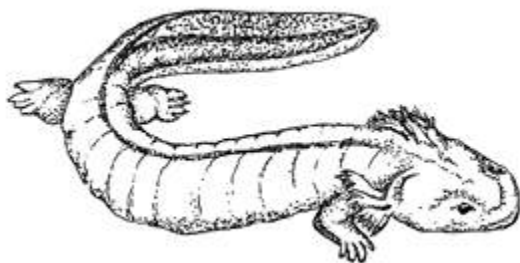
Ambystoma, larva, inhabits water



D

E

Water dog or mud puppy, *Necturus*, gills present in adult



F

Salamander with flat tail, *Notophthalmus*

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G

Salamander, *Siren*, with gills and front pair of legs present

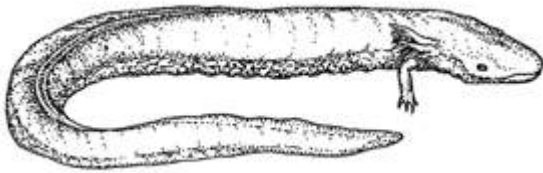


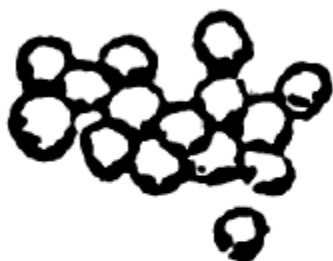
Plate 26. Bacteria and fungi. Diameter of most bacterial cells is less than 2 μm , although *Beggiatoa* (J) may range up to 16 μm in diameter and be of indefinite length. All freshwater inhabitants.

Bacterial cellular forms and arrangements:

A

Micrococcus

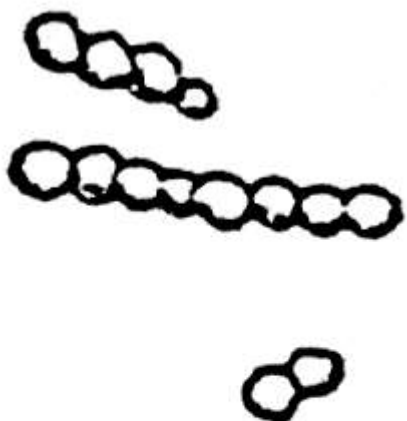
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A

B

Streptococcus



B

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C

Sarcina



C

D

Bacillus

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D

E

Vibrio



E

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F

Spirillum



F

Sewage organisms:

G

Sphaerotilus cells

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G

H

Sphaerotilus growth form



H

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I

Zoogloea growth form



I

J

Beggiatoa (sulfur bacterium)

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J

K

An actinomycete growth form from compost



K

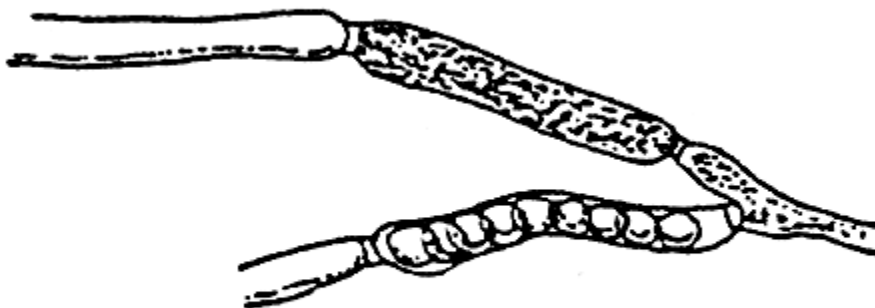
Fungi:

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L

Leptomitus, showing zoospores and cellulose plugs



L

M

Zoophagus with rotifer on mycelial peg (diam 3 μ)

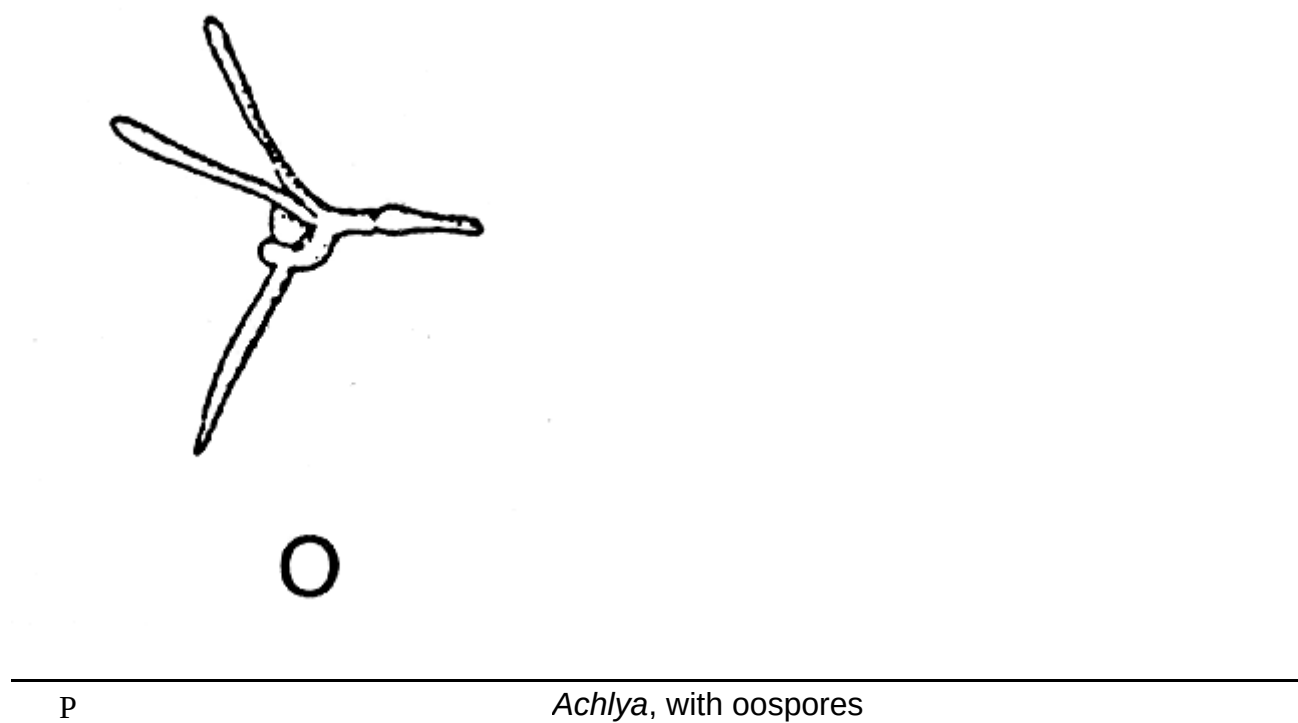
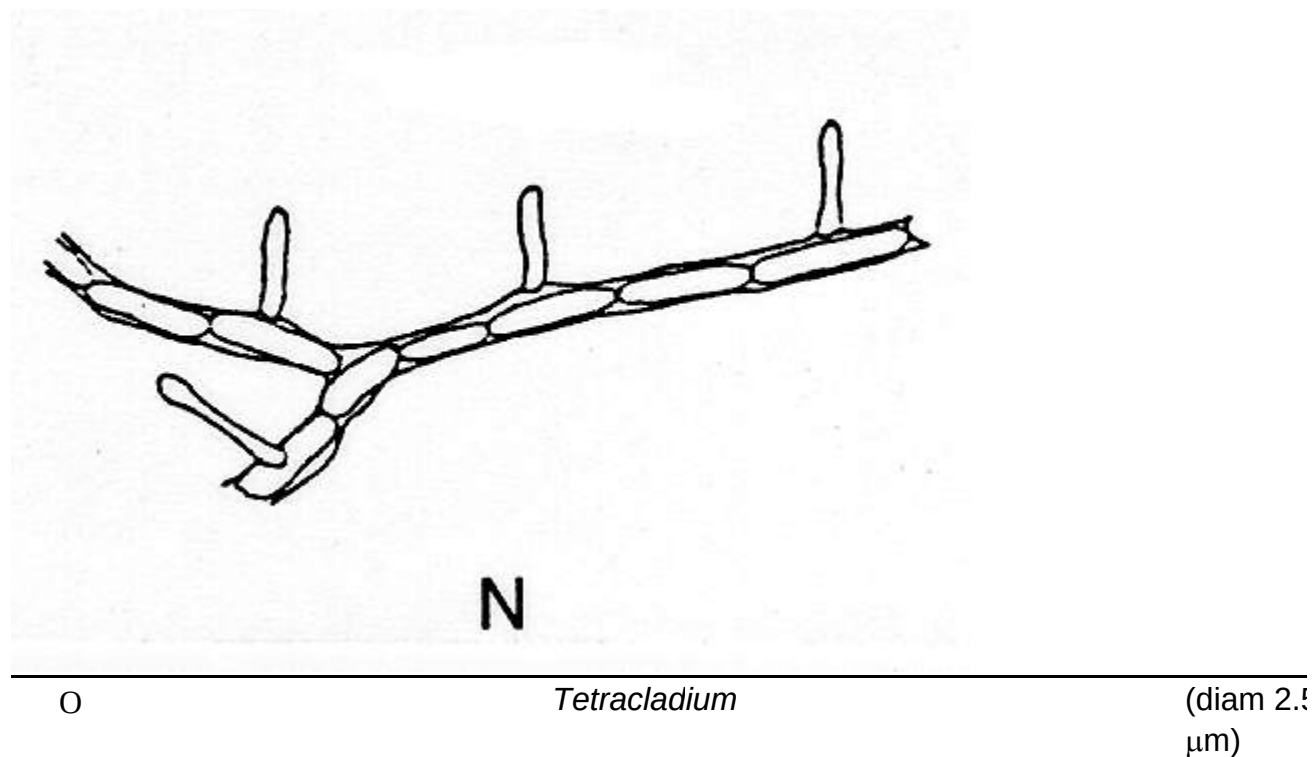


M

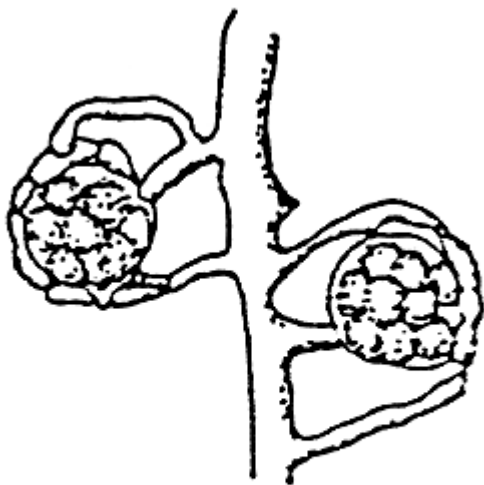
N

Zoophagus, showing mycelial pegs

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P

Q

Achlya, with encysted oospores

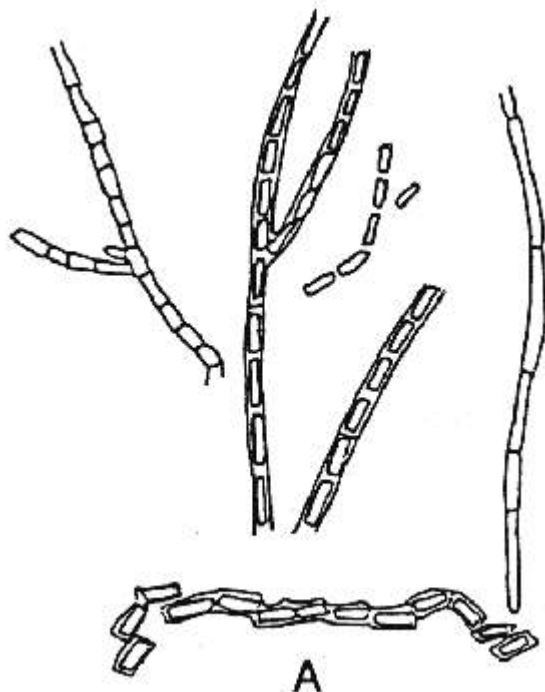


Q

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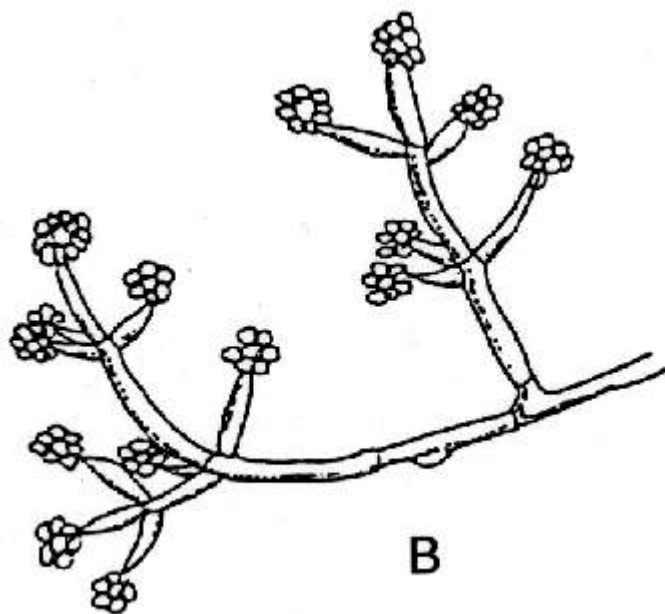
Plate 27. Fungi. All freshwater inhabitants.

A *Geotrichum candidum* colonies white



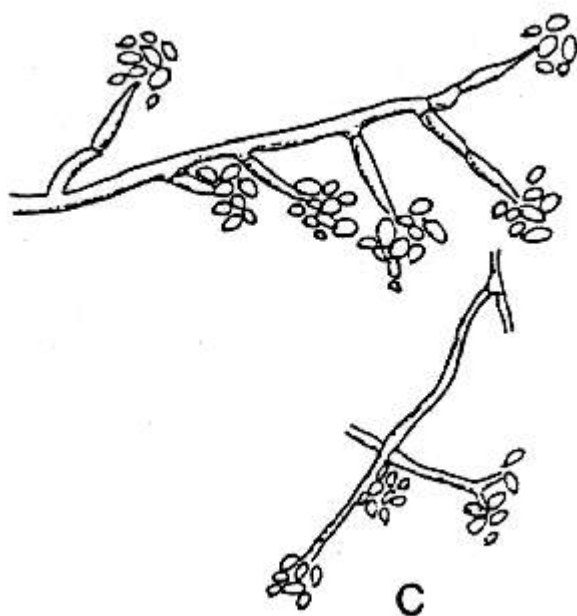
B *Trichoderma viride* colonies green

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C *Exophiala jeanselmei*

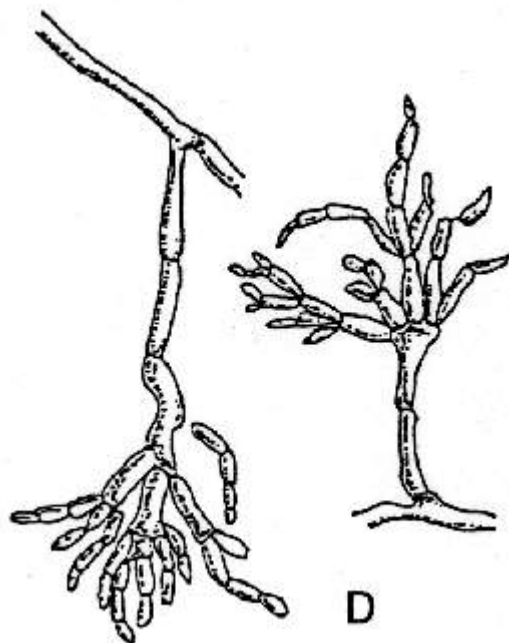
colonies black



D *Cladosporium cladosporioides*

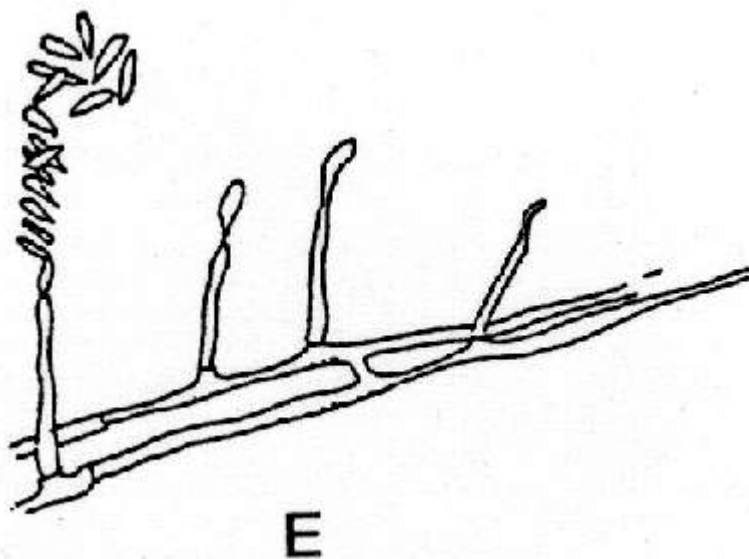
colonies black

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E *Fusarium* sp.

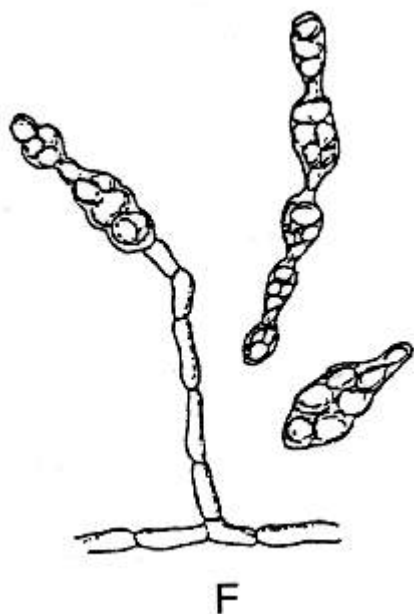
colonies orange



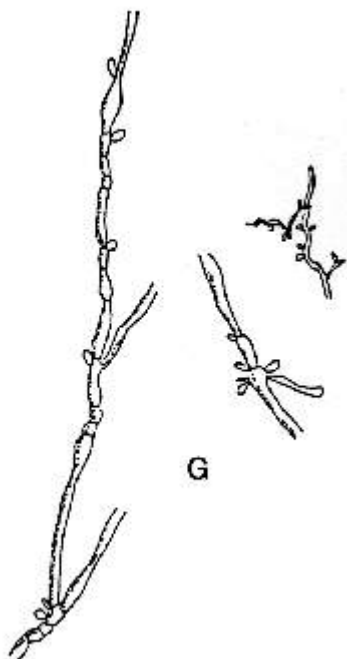
F *Alternaria alternata*

colonies gray

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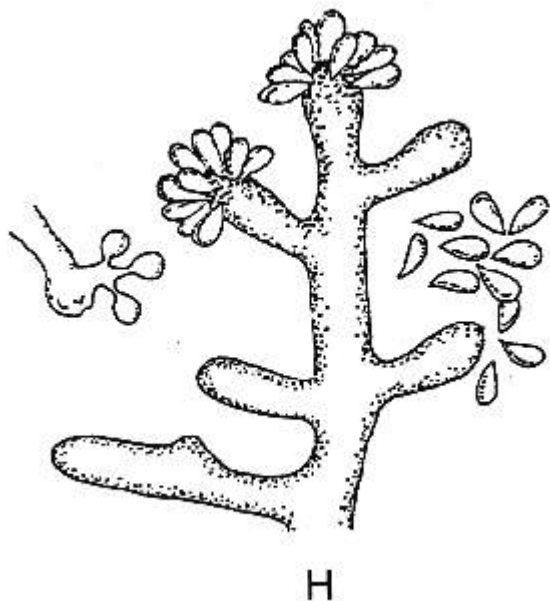


G *Trichosporon cutaneum* colonies white

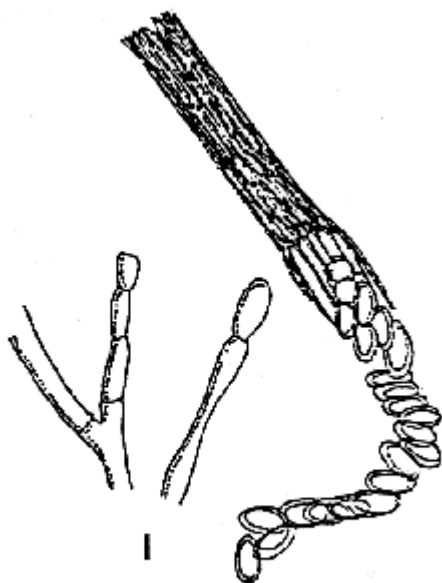


H *Pseudodallescheria boydii* colonies white

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I *Klusterskaya splendens*



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10900 C. Key for Identification of Freshwater Algae Common in Water Supplies and Polluted Waters*#(1) (Plate 1A, Plate 1B, Plate 28– Plate 35)

Beginning with 1a and 1b, choose one of the two contrasting statements and follow this procedure with the “a” and “b” statements of the number given at the end of the chosen statement. Continue until the name of the alga is given instead of another key number.

<i>Statement</i>		<i>Refer to Couplet No.</i>
1a.	Plastid (separate color body) absent; complete protoplast pigmented; generally blue-green; iodine starch test† negative (cyanobacteria, blue-green algae)	4
1b.	Plastid or plastids present; parts of protoplast free of some or all pigments; generally green, brown, red, etc., but not blue-green; iodine starch test† positive or negative	2
2a.	Cell wall permanently rigid (never showing evidence of collapse), and with 29 regular pattern of fine markings (striations, etc.); plastids brown to green; iodine starch test† negative; flagella absent; wall of two essentially similar halves, one placed over the other as a cover (diatoms)	
2b.	Cell wall, if present, capable of sagging, wrinkling, bulging, or rigidity, depending on existing turgor pressure of cell protoplast; regular pattern of fine markings on wall generally absent; plastids green, red, brown, etc.; iodine starch test† positive or negative; flagella present or absent; cell wall continuous and generally not of two parts	3
3a.	Cell or colony motile; flagella present (often not readily visible); anterior and posterior ends of cell different from one another in contents and often in shape (flagellate algae)	51

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<i>Statement</i>	<i>Refer to Couplet No.</i>
3b. Nonmotile; true flagella absent; ends of cells often not differentiated (green algae 77 and associated forms)	
1. Cyanobacteria (Blue-Green Algae)	
4a. Cells in filaments (or much elongated to form a thread)	5
4b. Cells not in (or as) filaments	23
5a. Heterocysts present	6
5b. Heterocysts absent	14
6a. Heterocyst located at one end of filament	7
6b. Heterocysts at various locations in filament	9
7a. Filaments radially arranged in a gelatinous bead	<i>Rivularia</i>
7b. Filaments isolated or irregularly grouped	8
8a. Filament gradually narrowed to one end	<i>Calothrix</i>
8b. Filament not gradually narrowed to one end	<i>Cylindrospermum</i>
9a. Filament unbranched	10
9b. Filament with occasional (false) branches	13
10a. Crosswalls in filament much closer together than width of <i>Nodularia</i> filament	
10b. Crosswalls in filament at least as far apart as width of filament	11

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<i>Statement</i>	<i>Refer to Couplet No.</i>
11a. Filaments normally in tight parallel clusters; heterocysts and spores cylindric to long oval in shape.	<i>Aphanizomenon</i>
11b. Filaments not in tight parallel clusters; heterocysts and spores often round to oval	12
12a. Filaments in a common gelatinous mass	<i>Nostoc</i>
12b. Filaments not in a common gelatinous mass	<i>Anabaena</i>
13a. False branches in pairs	<i>Scytonema</i>
13b. False branches, single	<i>Tolypothrix</i>
14a. Filament or elongated cell attached at one end, with one or more round cells (spores) at the other	<i>Chamaesiphon</i>
14b. Filament generally not attached at one end; no terminal spores present	15
15a. Filament with regular spiral form throughout	16
15b. Filament not spiral, or with spiral form limited to a portion of filament	17
16a. Filament septate	<i>Arthrospira</i>
16b. Filament nonseptate	<i>Spirulina</i>
17a. Filament very narrow, only 0.5 to 2.0 µm wide	<i>Schizothrix</i>
17b. Filament 3 to 95 µm wide	18
18a. Filaments loosely aggregated or not in clusters	19
18b. Filaments tightly aggregated and surrounded by a common gelatinous secretion that may be invisible	22
19a. Filament surrounded by wall-like sheath that frequently extends beyond the ends of the filament of cells; filament generally without movement	20
19b. Filament not surrounded by a wall-like sheath; filament may show movement	21

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<i>Statement</i>		<i>Refer to Couplet No.</i>
20a.	Cells separated from one another by a space	<i>Johannesbaptisti</i>
20b.	Cells in contact with adjacent cells	<i>Lyngbya</i>
21a.	All filaments short, with less than 20 cells; one or both ends of filament sharply pointed	<i>Raphidiopsis</i>
21b.	Filaments long, with more than 20 cells; filaments commonly without sharp-pointed ends	<i>Oscillatoria</i>
22a.	Filaments arranged in a tight, essentially parallel bundle	<i>Microcoleus</i>
22b.	Filaments arranged in irregular fashion, often forming a mat	<i>Phormidium</i>
23a.	Cells in a regular pattern of parallel rows, forming a plate	<i>Merismopedia</i>
23b.	Cells not regularly arranged to form a plate	24
24a.	Cells regularly arranged near surface of a spherical gelatinous bead	25
24b.	Gelatinous bead, if present, not spherical	26
25a.	Cells ovate to heart-shaped, connected to center of bead by colorless stalks	<i>Gomphosphaeria</i>
25b.	Cells round, without gelatinous stalks	<i>Coelosphaerium</i> type
26a.	Cells cylindric-oval	<i>Aphanothece</i>
26b.	Cells spherical	27
27a.	Two or more distinct layers of gelatinous sheath around each cell or cell cluster	<i>Gloeocapsa</i>

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<i>Statement</i>	<i>Refer to Couplet No.</i>
27b. Gelatinous sheath around cells not distinctly layered	28
28a. Cells isolated or in colonies of 2 to 32 cells	<i>Chroococcus</i>
28b. Cells in colonies composed of many cells	<i>Anacystis</i> (<i>Microcystis</i> , <i>Polycystis</i>)

2. Diatoms

29a. Front (valve) view circular in outline; markings radial in arrangement; cells may form a filament (centric diatoms)	30
29b. Front (valve) view elongate, not circular; transverse markings in one or two longitudinal rows; cells, if grouped, not forming a filament (pennate diatoms)	32
30a. Cells in persistent filaments with valve faces in contact; therefore, cells commonly seen in side (girdle) view	<i>Melosira</i>
30b. Cells isolated or in fragile filaments, often seen in front (valve) view	31
31a. Radial markings (striations), in valve view, extending from center to margin; short spines often present around margin (valve view)	<i>Stephanodiscus</i>
31b. Area of prominent radial markings, in valve view, limited to approximately outer half of circle, marginal spines generally absent	<i>Cyclotella</i>
32a. Cell longitudinally symmetrical in valve view	33

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<i>Statement</i>	<i>Refer to Couplet No.</i>
32b. Cell longitudinally unsymmetrical (two sides unequal in shape), at least in valve view	49
33a. Raphe at or near the edge of the valve	34
33b. Raphe or pseudoraphe median or submedian	35
34a. Marginal, keeled raphe areas lie opposite one another on the two valves <i>Hantzschia</i>	
34b. Marginal, keeled raphe areas lie diagonal to one another on the two valves <i>Nitzschia</i>	
35a. Cell transversely symmetrical in valve view	36
35b. Cell transversely unsymmetrical (two ends unequal in shape or size), at least in valve view	44
36a. Cell round-oval in valve view, not more than twice as long as it is wide <i>Cocconeis</i>	
36b. Cell elongate, more than twice as long as it is wide	37
37a. Cell flat (girdle face wide, valve face narrow) <i>Tabellaria</i>	
37b. Girdle and valve faces about equal in width	38
38a. Cell with several markings (septa) extending without interruption across the valve face; no marginal line of pores present <i>Diatoma</i>	
38b. Cross-markings (striations or costae) on valve surface, interrupted by either longitudinal space (pseudoraphe), or line (raphe), or line of pores (carinal dots)	39

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<i>Statement</i>			<i>Refer to Couplet No.</i>
39a.	Cells attached side by side to form a ribbon of several to many cells	<i>Fragilaria</i>	
39b.	Cells isolated or in pairs		40
40a.	Cell narrow, linear, often narrowed to both ends; true raphe absent	<i>Synedra</i>	
40b.	Cell commonly "boat-shape" in valve view; true raphe present		41
41a.	Cell longitudinally unsymmetrical in girdle view; sometimes with attachment stalk	<i>Achnanthes</i>	
41b.	Cell symmetrical in girdle as well as valve view; generally not attached		42
42a.	Area without striations extending as a transverse belt around middle of cell	<i>Stauroneis</i>	
42b.	No continuous clear belt around middle of cell		43
43a.	Cell with coarse transverse markings (costae), which appear as solid lines even under high magnification	<i>Pinnularia</i>	
43b.	Cell with fine transverse markings (striae), which appear as lines of dots under high magnification	<i>Navicula</i>	
44a.	Cells attached together at one end only to form radiating colony	<i>Asterionella</i>	
44b.	Cell not forming a loose radiating colony		45
45a.	Cells in fan-shaped colonies	<i>Meridion</i>	
45b.	Cells isolated or in pairs		46
46a.	Prominent wall markings in addition to striations present just below lateral margins on valve surface of cell	<i>Surirella</i>	

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<i>Statement</i>	<i>Refer to Couplet No.</i>
46b. Wall markings along sides of valve limited to striations	47
47a. Cell elongate, sides almost parallel except for terminal knobs	<i>Asterionella</i>
47b. Sides of cell converging toward one end	48
48a. Cells bent in girdle view	<i>Rhoicosphenia</i>
48b. Cells straight in girdle view	<i>Gomphonema</i>
49a. Valves with transverse septa or costae	<i>Epithemia</i>
49b. Valves with no transverse septa or costae	50
50a. Raphe located almost through center of valve	<i>Cymbella</i>
50b. Raphe excentric, near concave edge of valve	<i>Amphora</i>

3. Flagellate Algae

51a. Cell in a loose, rigid conical sac (lorica); isolated or in a branching colony	<i>Dinobryon</i>
51b. Case or sac, if present, not conical; colony, if present, not branching	52
52a. Cells isolated or in pairs	53
52b. Cells in a colony of four or more cells	71
53a. Prominent transverse groove encircles cell	54
53b. Cell without transverse groove	56
54a. Cell with prominent rigid projections, one forward and two or three on posterior end	<i>Ceratium</i>

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<i>Statement</i>		<i>Refer to Couplet No.</i>
54b.	Cell without several rigid polar projections	55
55a.	Portions above and below transverse groove about equal	<i>Peridinium</i>
55b.	Front portion distinctly larger than posterior portion	<i>Massartia</i>
56a.	Cell with long bristles extending from surface plates	<i>Mallomonas</i>
56b.	Cell without bristles and surface plates	57
57a.	Cell protoplast enclosed in loose, rigid covering (lorica)	58
57b.	Cell with tight membrane or wall but no loose, rigid covering	60
58a.	Lorica flattened; cell with two flagella	<i>Phacotus</i>
58b.	Lorica not flattened; cell with one flagellum	59
59a.	Lorica often opaque, generally dark brown to red; plastid green	<i>Trachelomonas</i>
59b.	Lorica often transparent, colorless to light brown; plastid light brown	<i>Chrysococcus</i>
60a.	Plastids brown to red to olive or blue-green	61
60b.	Plastids grass green	64
61a.	Plastids blue-green to blue	<i>Chroomonas</i>
61b.	Plastids brown to red to olive green	62
62a.	Plastid brown; one or two flagella	63
62b.	Plastids red, red-brown, or olive green; two flagella	<i>Rhodomonas</i>
63a.	Anterior end of cell oblique; two flagella	<i>Cryptomonas</i>
63b.	Anterior end of cell rounded or pointed; one flagellum	<i>Chromulina</i>
64a.	Cell with colorless rectangular wing	<i>Pteromonas</i>

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<i>Statement</i>	<i>Refer to Couplet No.</i>
64b. No wing extending from cell	65
65a. Cells flattened; margin rigid	<i>Phacus</i>
65b. Cell not flattened; margin rigid or flexible	66
66a. Pyrenoid present in the single plastid; no paramylon; margin not flexible; two or more flagella per cell	67
66b. Pyrenoid absent; paramylon present; several plastids per cell; margin flexible or rigid; one flagellum per cell	70
67a. Cells fusiform (tapering at each end)	<i>Chlorogonium</i>
67b. Cells not fusiform, generally almost spherical	68
68a. Plastids numerous	<i>Vacuolaria</i>
68b. Plastids few, commonly one	69
69a. Two flagella per cell	<i>Chlamydomonas</i>
69b. Four flagella per cell	<i>Carteria</i>
70a. Cell flexible in form; paramylon a capsule or disk; cell elongate	<i>Euglena</i>
70b. Cell rigid in form; paramylon ring-shaped; cell almost spherical	<i>Lepocinclis</i>
71a. Plastids brown	72
71b. Plastids green	73
72a. Cells in contact with one another	<i>Synura</i>
72b. Cells separated from one another by space	<i>Uroglenopsis</i>
73a. Colony flat, one cell thick	<i>Gonium</i>

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<i>Statement</i>	<i>Refer to Couplet No.</i>
73b. Colony rounded, more than one cell thick	74
74a. Cells in contact with one another	75
74b. Cells separated from one another by space	76
75a. Cells radially arranged	<i>Pandorina</i>
75b. Cells all facing one direction	<i>Pyrobotrys</i> <i>(Chlamydoobotrys)</i>
76a. Cells more than 400 per colony	<i>Volvox</i>
76b. Cells less than 75 per colony	<i>Eudorina</i>

4. Green Algae and Associated Forms

77a. Cells jointed together to form a net	<i>Hydrodictyon</i>	
77b. Cells not forming a net		78
78a. Cells attached side by side to form a plate or ribbon one cell wide and thick; number of cells commonly two, four, or eight	<i>Scenedesmus</i>	
78b. Cells not attached side by side		79
79a. Cells isolated or in nonfilamentous or nontubular thalli		80
79b. Cells in filaments or other tubular or threadlike thalli		111
80a. Cells isolated and narrowest at the center because of incomplete fissure (desmids)		81

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<i>Statement</i>			<i>Refer to Couplet No.</i>
	80b. Cells isolated or in clusters but without central fissure		84
81a.	Each half of cell with three spinelike or pointed knobular extensions	<i>Staurostrum</i>	
81b.	Cell margin with no such extensions		82
	82a. Semicells with a median incision or depression		83
	82b. Semicells with no median incision or depression	<i>Cosmarium</i>	
83a.	Margin with rounded lobes	<i>Euastrum</i>	
83b.	Margin with sharp-pointed teeth	<i>Micrasterias</i>	
	84a. Cells elongate		85
	84b. Cells round to oval or angular		92
85a.	Cell radiating from a central point	<i>Actinastrum</i>	
85b.	Cells isolated or in irregular clusters		86
	86a. Cells with terminal spines	<i>Schroederia</i>	
	86b. Cells without terminal spines		87
87a.	Cells with colorless attachment area at one end	<i>Characium</i>	
87b.	No attachment area at one end of cell		88
	88a. Plastids two per cell; unpigmented area across center of cell	<i>Closterium</i>	
	88b. Cell with plastid that continues across the center		89
89a.	Cell 5 to 10 times as long as it is broad		90
89b.	Cell 2 to 4 times as long as it is broad		91

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<i>Statement</i>	<i>Refer to Couplet No.</i>
90a. Pyrenoid absent, or one per cell	<i>Ankistrodesmus</i>
90b. Pyrenoids several per cell	<i>Closteriopsis</i>
91a. Cells semicircular; cell ends pointed but with no terminal spines	<i>Selenastrum</i>
91b. Cells arcuate but less than semicircular; cell ends pointed and each with a short spine	<i>Closteridium</i>
92a. Cells regularly arranged in a tight, flat colony	<i>Pediastrum</i>
92b. Cells not in a tight, flat regular colony	93
93a. Cells angular	94
93b. Cells round to oval	95
94a. Two or more spines at each angle	<i>Polyedriopsis</i>
94b. Spines none or less than two at each angle	<i>Tetraedron</i>
95a. Cells with long, sharp spines	96
95b. Long, sharp spines absent	99
96a. Cells round	97
96b. Cells oval	98
97a. Cells isolated	<i>Golenkinia</i>
97b. Cells in colonies	<i>Micractinium</i>
98a. Each cell end has one spine	<i>Diacanthos</i>
98b. Each cell end has more than one spine	<i>Chodatella</i>
99a. Colony of definite regular form, round to oval	100
99b. Colony, if present, not a definite oval or sphere; or cells may be isolated	104

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<i>Statement</i>		<i>Refer to Couplet No.</i>
100a. Colony a tight sphere of cells		101
100b. Colony a loose sphere of cells enclosed by a common membrane		102
101a. Sphere solid, slightly irregular, no connecting processes between cells	<i>Planktosphaeria</i>	
101b. Sphere hollow, regular; short connecting processes between cells	<i>Coelastrum</i>	
102a. Cells round		103
102b. Cells oval	<i>Oocystis</i>	
103a. Cells connected to center of colony by branching stalk	<i>Dictyosphaerium</i>	
103b. No stalk connecting cells	<i>Sphaerocystis</i>	
104a. Oval cells, enclosed in a somewhat spherical, often orange-colored matrix	<i>Botryococcus</i>	
104b. Cells round, isolated or in colorless matrix		105
105a. Adjoining cells with straight, flat walls between their protoplasts		106
105b. Adjoining cells with rounded walls between their protoplasts		107
106a. Cells embedded in a common gelatinous matrix	<i>Palmella</i>	
106b. No matrix or sheath outside of cell walls	<i>Phytoconis (Protococcus)</i>	
107a. Cells loosely arranged in a large gelatinous matrix	<i>Tetraspora</i>	
107b. Cells isolated or tightly grouped in a small colony		108
108a. Cells located inside of protozoa	<i>Zoochlorella</i>	
108b. Cells not inside of protozoa		109

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<i>Statement</i>	<i>Refer to Couplet No.</i>
109a. Plastid filling 2/3 or less of cell	110
109b. Plastid filling ¾ or more of cell	<i>Chlorococcum</i>
110a. Cell diameter 2 µm or less; reproduction by cell division	<i>Nannochloris</i>
110b. Cell diameter 2.5 µm or more; reproduction by internal spores	<i>Chlorella</i>
111a. Cells attached end to end in an unbranched filament	112
111b. Thallus branched, or more than one cell wide	119
112a. Plastids in form of one or more marginal spiral ribbons	<i>Spirogyra</i>
112b. Plastids not in form of spiral ribbons	113
113a. Filaments, when breaking, separating through middle of cells	114
113b. Filaments, when breaking, separating irregularly or at ends of cells	115
114a. Starch test positive; cell margin straight; one plastid, granular	<i>Microspora</i>
114b. Starch test negative; cell margin slightly bulging; several plastids	<i>Tribonema</i>
115a. Marginal indentations between cells	<i>Desmidium</i>
115b. No marginal indentations between cells	116
116a. Plastids, two per cell	<i>Zygnema</i>
116b. Plastid, one per cell (sometimes appearing numerous)	117
117a. Some cells with walls having transverse wrinkles near one end; plastid an irregular net	<i>Oedogonium</i>
117b. No apical wrinkles in wall; plastid not porous	118

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<i>Statement</i>	<i>Refer to Couplet No.</i>
118a. Plastid a flat or twisted axial ribbon	<i>Mougeotia</i>
118b. Plastid an arcuate marginal band	<i>Ulothrix</i>
119a. Thallus a flat plate of cells	<i>Hildenbrandia</i>
119b. Thallus otherwise	120
120a. Thallus a long tube without crosswalls	<i>Vaucheria</i>
120b. Thallus otherwise	121
121a. Thallus a leathery strand with regularly spaced swellings and a continuous surface membrane of cells	<i>Lemanea</i>
121b. Thallus otherwise	122
122a. Filament unbranched	<i>Schizomeris</i>
122b. Filament branched	123
123a. Branches in whorls (clusters)	124
123b. Branches single or in pairs	126
124a. Thallus embedded in gelatinous matrix	<i>Batrachospermum</i>
124b. Thallus not embedded in gelatinous matrix	125
125a. Main filament one cell thick	<i>Nitella</i>
125b. Main filament three cells thick	<i>Chara</i>
126a. Most of filament surrounded by a layer of cells	<i>Compsopogon</i>
126b. Filament not surrounded by a layer of cells	127
127a. End cell of branches with a rounded or blunt-pointed tip	128

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<i>Statement</i>	<i>Refer to Couplet No.</i>
127b. End cell of branches with a sharp-pointed tip	130
128a. Plastids green; starch test positive	129
128b. Plastids red; starch test negative <i>Audouinella</i>	
129a. Some cells dense, swollen, dark green (spores); other cells light green, cylindric <i>Pithophora</i>	
129b. All cells essentially alike, light to medium green, cylindric <i>Cladophora</i>	
130a. Filaments embedded in gelatinous matrix	131
130b. Filaments not embedded in gelatinous matrix	132
131a. Cells of main filament much wider than even the basal cells of branches <i>Draparnaldia</i>	
131b. No abrupt change in width of cells from main filament to branches <i>Chaetophora</i>	
132a. Branches very short, with no cross-walls <i>Rhizoclonium</i>	
132b. Branches long, with cross-walls	133
133a. Branches ending in an abrupt spine having a bulbous base <i>Bulbochaete</i>	
133b. Branches gradually reduced in width, ending in a long pointed cell, with or without color <i>Stigeoclonium</i>	

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Organism	Plate
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Organism	Plate
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Organism	Plate
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Organism	Plate
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<i>Nephtys</i>	10
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<i>Petromyzon</i>	24
<i>Phacotus</i>	31
<i>Phacus</i>	30, 32
Phaeophyta	2A, 31
<i>Philodina</i>	8
<i>Phormidium</i>	30, 33
Phoronidea	22
<i>Phoronis</i>	22
<i>Physa</i>	19
<i>Phytoconis</i>	32
Pickerelweed	3B
Pilidae	19
<i>Pinnularia</i>	31
<i>Pista</i>	10
<i>Pithopora</i>	1B
<i>Planaria</i>	9
<i>Planktosphaeria</i>	34

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Organism	Plate
Planorbidae	19
Plants, higher	3A, 3B, 3C
<i>Platydorina</i>	4A
Platyhelminthes	9
Plecoptera	13
<i>Plectus</i>	9
<i>Pleodorina</i>	4B
<i>Pleurobema</i>	21
<i>Pleurocera</i>	19
<i>Pleuronema</i>	6
<i>Plocamium</i>	2B
<i>Polinices</i>	20
<i>Polyarthra</i>	8
Polychaeta	9, 10
<i>Polydora</i>	10
<i>Polyedriopsis</i>	34
<i>Polymesoda</i>	21
<i>Polyphemus</i>	11
Polyplacophora	20
<i>Pomacea</i>	19
Pond snail	19
Pondweed	3B
<i>Pontederia</i>	3B

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Organism	Plate
Porifera	7
<i>Porphyra</i>	35
<i>Potamogeton</i>	3B
Pouch snail	19
<i>Prasiola</i>	35
<i>Proales</i>	8
<i>Proptera</i>	21
<i>Prorocentrum</i>	35
Protozoa	4A, 4B, 5A, 5B
Psammobiidae	21
<i>Psephenus</i>	17
<i>Pseudodallescheria</i>	27
<i>Psychoda</i>	16
Pteridophyta	3A
<i>Pteromonas</i>	4A, 34
<i>Pteronarcys</i>	13
Purple sponge	7
Pycnogonid	12
<i>Pyrobotrys</i>	30
Quahog clam	21
Rainbow trout	24
<i>Rana</i>	25
<i>Rangia</i>	21

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Organism	Plate
Rat-tailed maggot	16
Ray	24
<i>Rhizoclonium</i>	31
<i>Rhizosolenia</i>	1B
<i>Rhodoglossum</i>	35
<i>Rhodomonas</i>	31
Rhodophyta	2B
Riffle beetle	17
River snail	19
<i>Rivularia</i>	29
Rockweed	2A
Rotifers	8
Roundworms	9
<i>Sabella</i>	10
Salamanders	25
Salientia	25
<i>Salmo</i>	24
<i>Salvinia</i>	3A
Sand dollar	23
Sand starfish	23
Sarcina	26
<i>Sargassum</i>	2A
Scale worm	10

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Organism	Plate
Scaphopoda	20
<i>Scenedesmus</i>	32, 34
<i>Schizomeris</i>	1A
<i>Schizothrix</i>	34
<i>Schroederia</i>	34
Scud	12
<i>Scytosiphon</i>	35
Sea cucumbers	23
Sea lettuce	2A
Sea slug	20
Sea spider	12
Sea urchin	23
Segmented worms	9, 10
<i>Selenastrum</i>	1A
Sewage fly	16
Shrimp	12
<i>Sialis</i>	15
<i>Sigara</i>	18
<i>Simulium</i>	16
Simuliidae	16
<i>Siren</i>	25
<i>Skeletonema</i>	1B, 35
Sludgeworm	9

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Organism	Plate
Snails	19, 20
Soft-rayed fish	24
Soft-shelled clam	21
Sowbug	12
Spectacle case	21
Spermatophytes	2B, 3A, 3B, 3C
<i>Sphaerium</i>	21
<i>Sphaerocystis</i>	32
<i>Sphaerotilus</i>	26
Spicule types	7
Spike rush	3C
Spionid	10
Spirillum	26
<i>Spirodela</i>	3A
<i>Spirogyra</i>	29, 30
<i>Spirontocaris</i>	12
Spiny-rayed fish	24
<i>Spirulina</i>	34, 35
Sponges	7
Sponge weed	2A
Springtail	22
Starfishes	23
Statoblast, <i>Pectinatella</i>	22

Standard Methods for the Examination of Water and Wastewater

Organism	Plate
<i>Staurastrum</i>	28, 31
<i>Stauroneis</i>	32
<i>Stenelmis</i>	17
<i>Stenonema</i>	13
<i>Stenoplax</i>	20
<i>Stephanodiscus</i>	32
<i>Stephanoptera</i>	35
<i>Stichococcus</i>	1B, 35
<i>Stigeoclonium</i>	29, 33
Stingray	24
Stoneflies	13
Streptococcus	26
<i>Strongylocentrotus</i>	23
<i>Stylonychia</i>	6
<i>Surirella</i>	31
Sweet flag	3C
Syllids	10
<i>Synchaeta</i>	8
<i>Synedra</i>	28, 29
<i>Synura</i>	28
Syrphidae	16
<i>Tabanus</i>	16
<i>Tabellaria</i>	28, 29

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Organism	Plate
Tadpole	25
<i>Tagelus</i>	21
Tanaidacea	12
<i>Tarebia</i>	19
Terebellid	10
<i>Tetracladium</i>	26
<i>Tetraedron</i>	30
<i>Tetramitus</i>	4B
<i>Tetraspora</i>	32
<i>Tetrastrum</i>	1A
Thiaeiidae	19
<i>Thyrone</i>	23
<i>Tigriopus</i>	11
<i>Tintinnidium</i>	6
<i>Tipula</i>	16
Toads	25
<i>Tolypothrix</i>	32
<i>Trachelomonas</i>	29
<i>Triaenodes</i>	15
<i>Tribonema</i>	29
<i>Trichocerca</i>	8
<i>Trichoderma</i>	27
<i>Trichodesmium</i>	35

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Organism	Plate
<i>Trichosporon</i>	27
<i>Tripyla</i>	9
<i>Trochospongilla</i> gemmules	7
Trout	24
<i>Tubifex</i>	9
Tusk shell	20
<i>Typha</i>	3C
<i>Typosyllis</i>	10
<i>Uca</i>	12
<i>Ulothrix</i>	31, 33
<i>Ulva</i>	2A, 35
Unionidae	21
<i>Uroglenopsis</i>	21, 28
<i>Vacuolaria</i>	34
<i>Vallisneria</i>	2B
<i>Valvata</i>	19
Vase sponge	7
<i>Vaucheria</i>	33
Veneridae	21
<i>Vibrio</i>	26
Viviparidae	19
<i>Volvox</i>	28
<i>Vorticella</i>	6

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Organism	Plate
Water boatman	18
Water dog	25
Water fern	3A
Water hyacinth	3A
Watermeal	3A
Water milfoil	3B
Water mite	22
Water penny	17
Water scavenger beetle	17
Water strider	18
Water velvet	3A
Waterweed	3B
<i>Westella</i>	1A
Whirligig beetle	17
Winged lampshell	21
<i>Wolffia</i>	3A
Worms	9, 10
Zebra mussel	21
<i>Zoobotryon</i>	22
<i>Zoogloea</i>	26
<i>Zoophagus</i>	26
<i>Zostera</i>	2B
<i>Zygnema</i>	32

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Organism	Plate

10900 E. Selected Taxonomic References

The most useful references for the nonspecialist are listed below. These references are primarily regional and will aid the biologist in the identification of both freshwater and marine plants and animals. Each reference is listed once under the broadest classification, i.e., a general reference to the identification of invertebrates is listed under “Invertebrates, General” and is not repeated under each individual phylum. As a rule, more academic and specialized reports on a single genus or family are not listed; however, these often are listed in the bibliography of a cited reference.

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Endnotes

1 (Popup - Footnote)

* Source: PALMER, C.M. 1977. Algae and Water Pollution. Publ. No. 600/9-77-036, U.S. Environmental Protection Agency, Cincinnati, Ohio. † Add 1 drop of Lugol's (iodine) solution, diluted 1:1 with distilled water. In about 1 min, if positive, starch is stained blue and later black. Other structures (such as nucleus, plastids, cell wall) also may stain, but turn brown to yellow.

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ALGAE COLOR PLATES

Plate 28. Taste and odor algae.

Asterionella



ASTERIONELLA

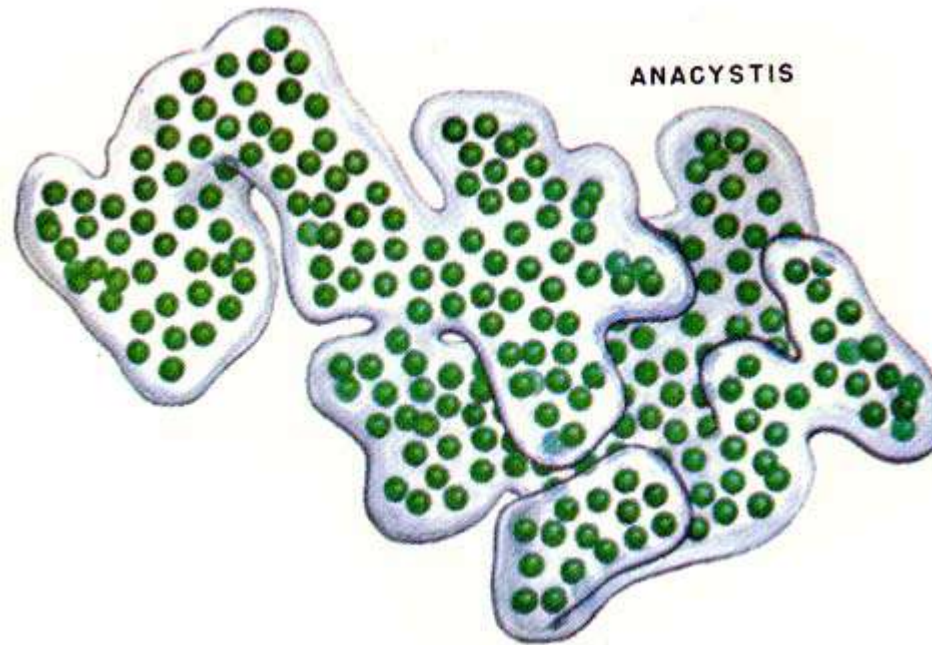
Anabaena

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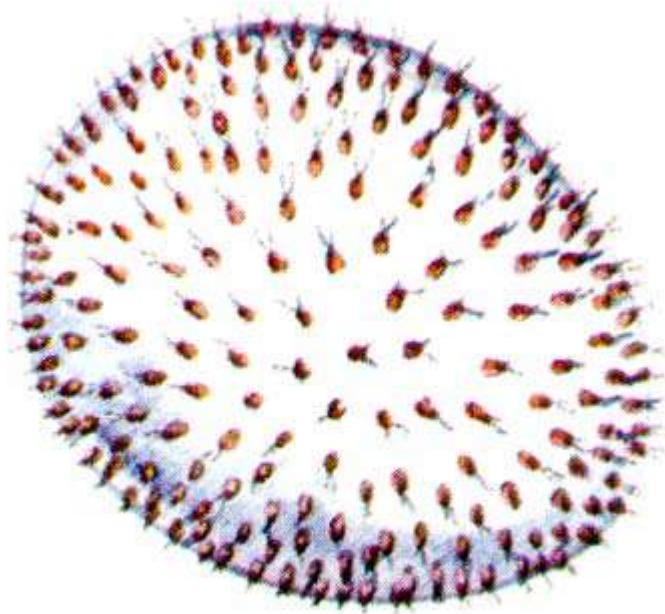
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Uroglenopsis

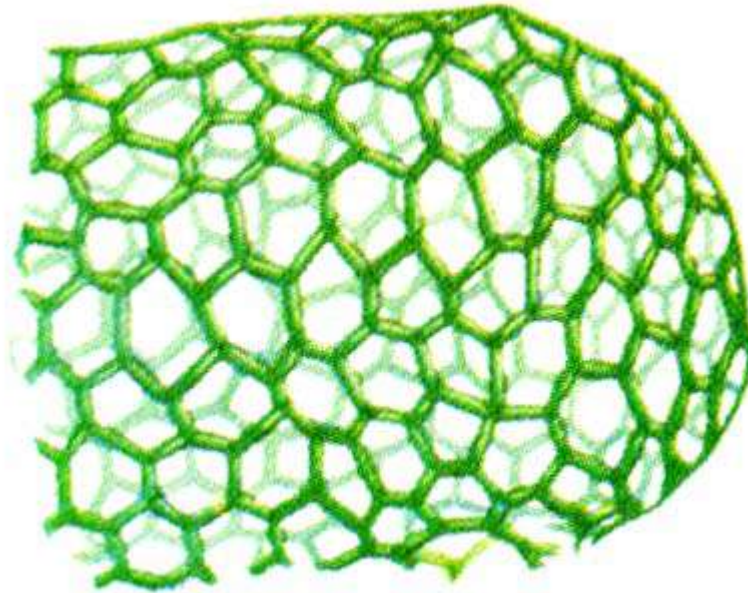
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UROGLENOPSIS

Hydrodictyon

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HYDRODICTYON

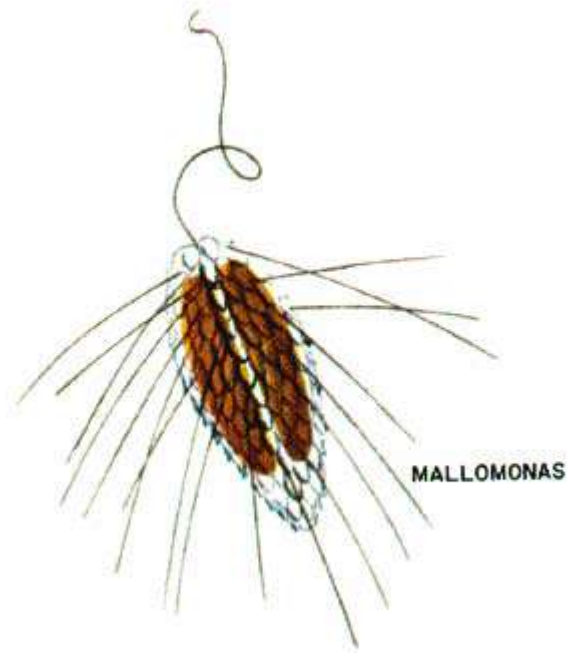
Synedra

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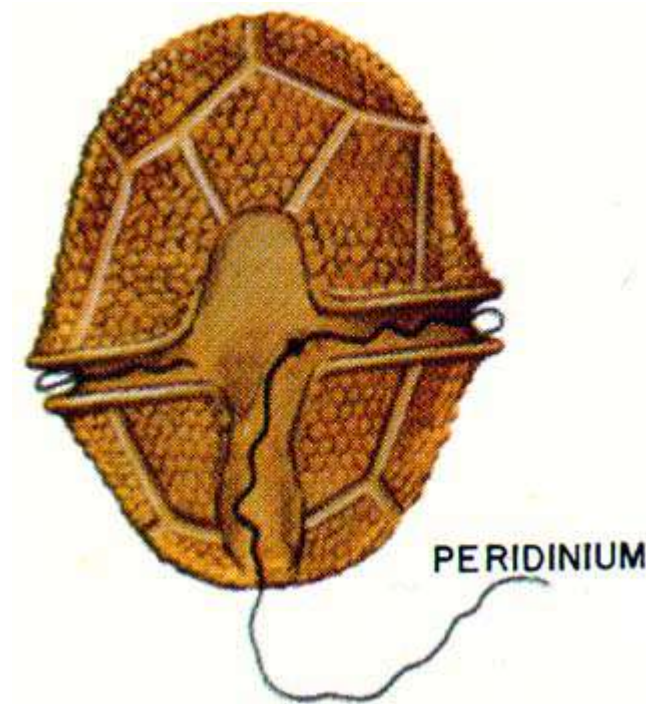
Mallomonas

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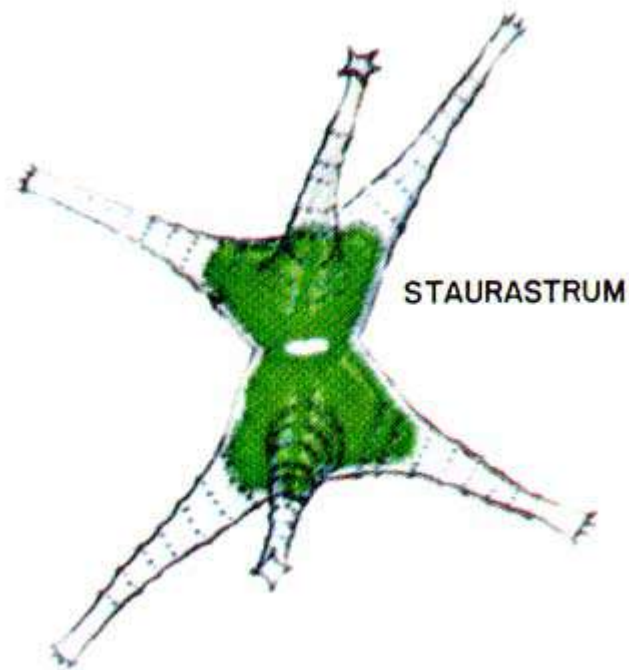
Peridinium

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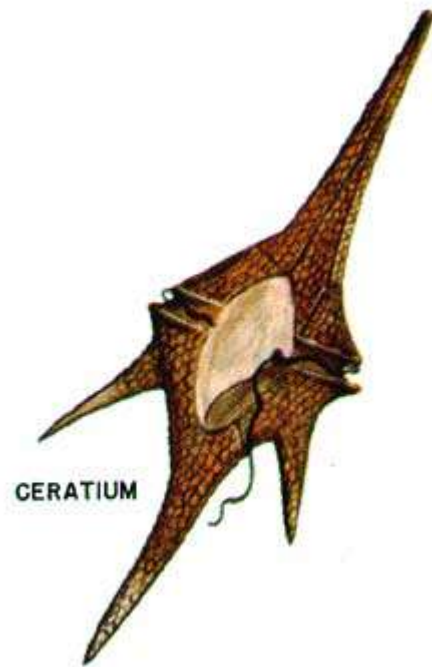
Staurastrum

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Ceratium

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Aphanizomenon

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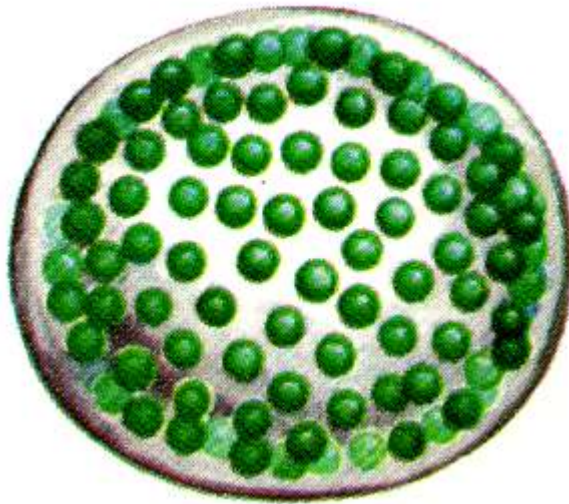
Nitella

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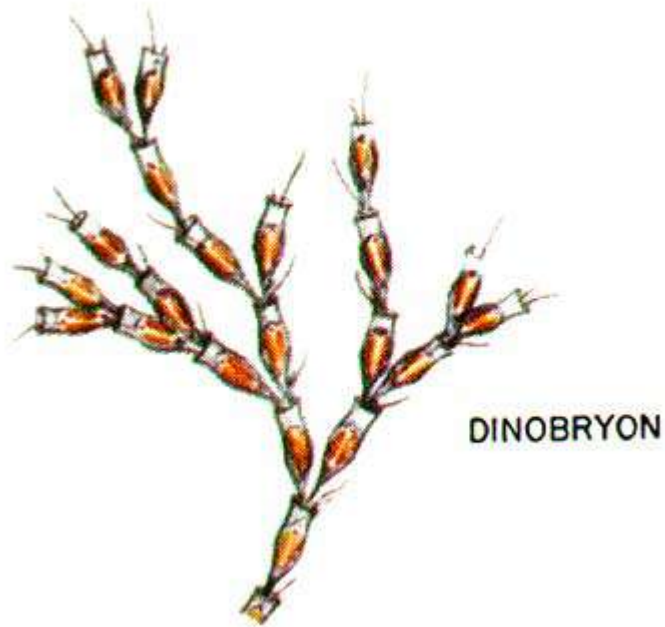
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GOMPHOSPHAERIA



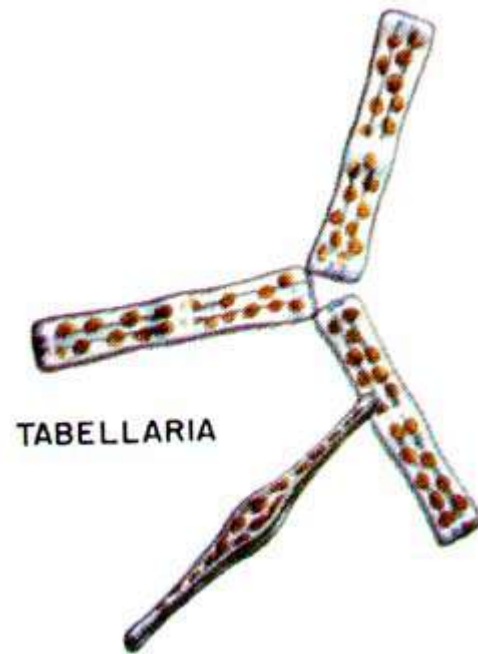
Dinobryon

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Tabellaria

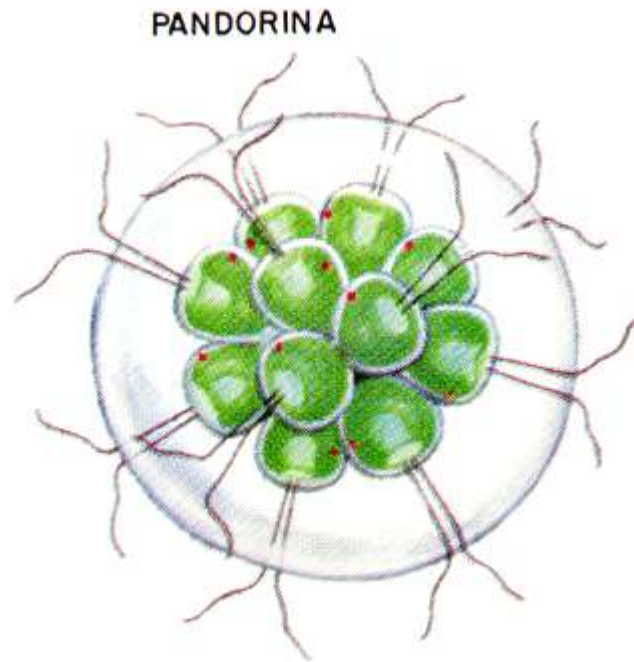
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TABELLARIA

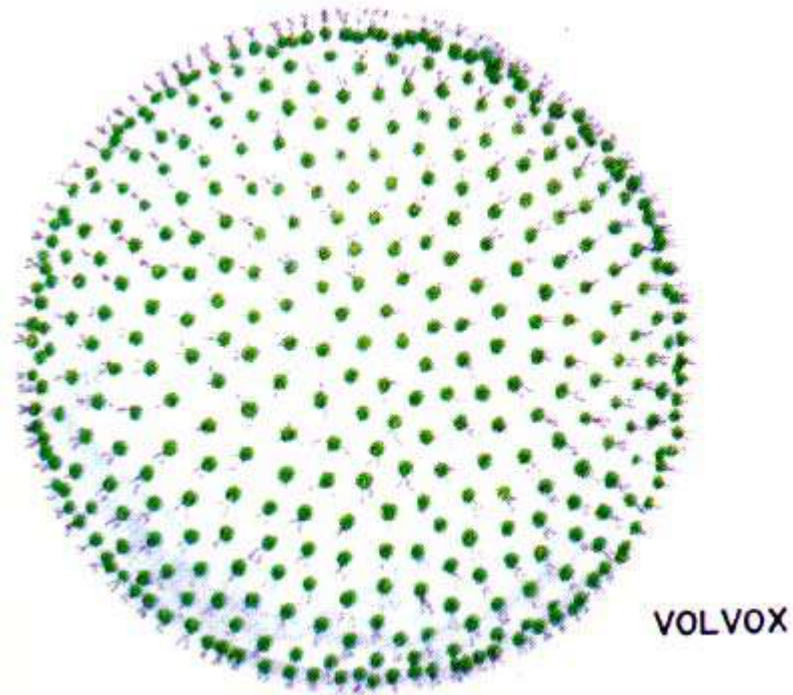
Pandorina

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Volvox

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Synura

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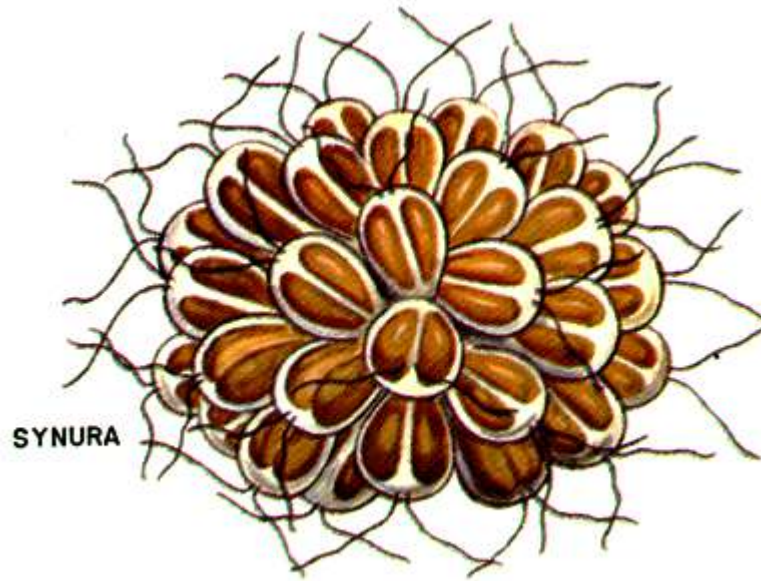
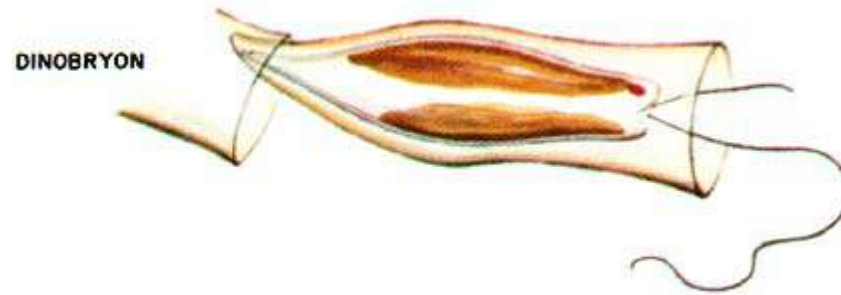


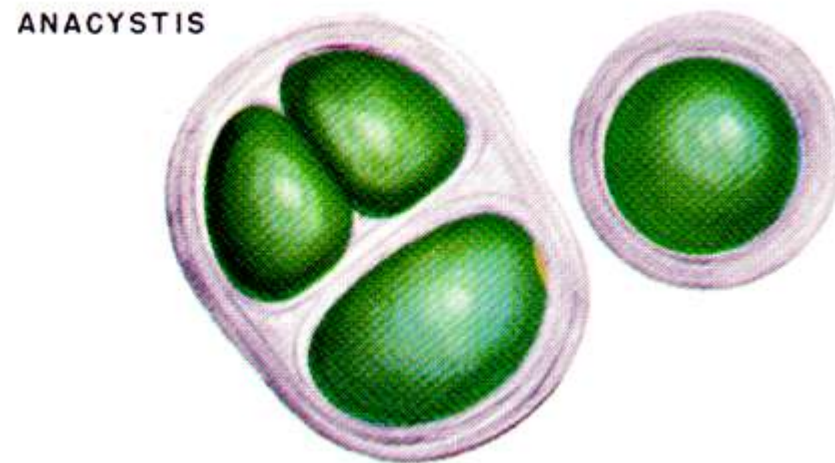
Plate 29. Filter- and screen-clogging algae.

Dinobryon

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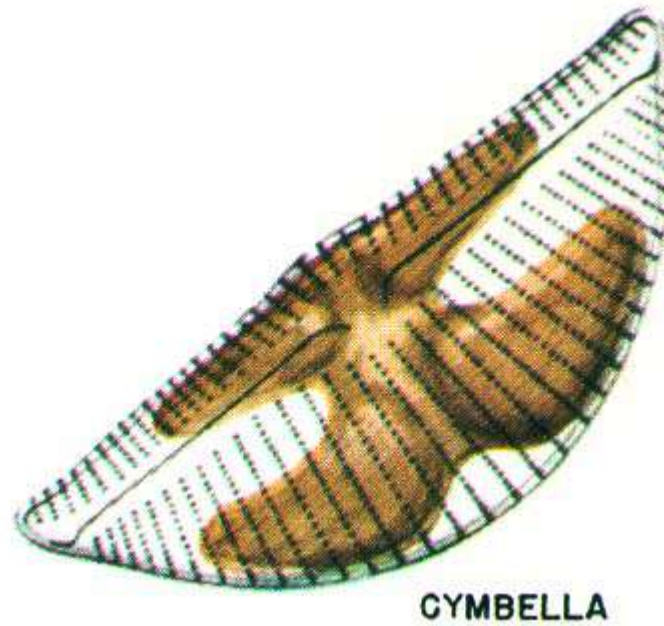


Anacystis



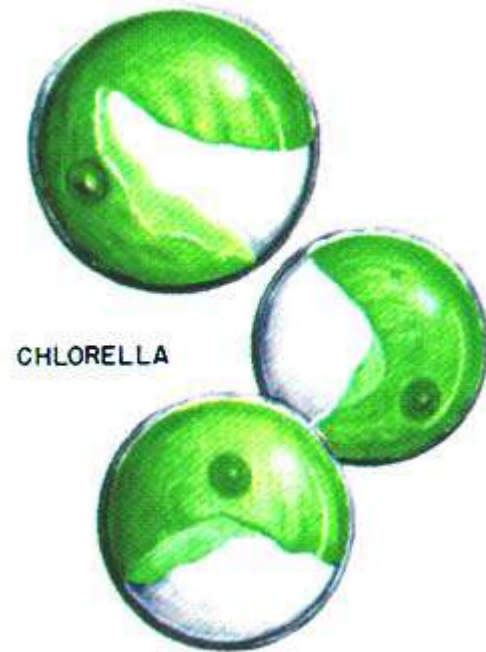
Cymbella

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Chlorella

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CHLORELLA

Tribonema



TRIBONEMA

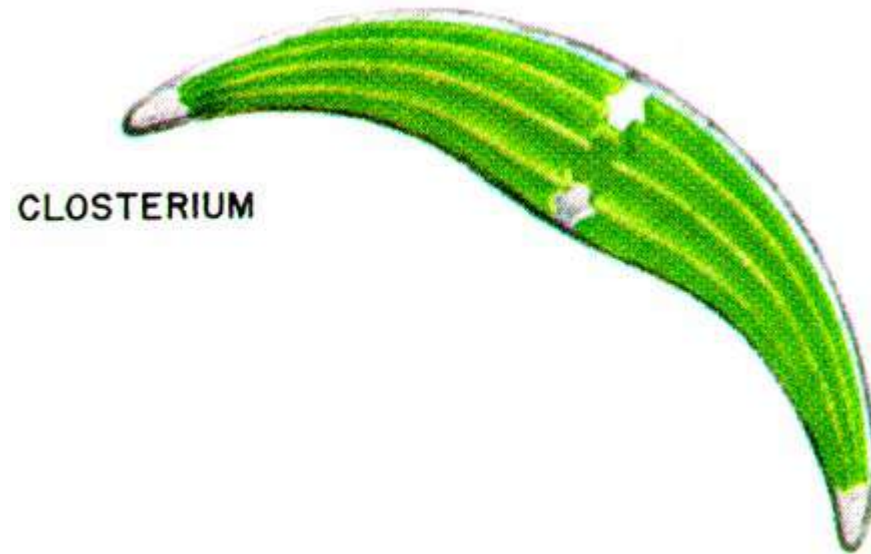
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Synedra



Closterium

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Melosira



Rivularia

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RIVULARIA

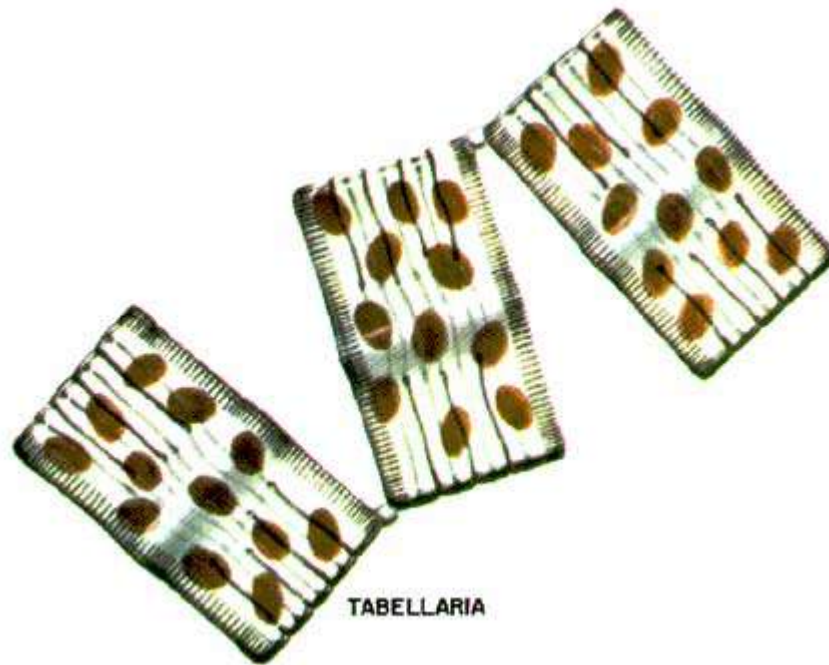
Cyclotella

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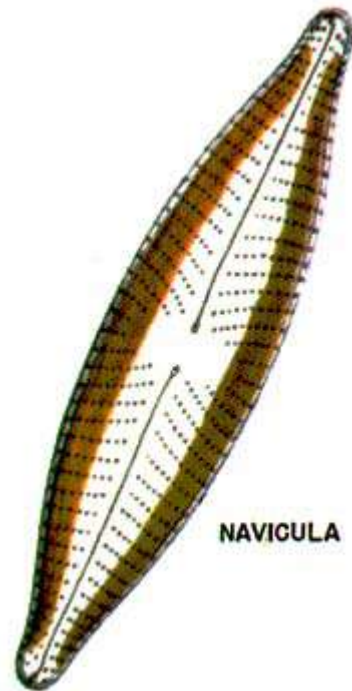
Tabellaria

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Navicula

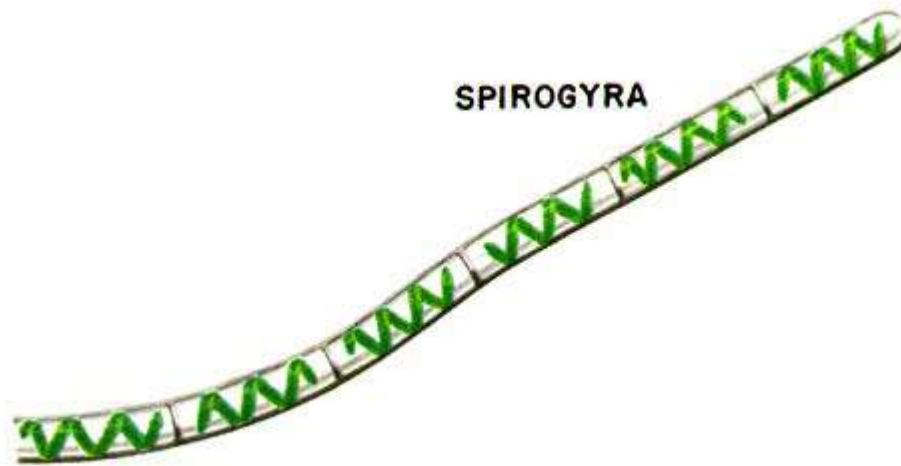
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NAVICULA

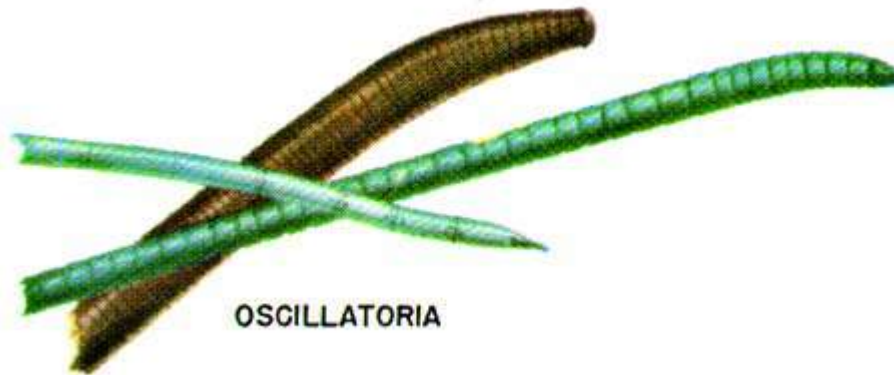
Spirogyra

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SPIROGYRA

Oscillatoria



OSCILLATORIA

Trachelomonas

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TRACHELOMONAS



Asterionella

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Palmella

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Fragilaria

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Anabaena

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Diatoma

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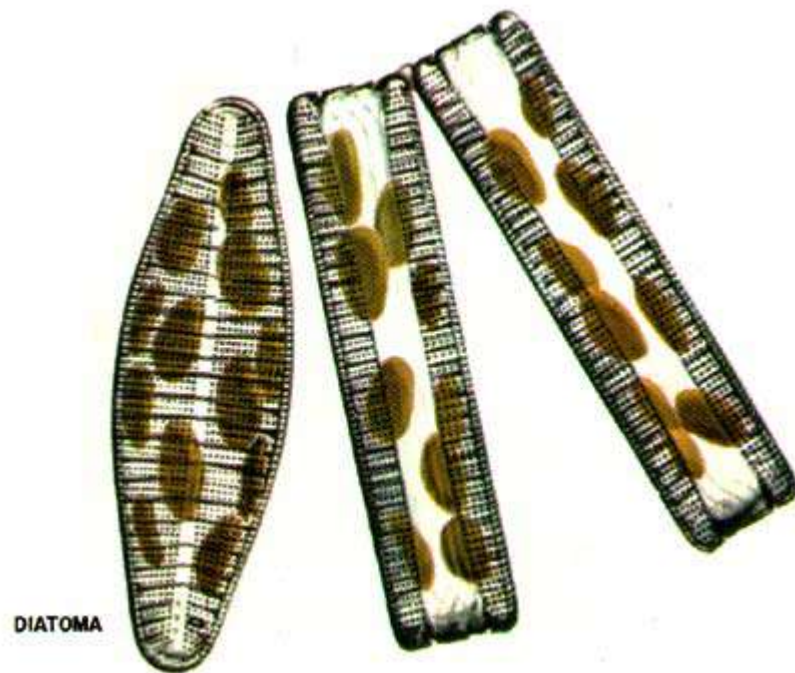
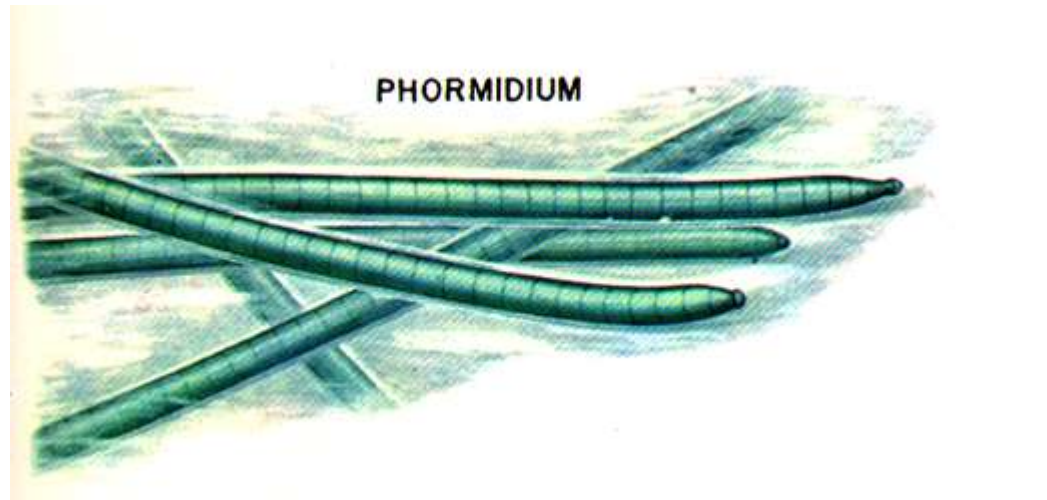


Plate 30. Fresh-water pollution algae.

Phormidium

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Merismopedia

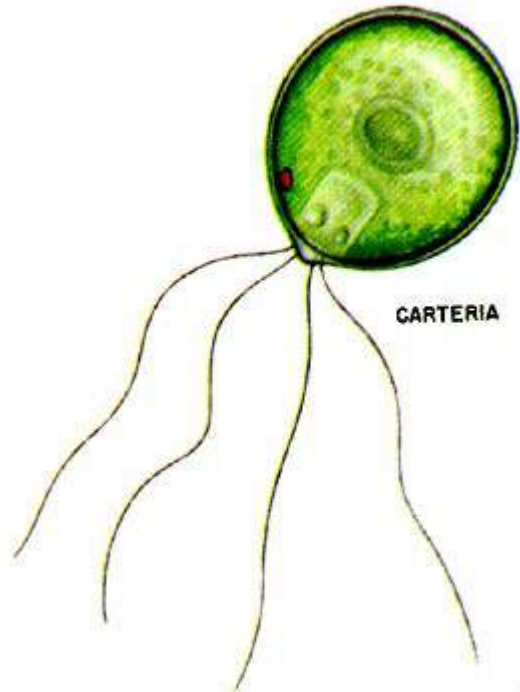
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MERISMOPEDIA



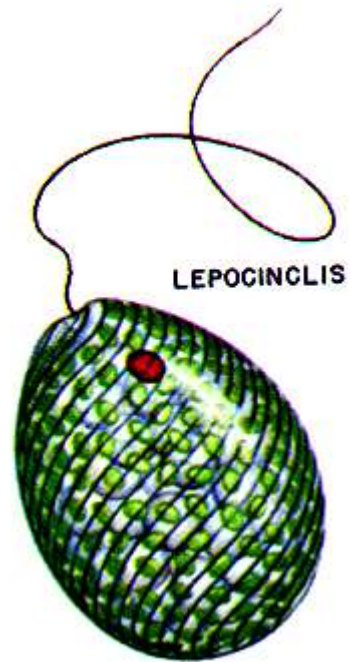
Carteria

Standard Methods for the Examination of Water and Wastewater



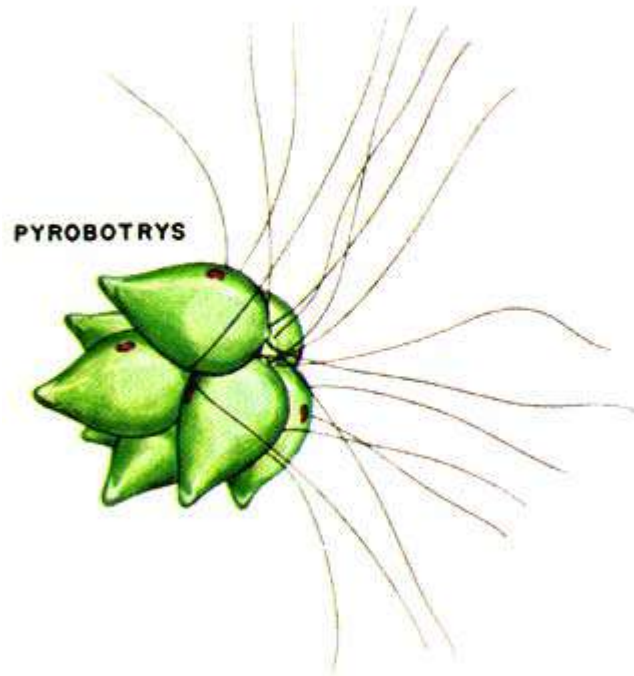
Lepocinclis

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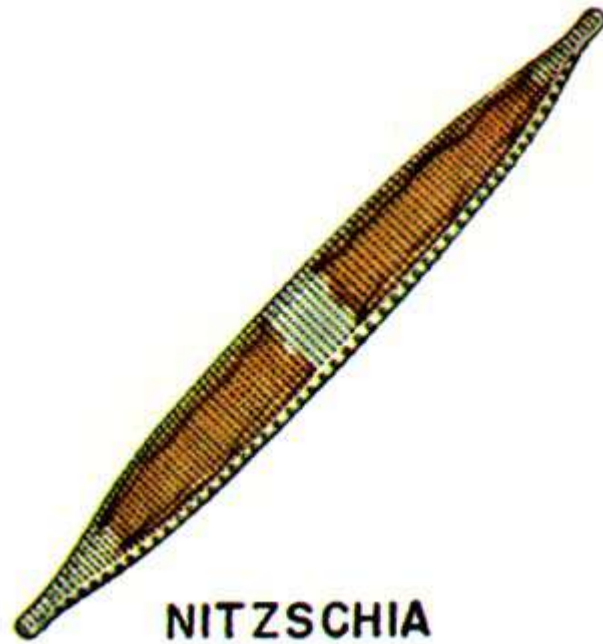
Pyrobotrys

Standard Methods for the Examination of Water and Wastewater



Nitzschia

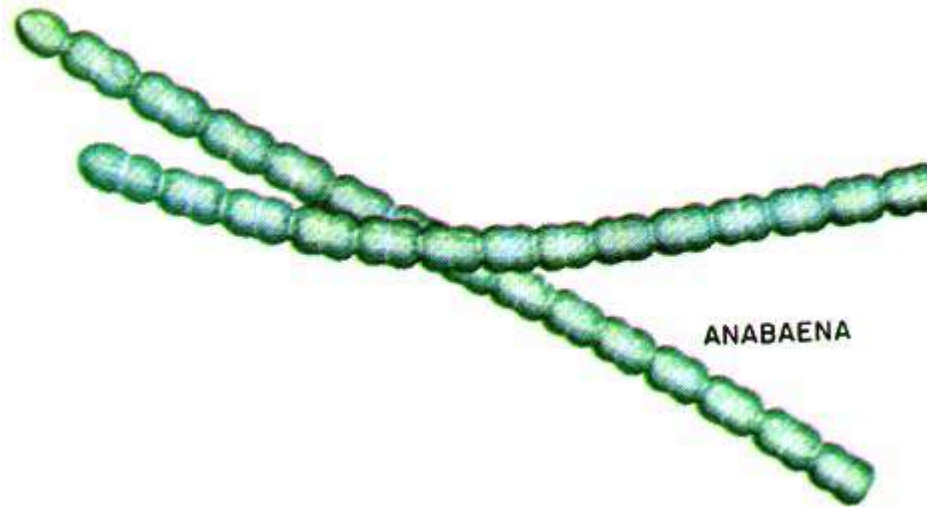
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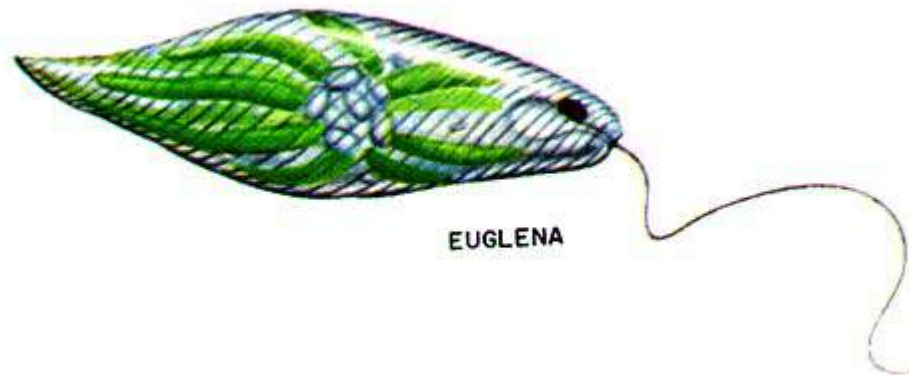
NITZSCHIA

Anabaena

Standard Methods for the Examination of Water and Wastewater

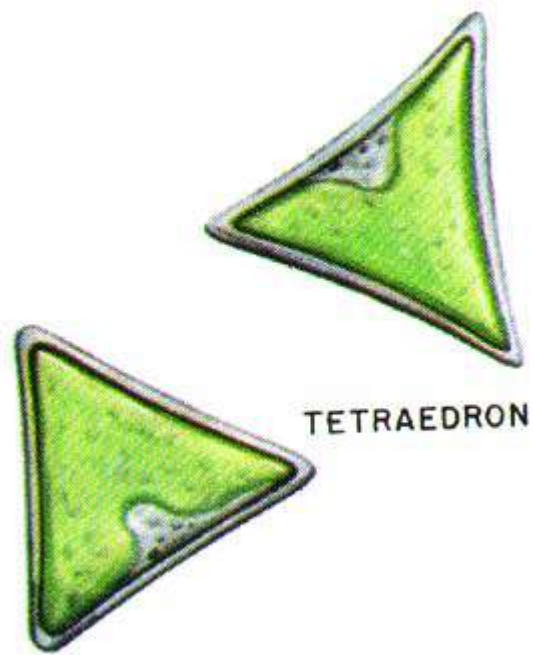


Euglena



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Tetraedron



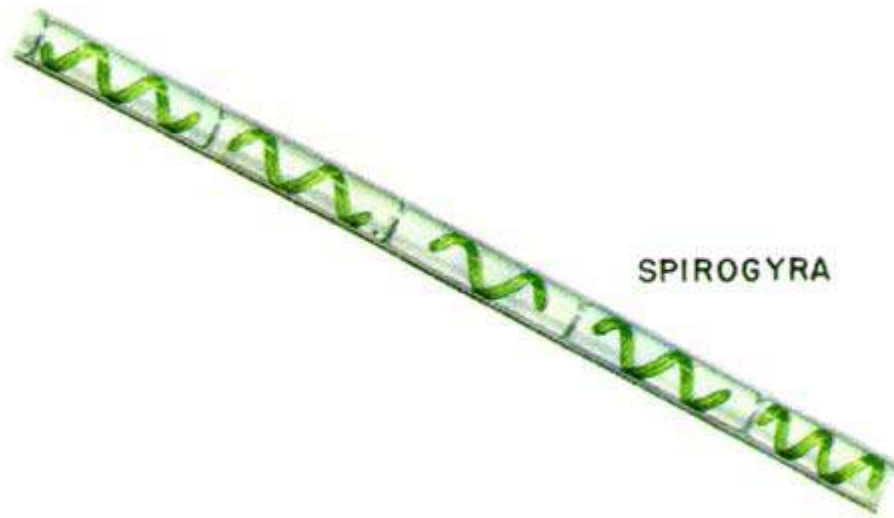
Chlorococcum

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Spirogyra

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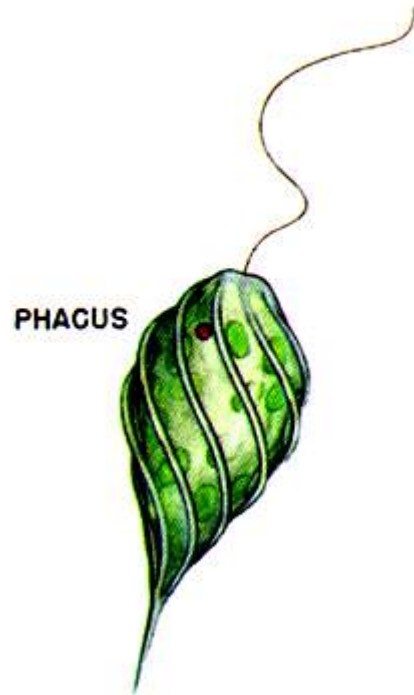
Oscillatoria

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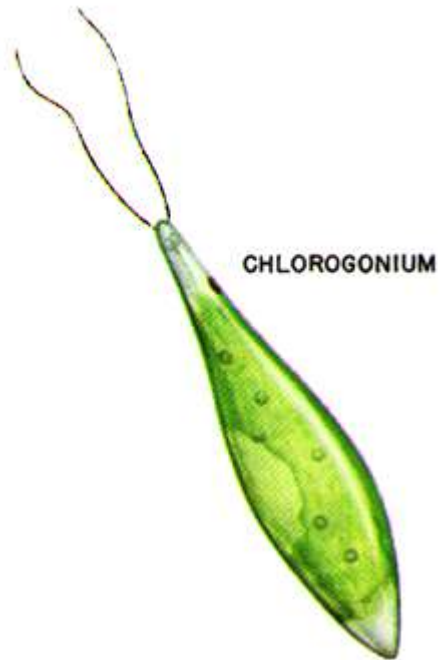
Phacus

Standard Methods for the Examination of Water and Wastewater



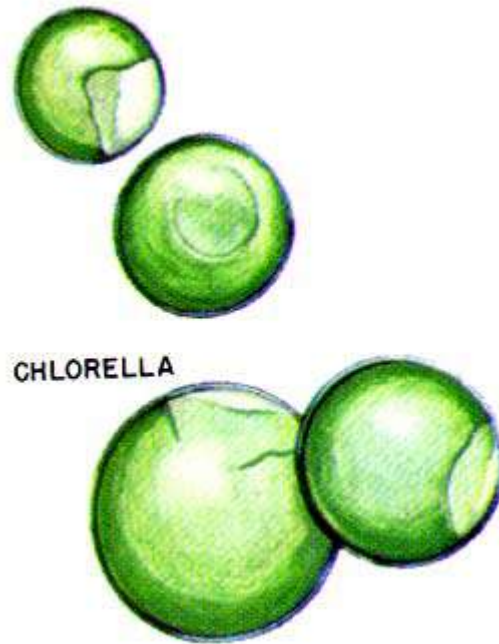
Chlorogonium

Standard Methods for the Examination of Water and Wastewater



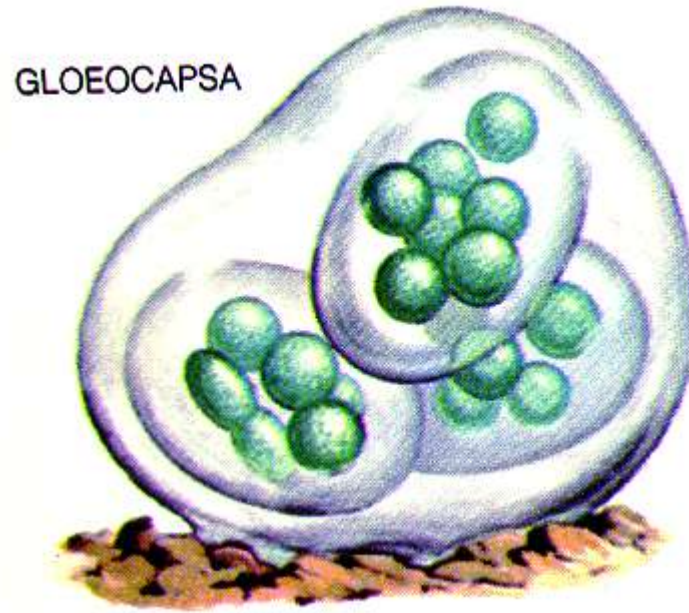
Chlorella

Standard Methods for the Examination of Water and Wastewater



Gloeocapsa

Standard Methods for the Examination of Water and Wastewater



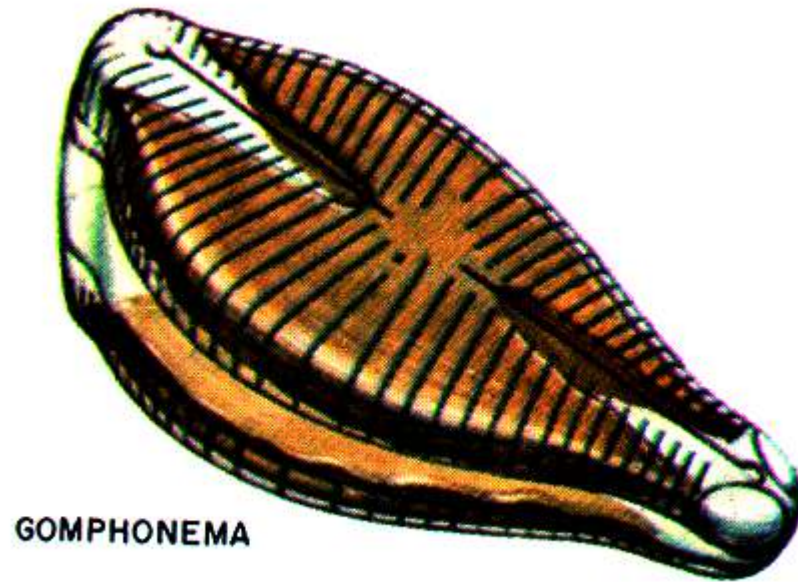
Stigeoclonium

Standard Methods for the Examination of Water and Wastewater



Gomphonema

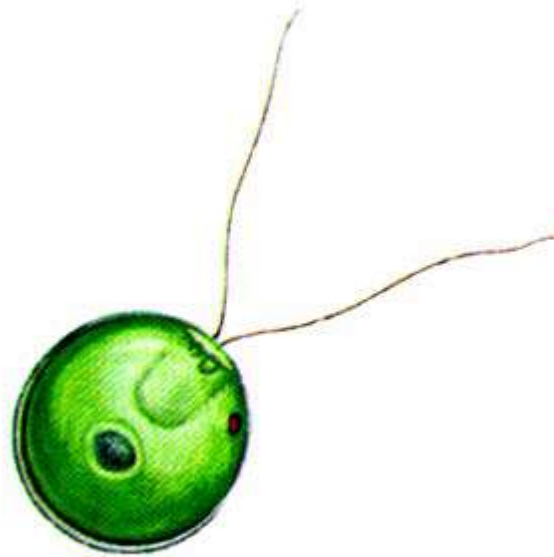
Standard Methods for the Examination of Water and Wastewater



GOMPHONEMA

Chlamydomonas

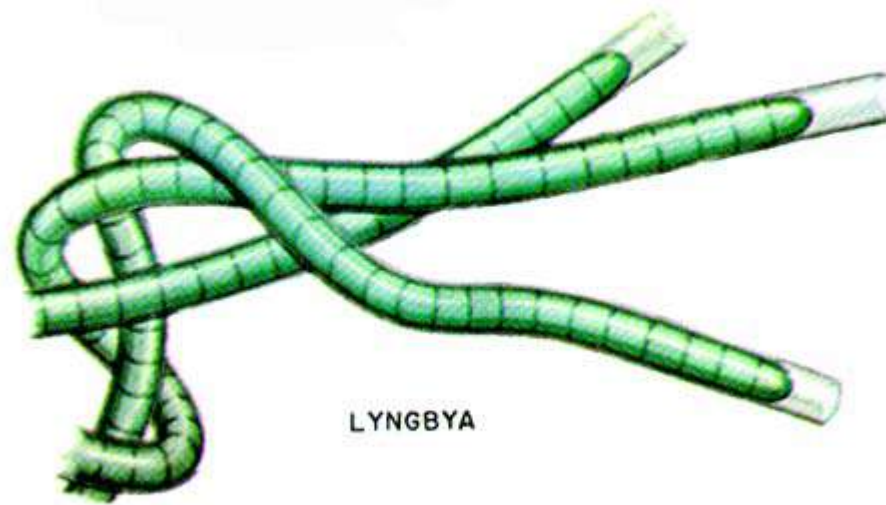
Standard Methods for the Examination of Water and Wastewater



CHLAMYDOMONAS

Lyngbya

Standard Methods for the Examination of Water and Wastewater



Arthrospira

Standard Methods for the Examination of Water and Wastewater

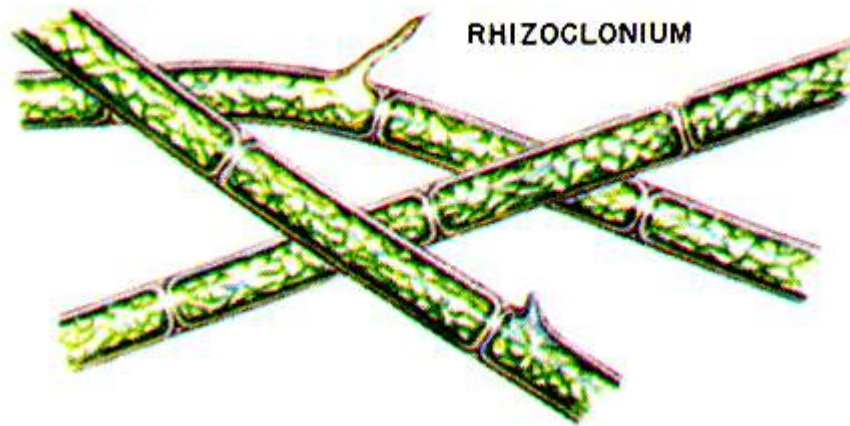


ARTHROSPIRA

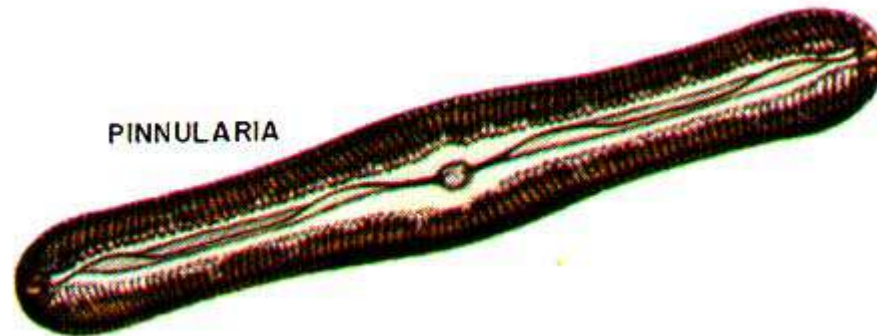
Plate 31. Clean-water algae.

Rhizoclonium

Standard Methods for the Examination of Water and Wastewater



Pinnularia



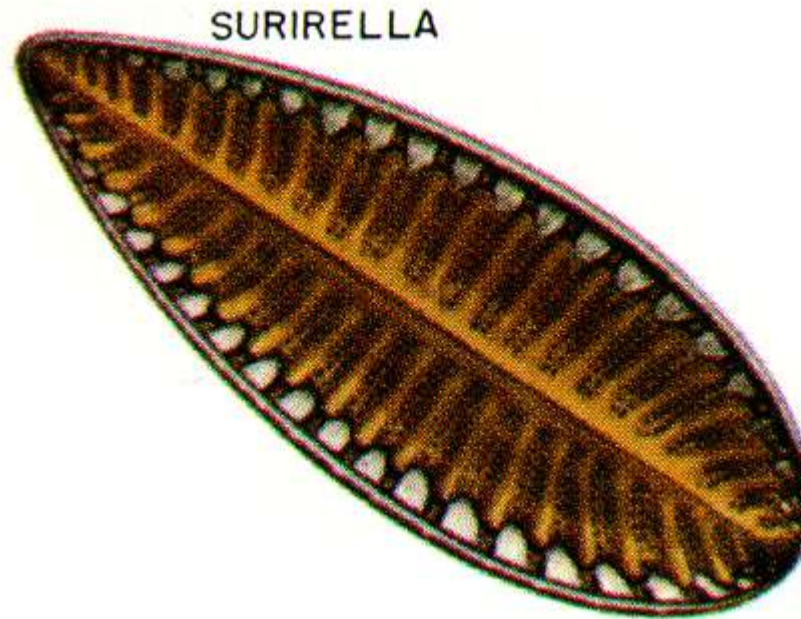
Cladophora

Standard Methods for the Examination of Water and Wastewater



Surirella

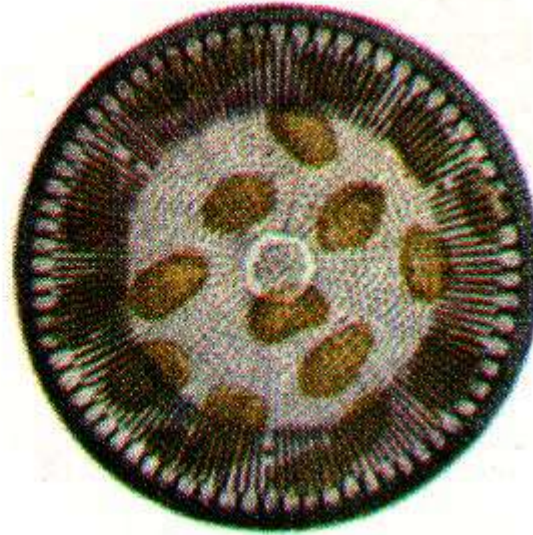
Standard Methods for the Examination of Water and Wastewater



Cyclotella

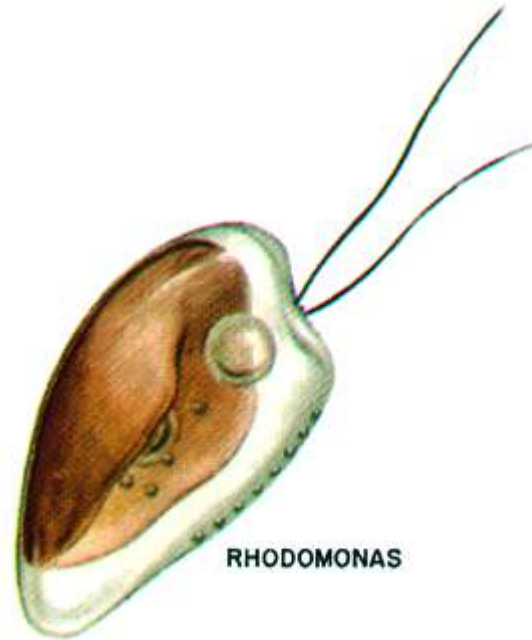
Standard Methods for the Examination of Water and Wastewater

CYCLOTELLA



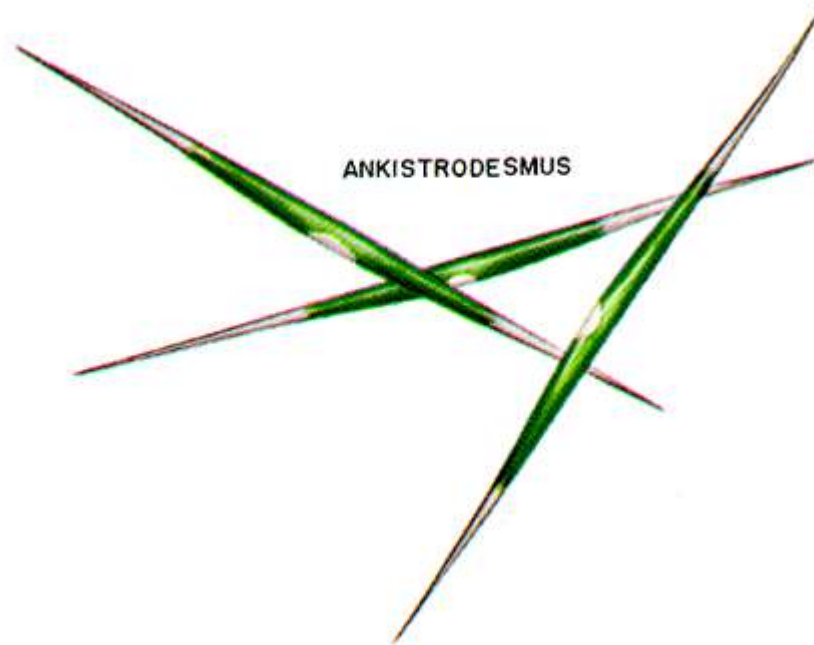
Rhodomonas

Standard Methods for the Examination of Water and Wastewater



Ankistrodesmus

Standard Methods for the Examination of Water and Wastewater



Chrysococcus

Standard Methods for the Examination of Water and Wastewater



Merismopedia

Standard Methods for the Examination of Water and Wastewater



Aphanotheca

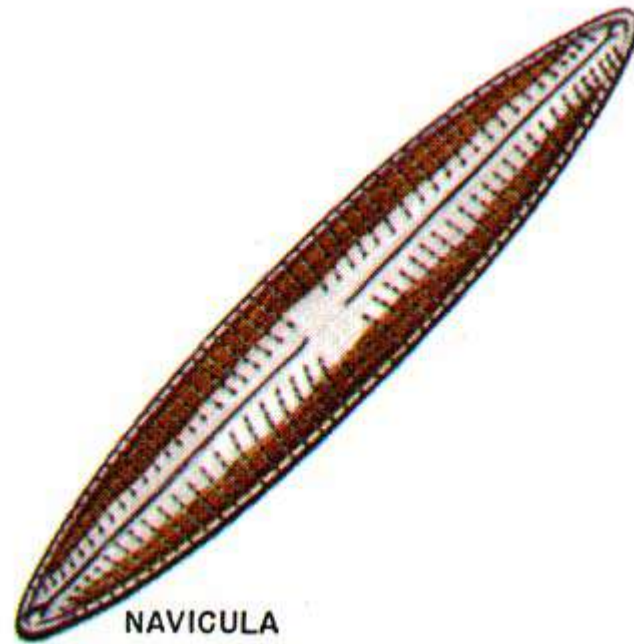
Standard Methods for the Examination of Water and Wastewater



APHANOTHECA

Navicula

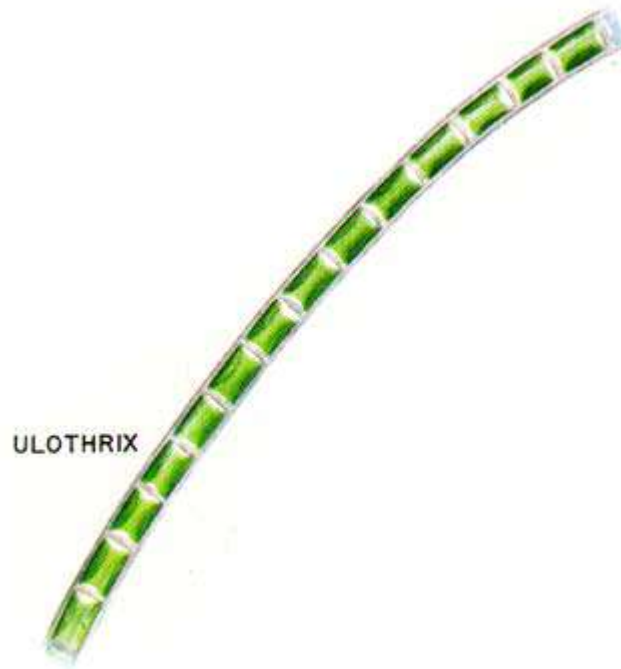
Standard Methods for the Examination of Water and Wastewater



NAVICULA

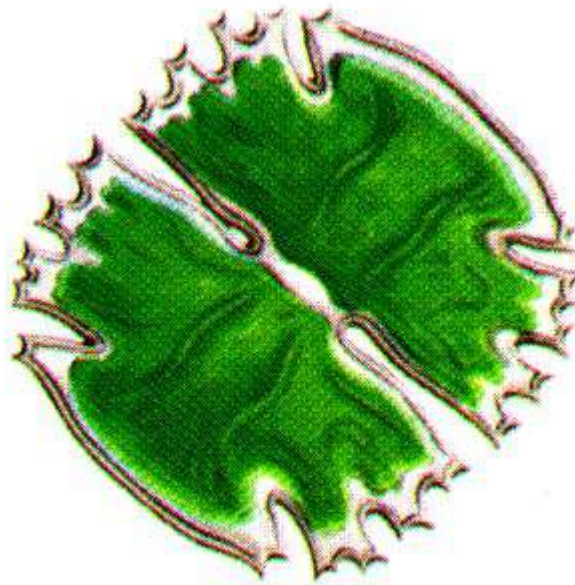
Ulothrix

Standard Methods for the Examination of Water and Wastewater



Micrasterias

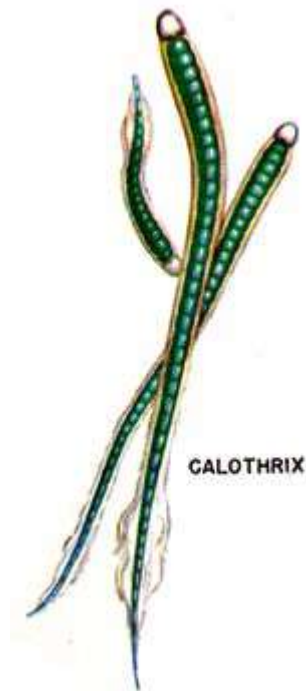
Standard Methods for the Examination of Water and Wastewater



MICRASTERIAS

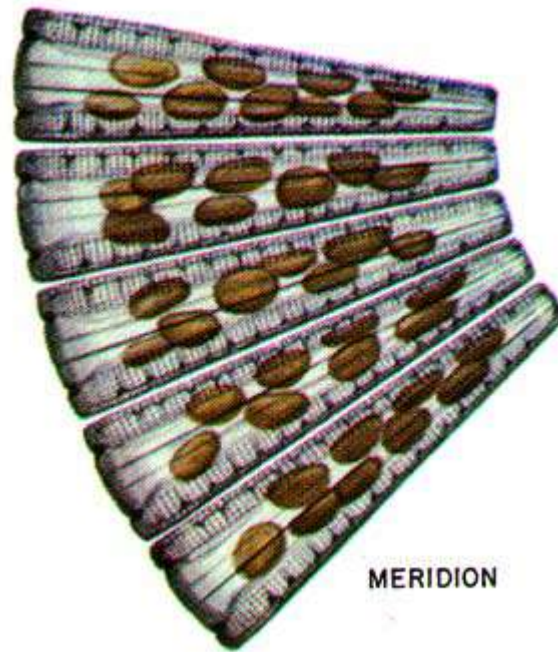
Calothrix

Standard Methods for the Examination of Water and Wastewater



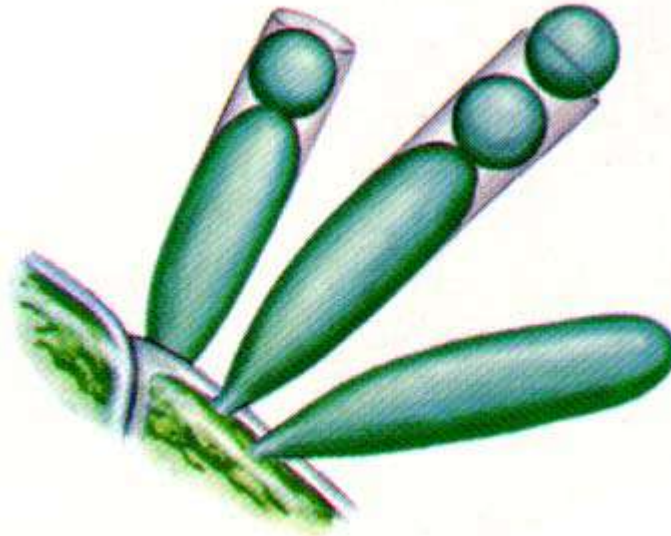
Meridion

Standard Methods for the Examination of Water and Wastewater



Chamaesiphon

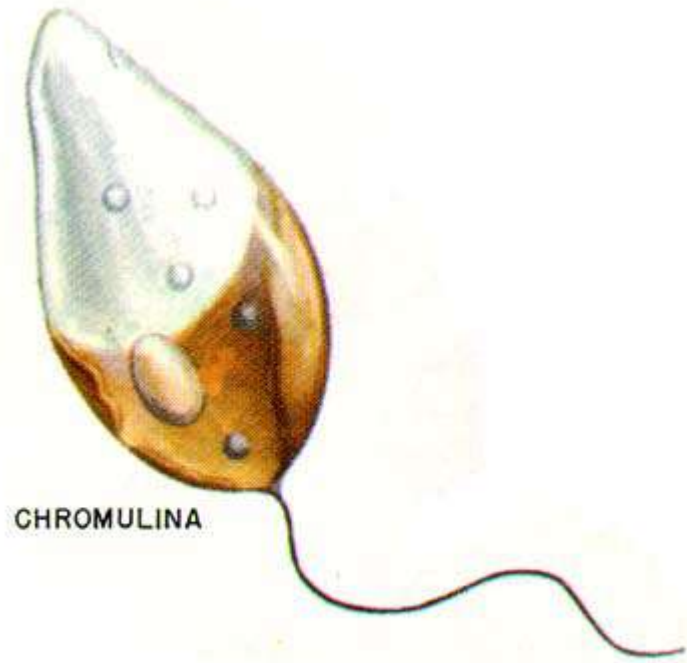
Standard Methods for the Examination of Water and Wastewater



CHAMAESIPHON

Chromulina

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Hildenbrandia

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Phacotus

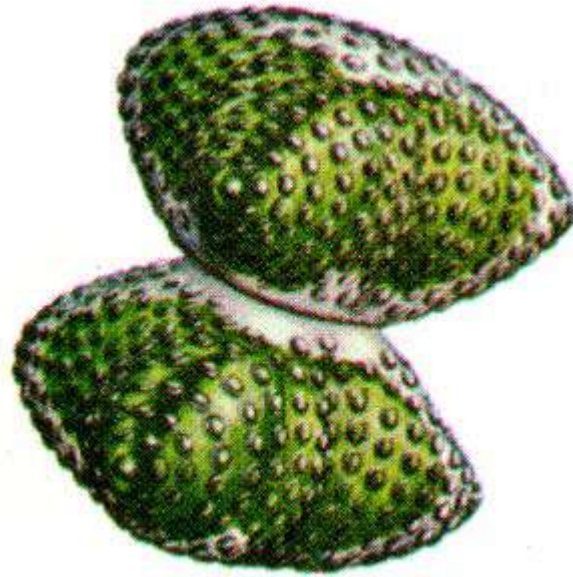
Standard Methods for the Examination of Water and Wastewater



PHACOTUS

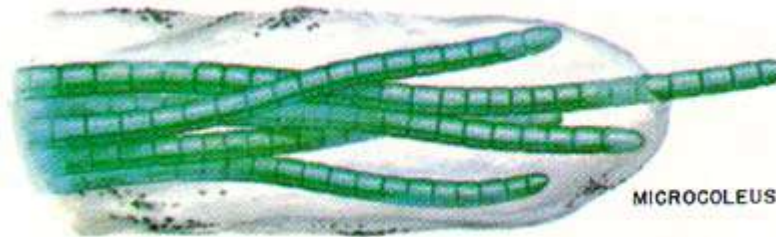
Staurostrum

Standard Methods for the Examination of Water and Wastewater



STAURASTRUM

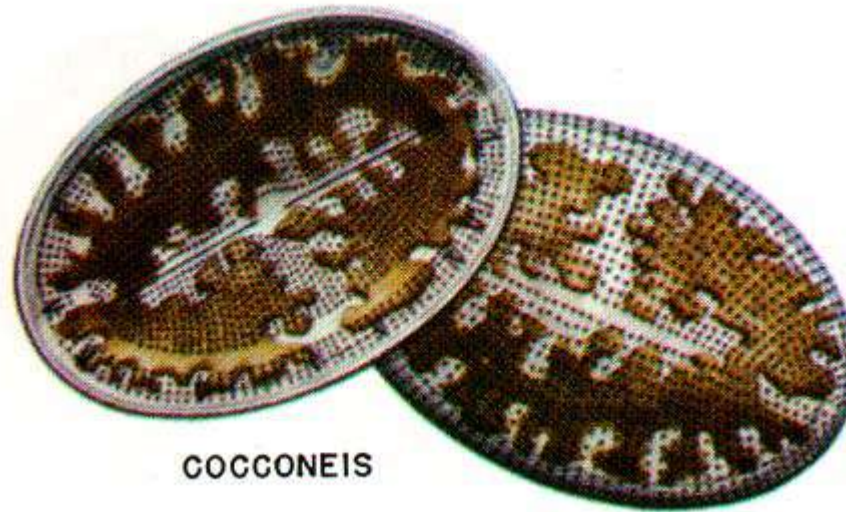
Microcoleus



MICROCOLEUS

Standard Methods for the Examination of Water and Wastewater

Cocconeis



Lemanea

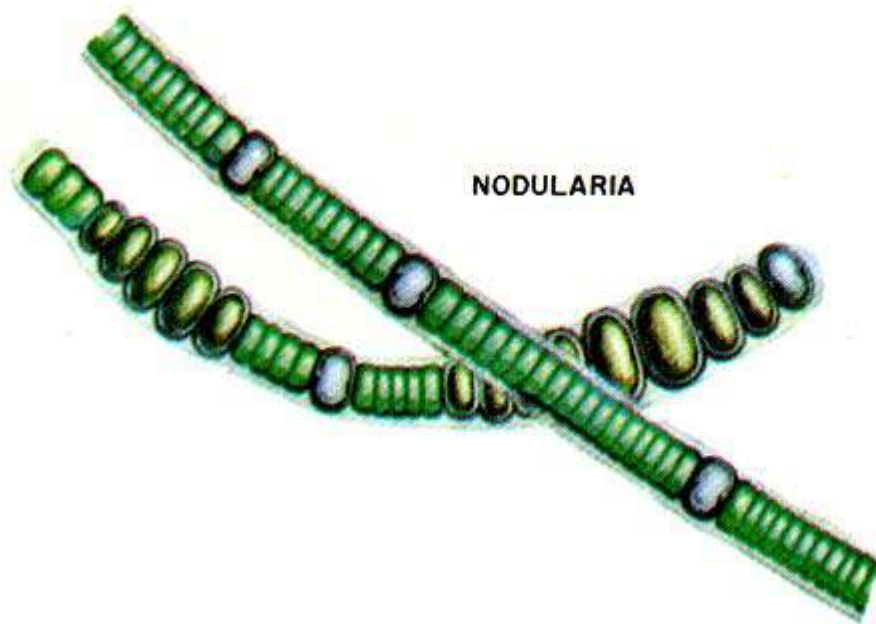
Standard Methods for the Examination of Water and Wastewater



Plate 32. Plankton and other surface-water algae.

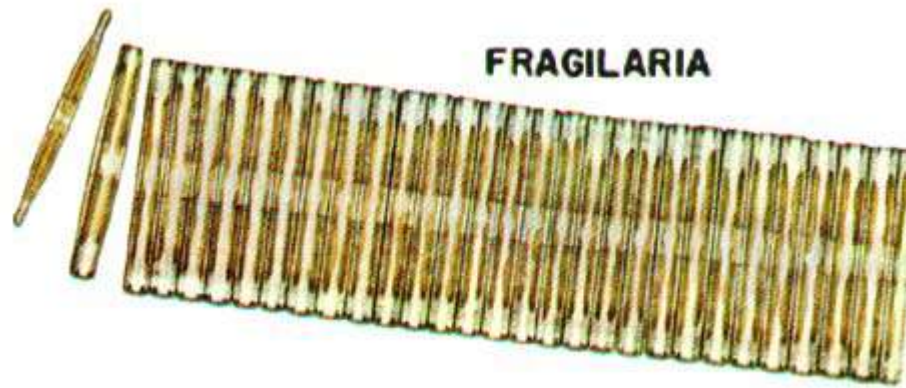
Nodularia

Standard Methods for the Examination of Water and Wastewater



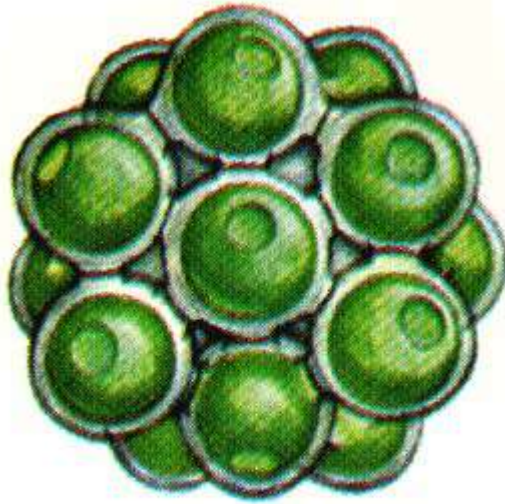
Fragilaria

Standard Methods for the Examination of Water and Wastewater



Coelastrum

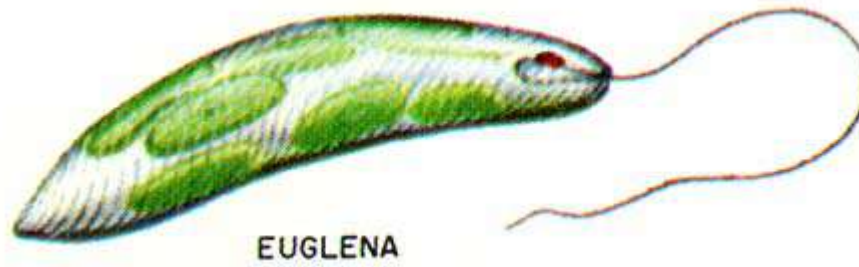
Standard Methods for the Examination of Water and Wastewater



COELASTRUM

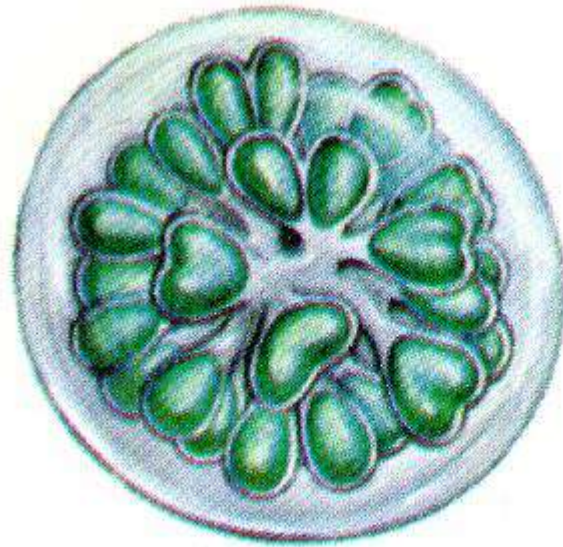
Euglena

Standard Methods for the Examination of Water and Wastewater



Gomphosphaeria

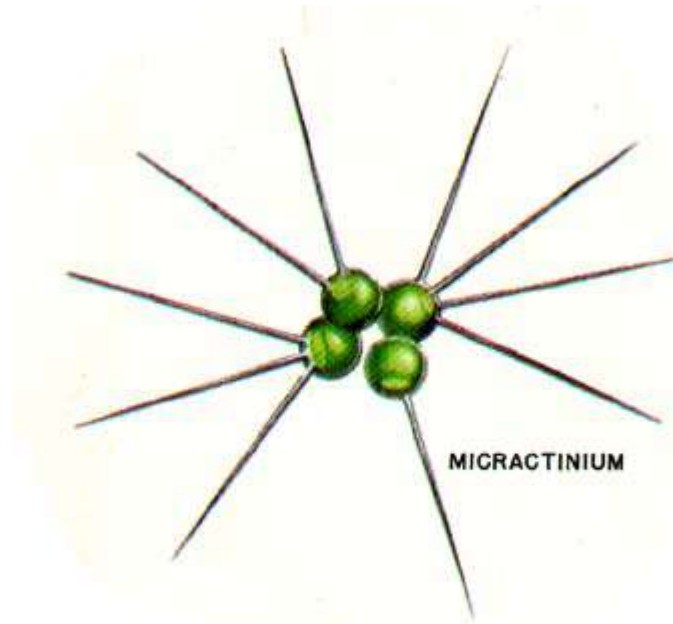
Standard Methods for the Examination of Water and Wastewater



GOMPHOSPHAERIA

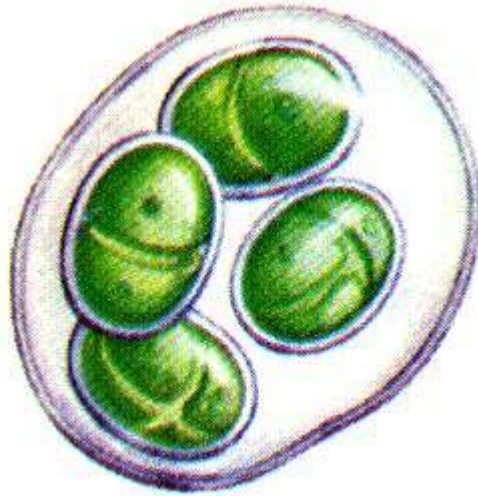
Micractinium

Standard Methods for the Examination of Water and Wastewater



Oocystis

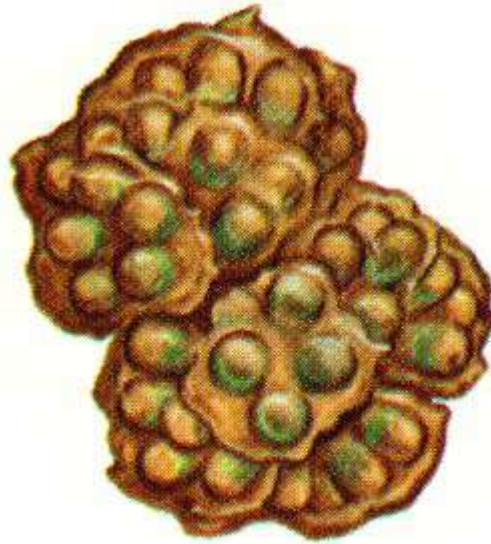
Standard Methods for the Examination of Water and Wastewater



OOCYSTIS

Botryococcus

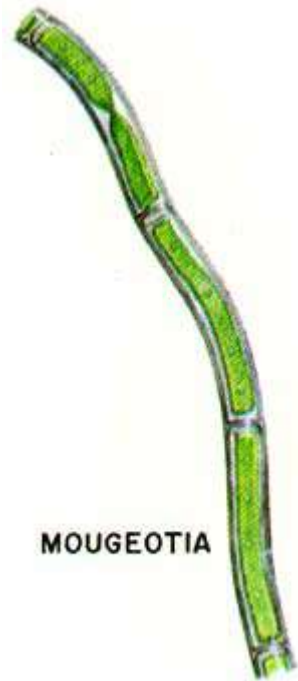
Standard Methods for the Examination of Water and Wastewater



BOTRYOCOCCUS

Mougeotia

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MOUGEOTIA

Euastrum

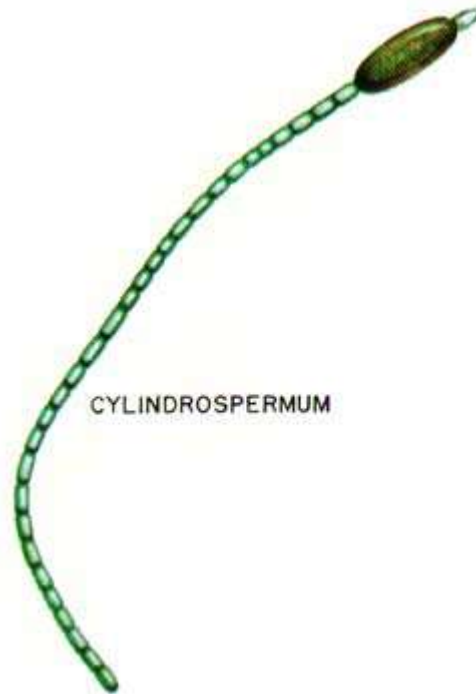
Standard Methods for the Examination of Water and Wastewater



EUASTRUM

Cylindrospermum

Standard Methods for the Examination of Water and Wastewater



Phacus

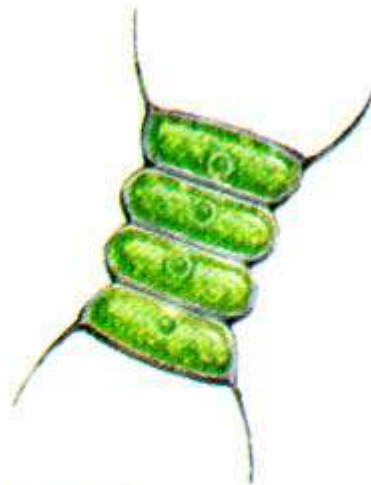
Standard Methods for the Examination of Water and Wastewater



PHACUS

Scenedesmus

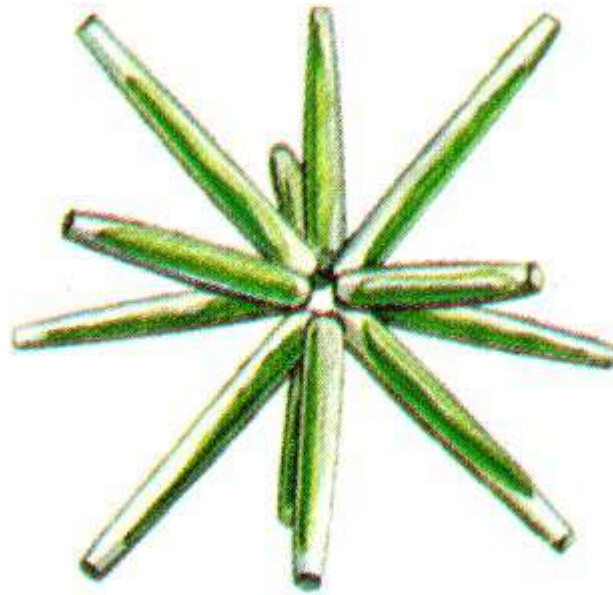
Standard Methods for the Examination of Water and Wastewater



SCENEDESMUS

Actinastrum

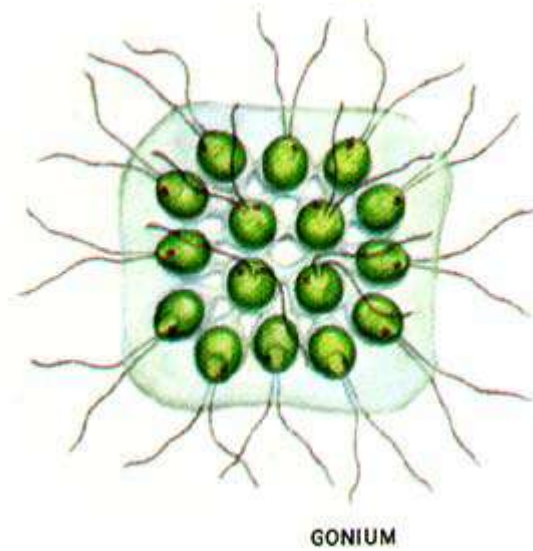
Standard Methods for the Examination of Water and Wastewater



ACTINASTRUM

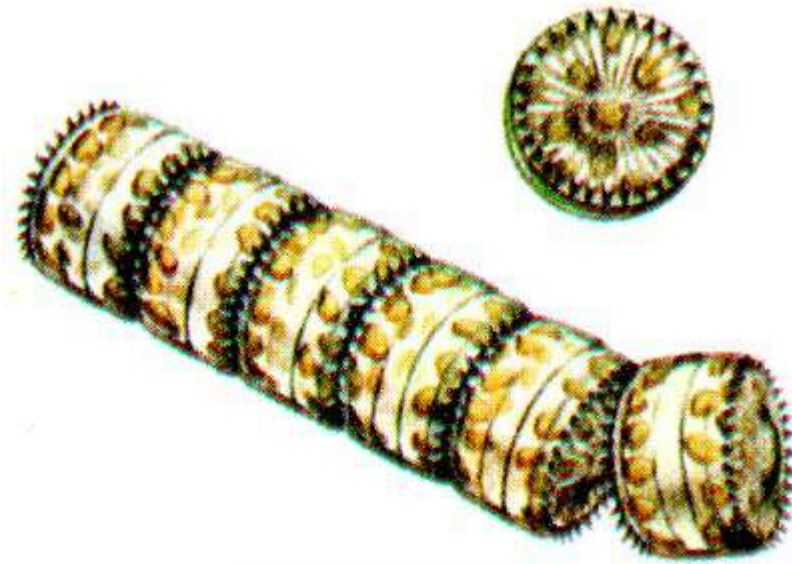
Gonium

Standard Methods for the Examination of Water and Wastewater



Stephanodiscus

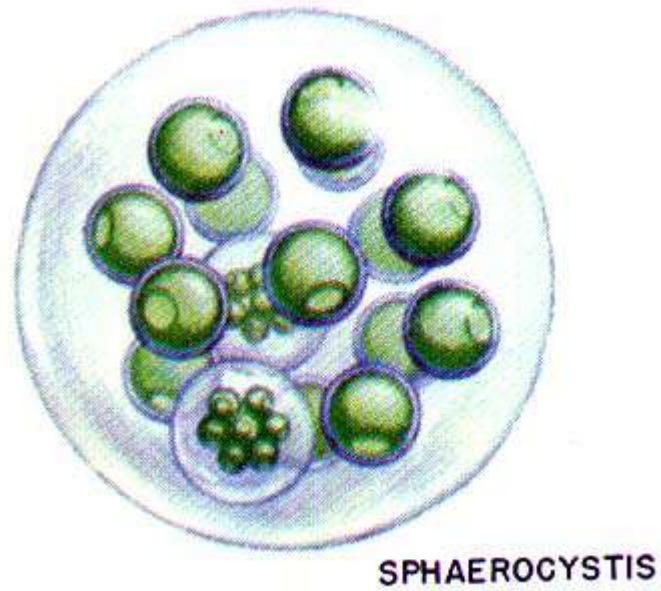
Standard Methods for the Examination of Water and Wastewater



STEPHANODISCUS

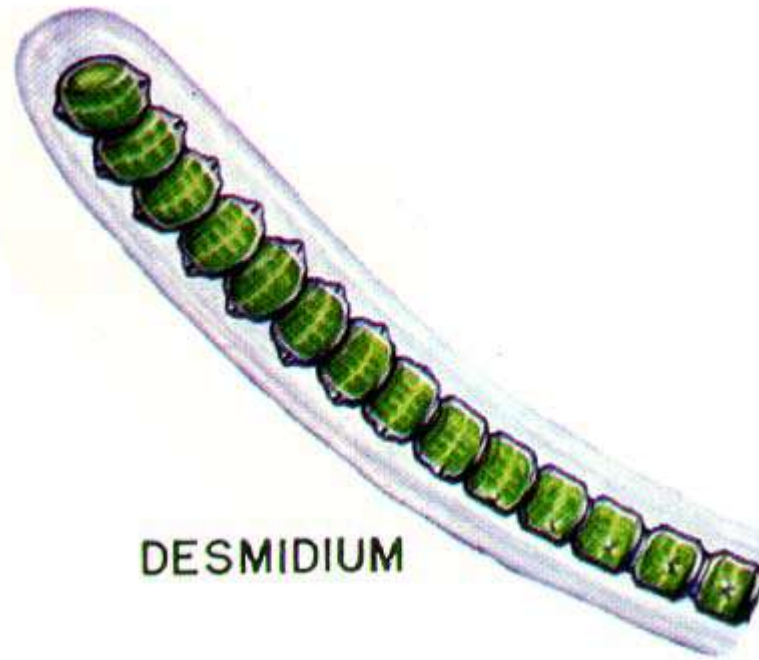
Sphaerocystis

Standard Methods for the Examination of Water and Wastewater



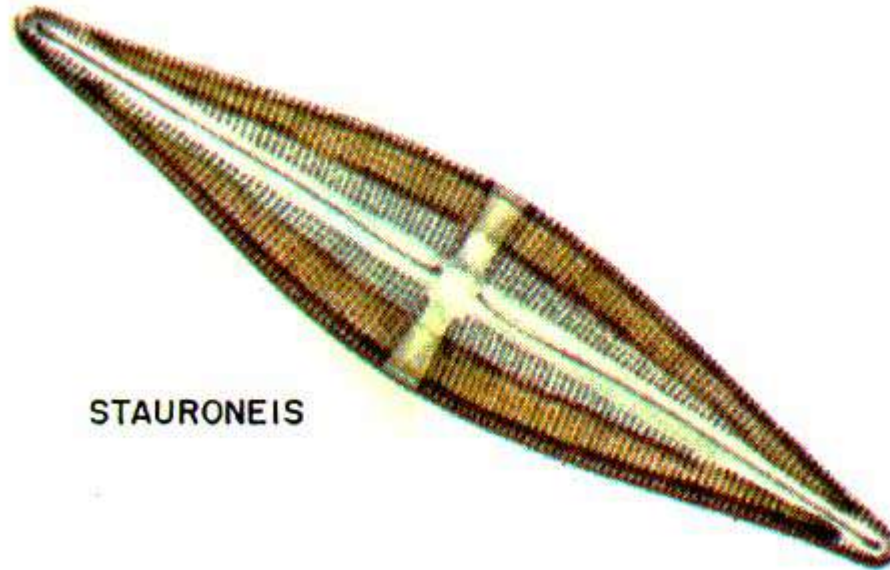
Desmidium

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Stauroneis

Standard Methods for the Examination of Water and Wastewater



STAURONEIS

Zygnema

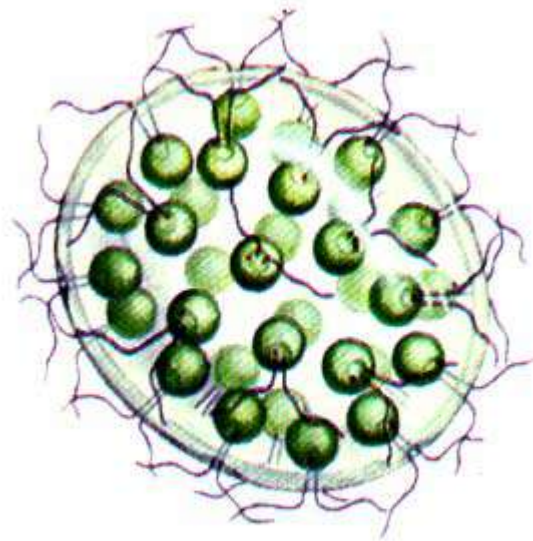
Standard Methods for the Examination of Water and Wastewater



ZYGNEMA

Eudorina

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EUDORINA

Pediastrum

Standard Methods for the Examination of Water and Wastewater

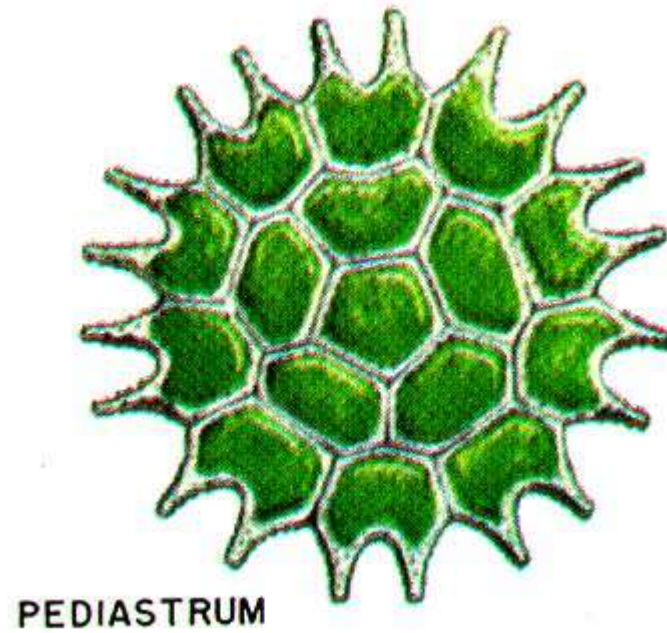
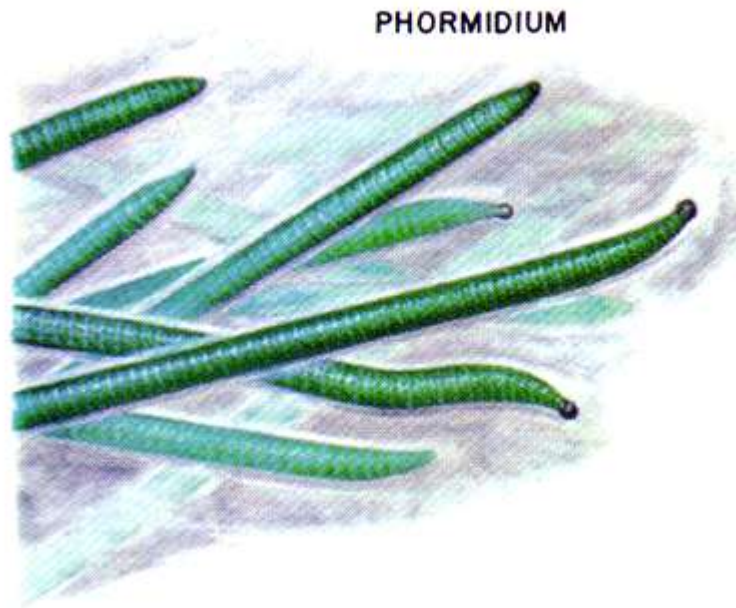


Plate 33. Algae growing on surfaces.

Phormidium

Standard Methods for the Examination of Water and Wastewater

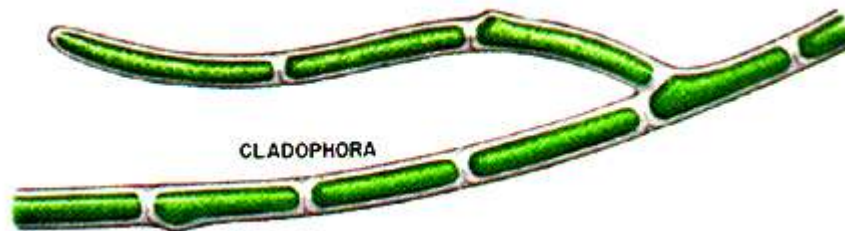


Ulothrix

Standard Methods for the Examination of Water and Wastewater



Cladophora



Standard Methods for the Examination of Water and Wastewater

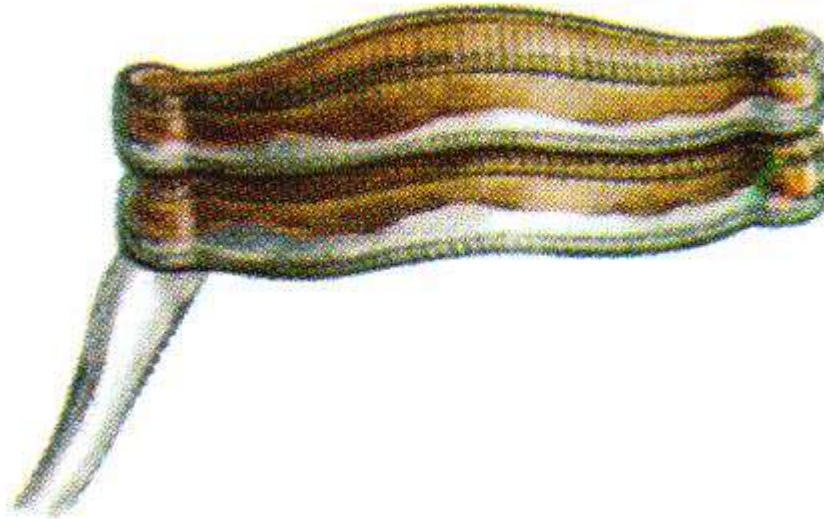
Gomphonema



Achnanthes

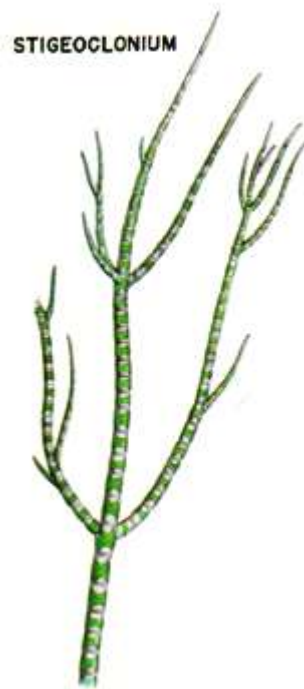
Standard Methods for the Examination of Water and Wastewater

ACHNANTHES



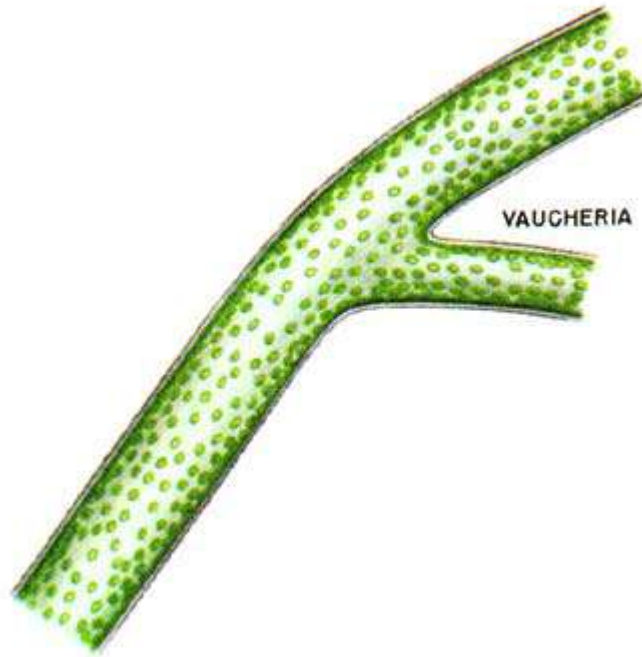
Stigeoclonium

Standard Methods for the Examination of Water and Wastewater



Vaucheria

Standard Methods for the Examination of Water and Wastewater



Tetraspora

Standard Methods for the Examination of Water and Wastewater



TETRASPORA

Audouinella

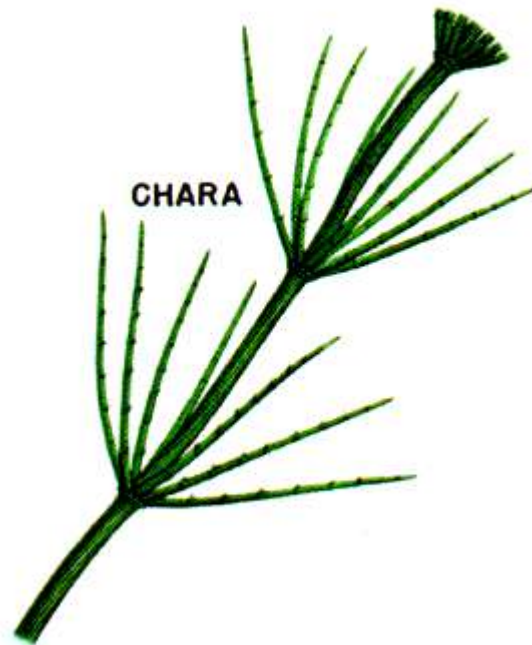
Standard Methods for the Examination of Water and Wastewater



AUDOUINELLA

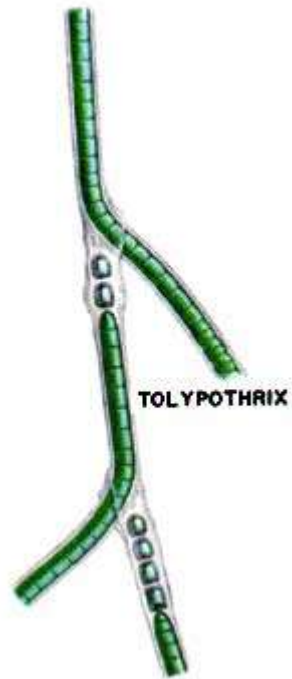
Chara

Standard Methods for the Examination of Water and Wastewater



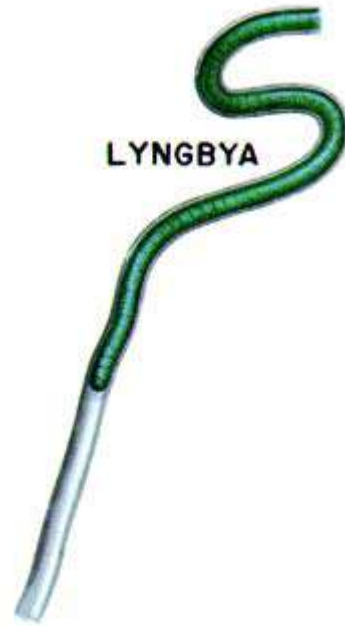
Tolypothrix

Standard Methods for the Examination of Water and Wastewater



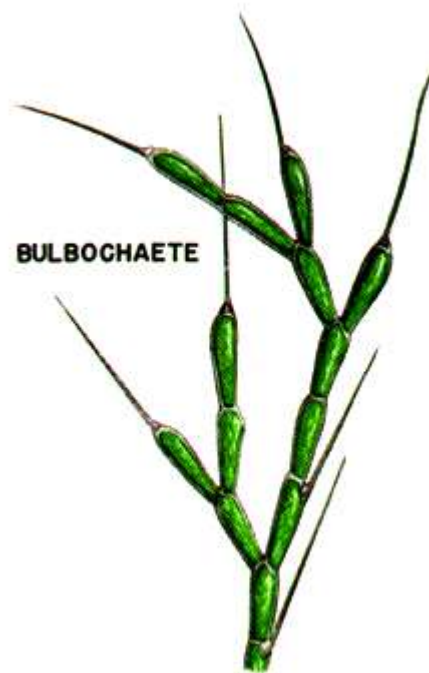
Lyngbya

Standard Methods for the Examination of Water and Wastewater



Bulbochaete

Standard Methods for the Examination of Water and Wastewater



Microspora

Standard Methods for the Examination of Water and Wastewater



MICROSPORA

Compsopogon

Standard Methods for the Examination of Water and Wastewater



Batrachospermum

Standard Methods for the Examination of Water and Wastewater



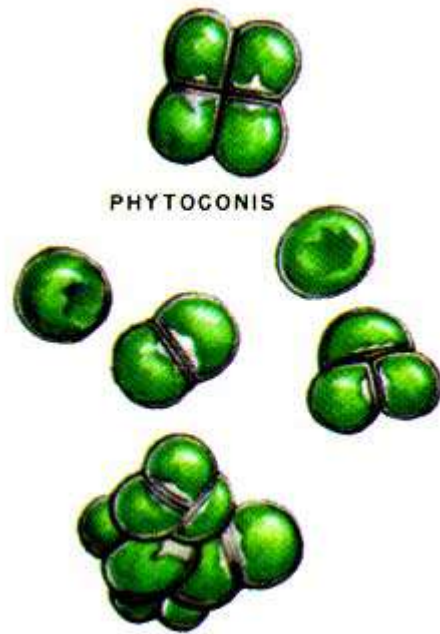
Cymbella

Standard Methods for the Examination of Water and Wastewater



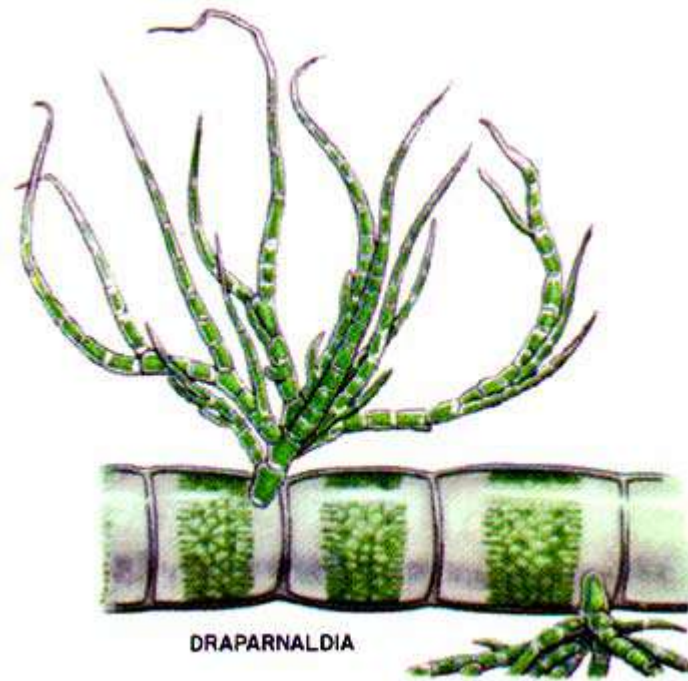
Phytoconis

Standard Methods for the Examination of Water and Wastewater



Draparnaldia

Standard Methods for the Examination of Water and Wastewater



Oedogonium

Standard Methods for the Examination of Water and Wastewater



Chaetophora

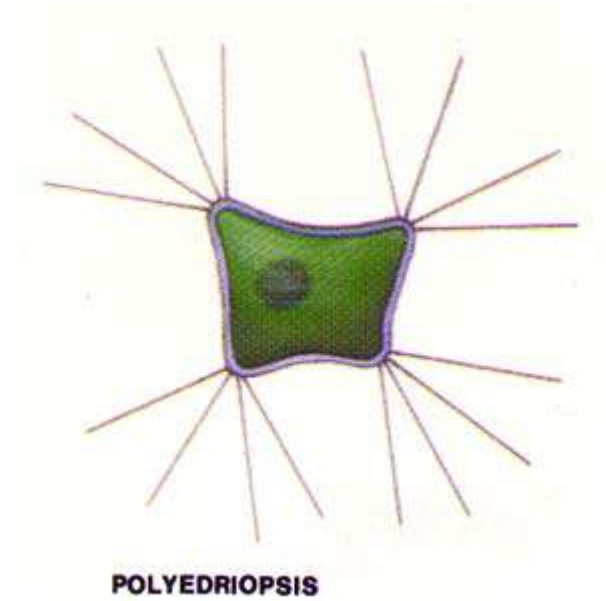
Standard Methods for the Examination of Water and Wastewater



Plate 34. Wastewater-treatment-pond algae.

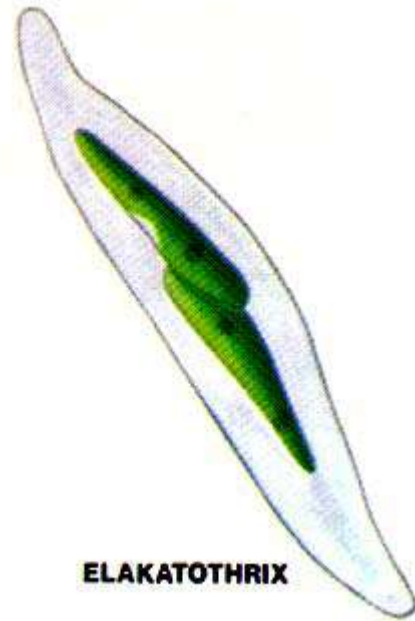
Polyedriopsis

Standard Methods for the Examination of Water and Wastewater



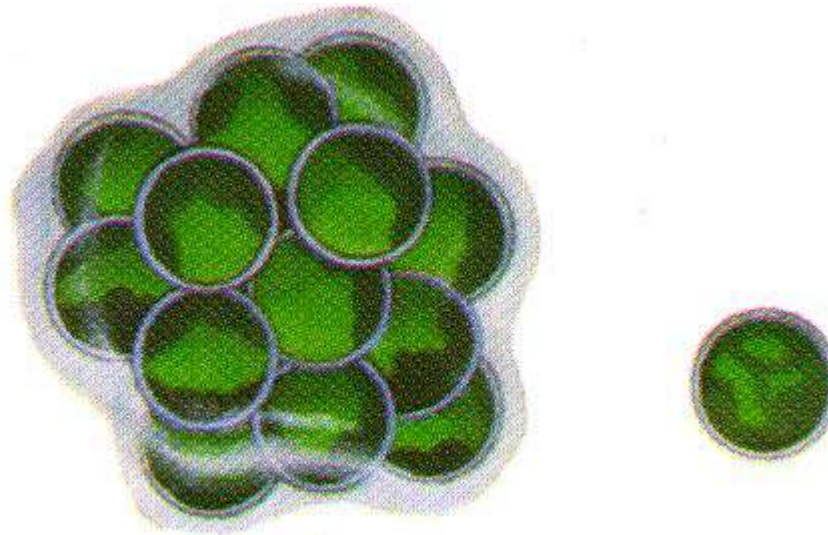
Elakotothrix

Standard Methods for the Examination of Water and Wastewater



Planktosphaeria

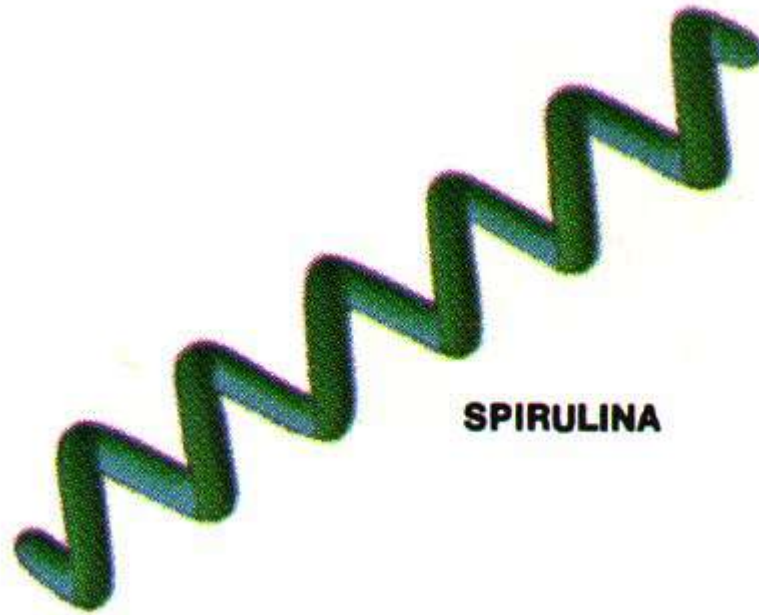
Standard Methods for the Examination of Water and Wastewater



PLANKTOSPHAERIA

Spirulina

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Ourococcus

Standard Methods for the Examination of Water and Wastewater



OUROCOCCUS

Diacanthos

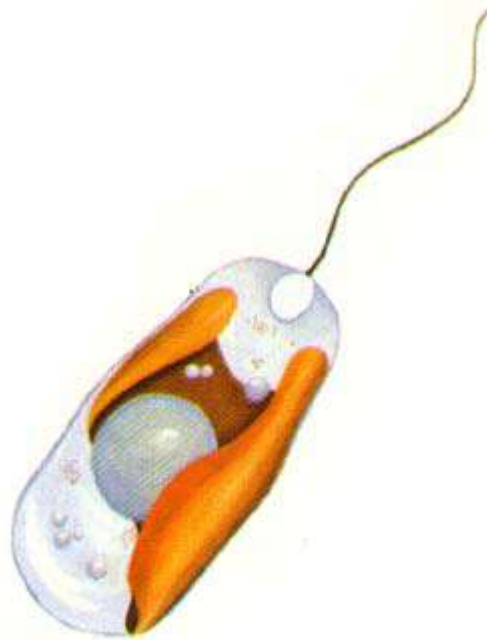
Standard Methods for the Examination of Water and Wastewater



DIACANTHOS

Chromulina

Standard Methods for the Examination of Water and Wastewater



CHROMULINA

Vacuolaria

Standard Methods for the Examination of Water and Wastewater



Closteridium

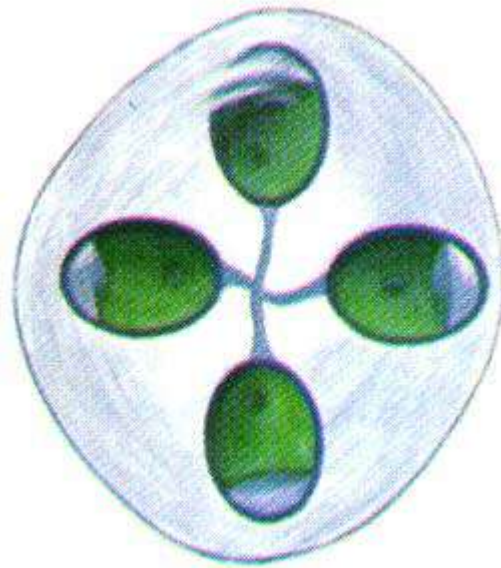
Standard Methods for the Examination of Water and Wastewater



CLOSTERIDIUM

Dictyosphaerium

Standard Methods for the Examination of Water and Wastewater



DICTYOSPHAERIUM

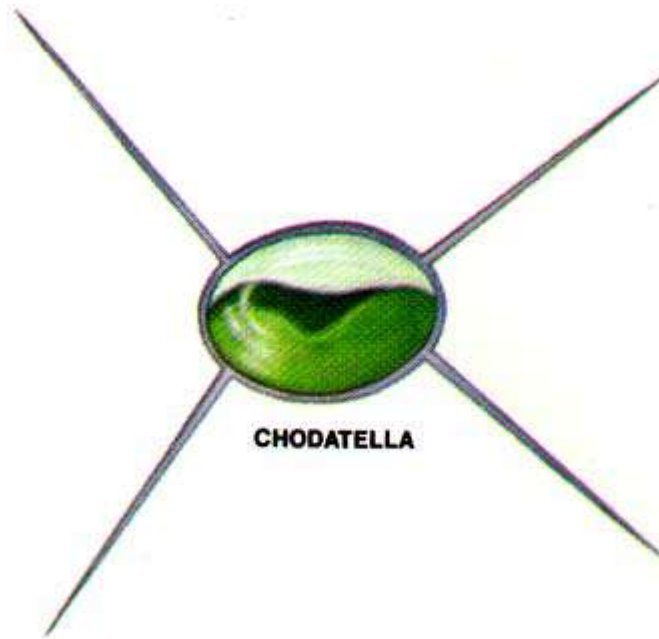
Chroomonas

Standard Methods for the Examination of Water and Wastewater



Chodatella

Standard Methods for the Examination of Water and Wastewater



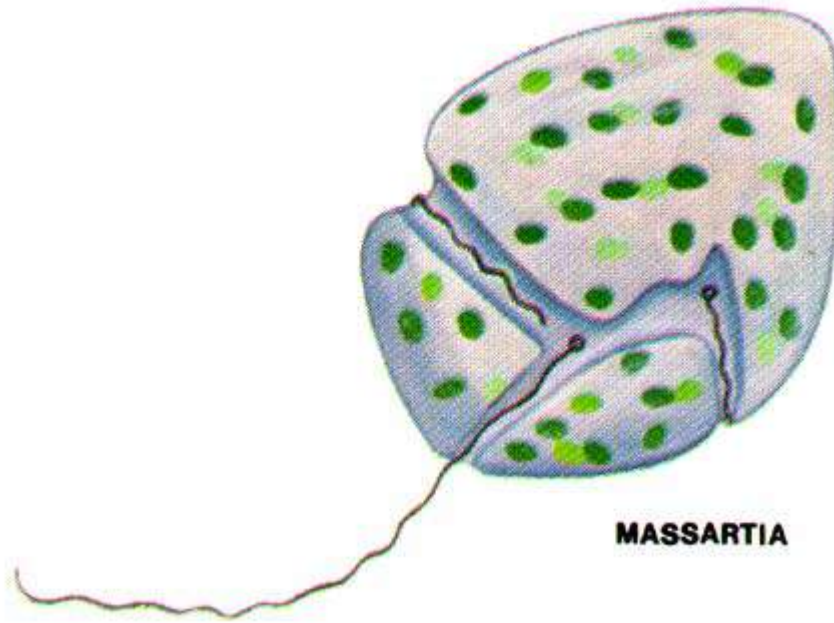
Ankistrodesmus

Standard Methods for the Examination of Water and Wastewater



Massartia

Standard Methods for the Examination of Water and Wastewater



Cryptomonas

Standard Methods for the Examination of Water and Wastewater



CRYPTOMONAS

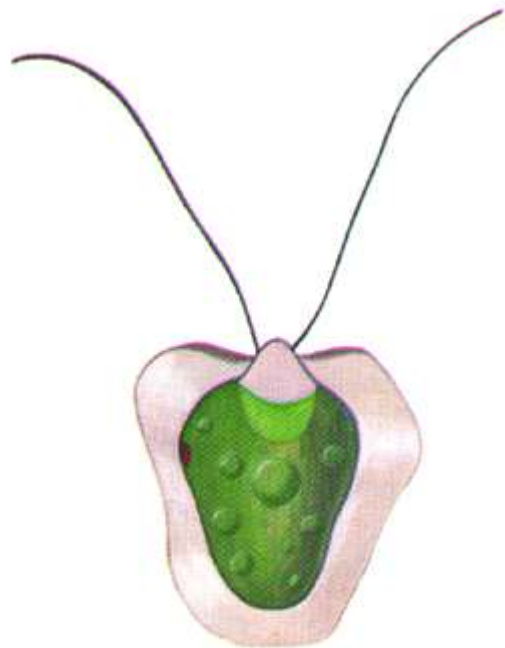
Closteriopsis



CLOSTERIOPSIS

Pteromonas

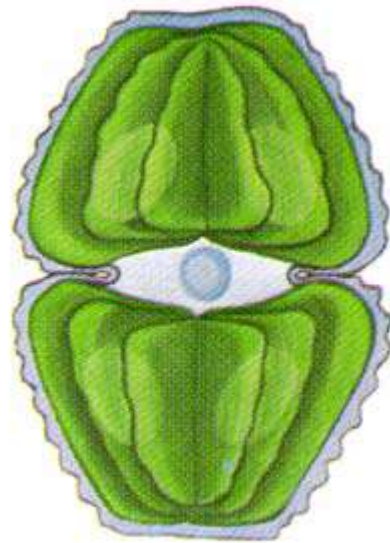
Standard Methods for the Examination of Water and Wastewater



PTEROMONAS

Cosmarium

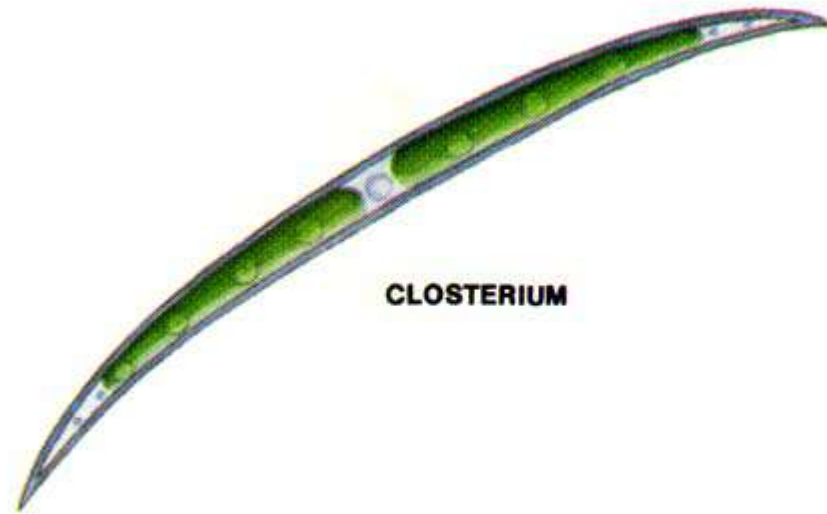
Standard Methods for the Examination of Water and Wastewater



COSMARIUM

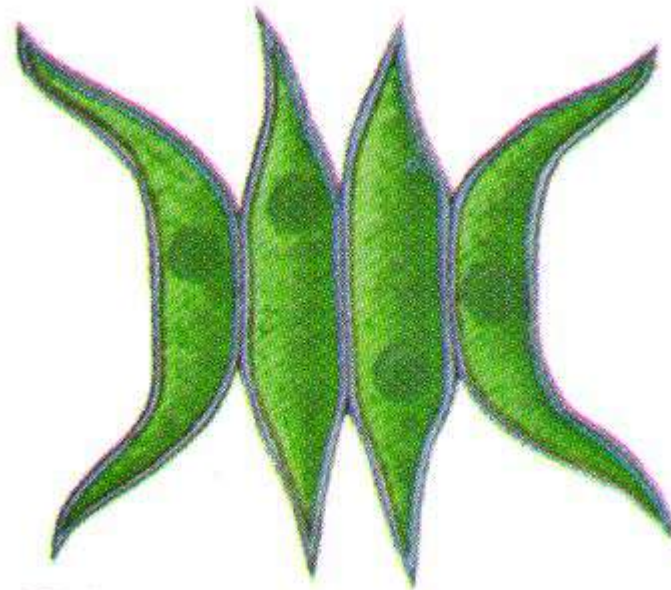
Closterium

Standard Methods for the Examination of Water and Wastewater



Scenedesmus

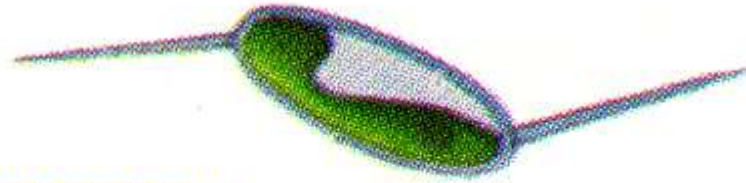
Standard Methods for the Examination of Water and Wastewater



SCENEDESMUS

Schroederia

Standard Methods for the Examination of Water and Wastewater



SCHROEDERIA

Chlamydomonas

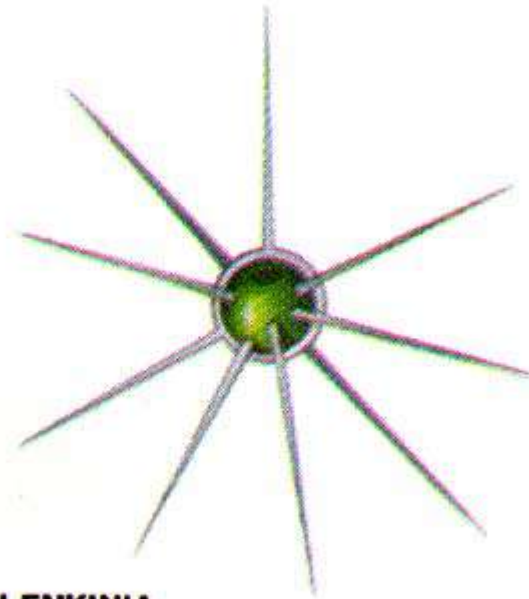
Standard Methods for the Examination of Water and Wastewater



CHLAMYDOMONAS

Golenkinia

Standard Methods for the Examination of Water and Wastewater



GOLENKINIA

Schizothrix

Standard Methods for the Examination of Water and Wastewater

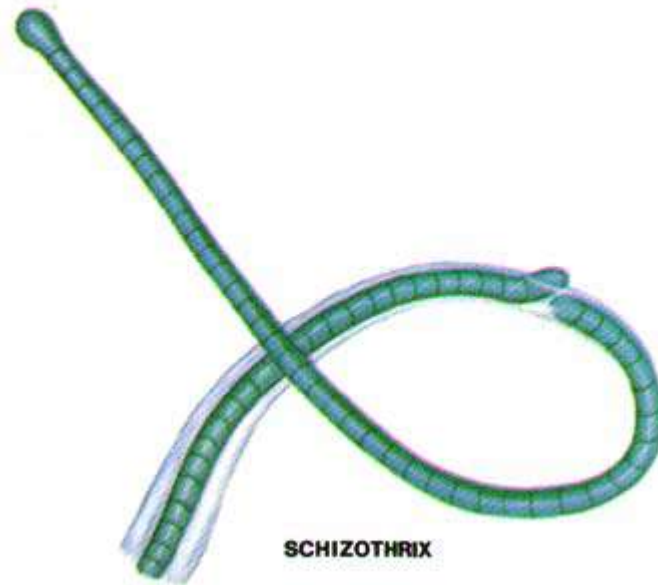


Plate 35. Estuarine pollution algae.

Spirulina

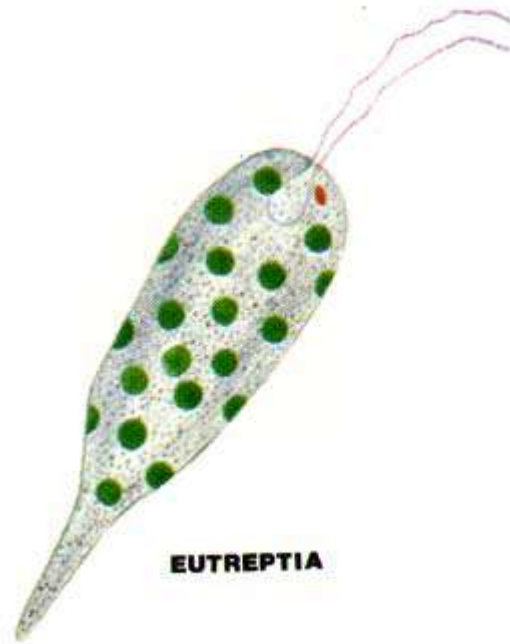
Standard Methods for the Examination of Water and Wastewater



SPIRULINA

Eutreptia

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Asterionella

Standard Methods for the Examination of Water and Wastewater



ASTERIONELLA

Porphyra

Standard Methods for the Examination of Water and Wastewater



Stephanoptera

Standard Methods for the Examination of Water and Wastewater



STEPHANOPTERA

Scytosiphon

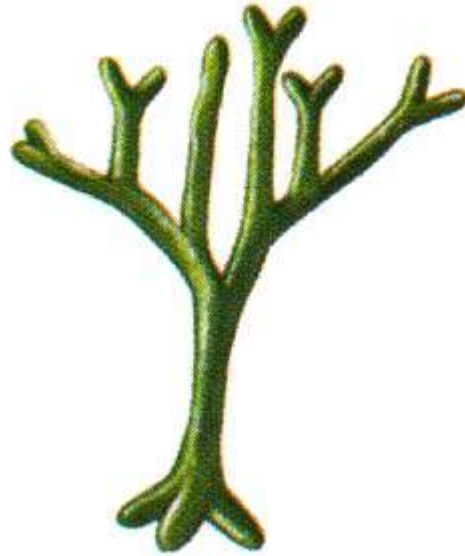
Standard Methods for the Examination of Water and Wastewater



SCYTOSIPHON

Codium

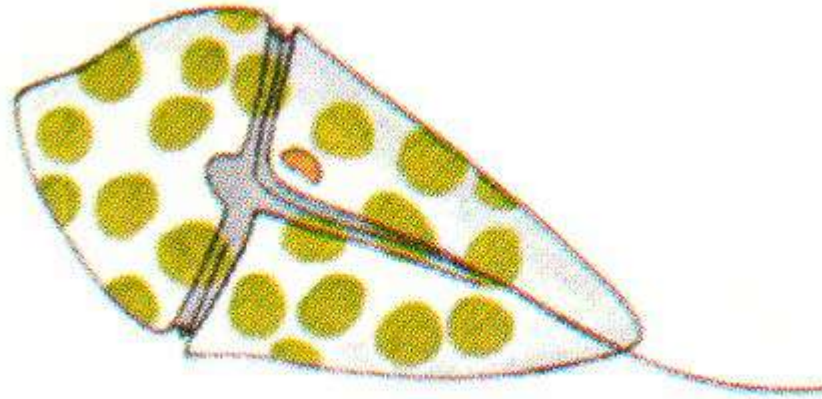
Standard Methods for the Examination of Water and Wastewater



CODIUM

Amphidinium

Standard Methods for the Examination of Water and Wastewater



AMPHIDINIUM

Trichodesmium

Standard Methods for the Examination of Water and Wastewater



TRICHODESMIUM

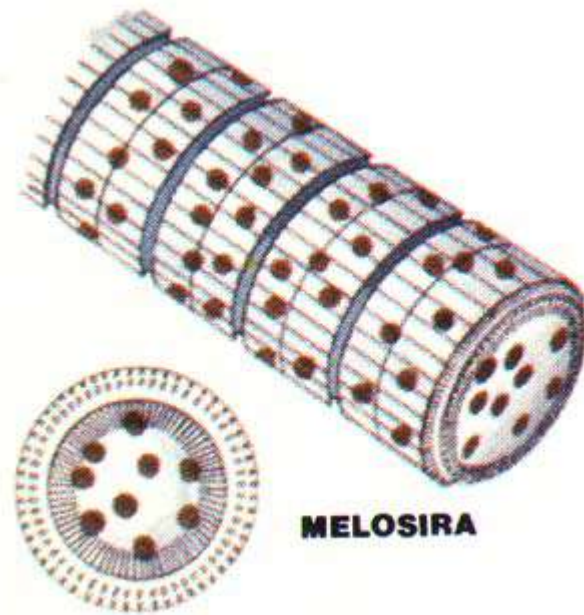
Pelvetia

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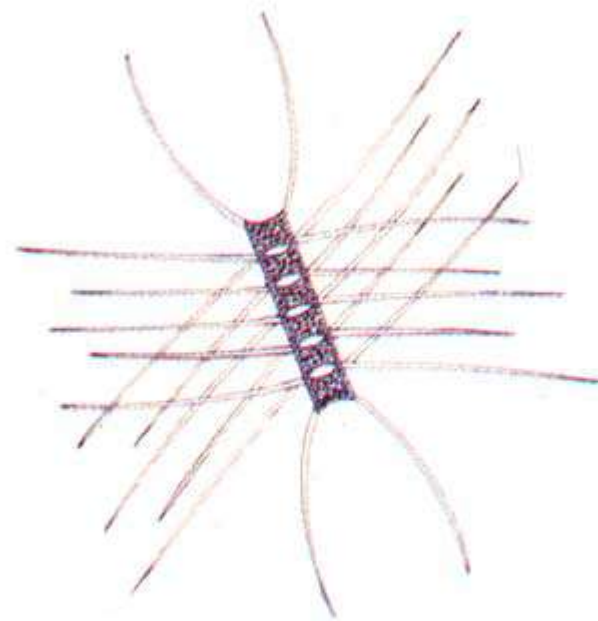
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CHAETOCEROS

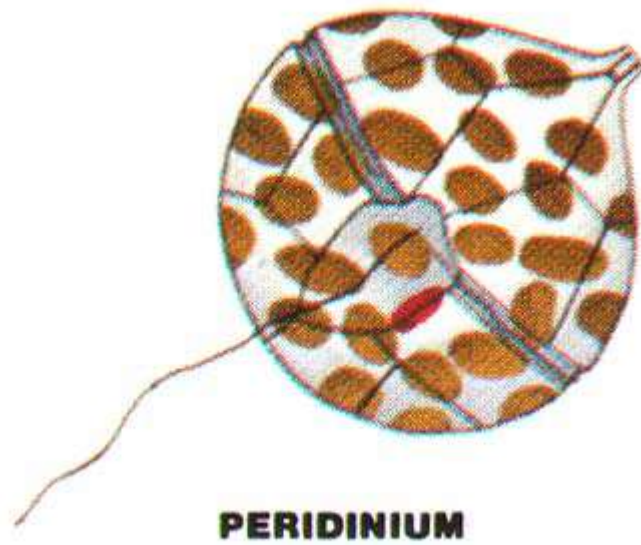
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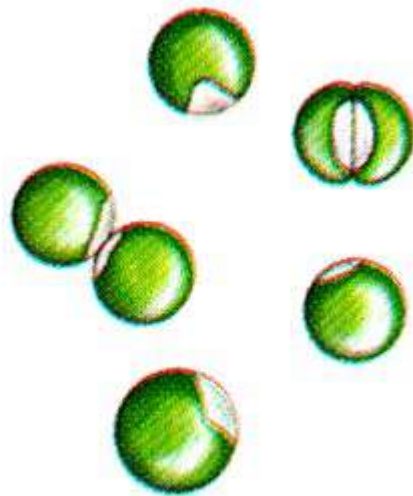
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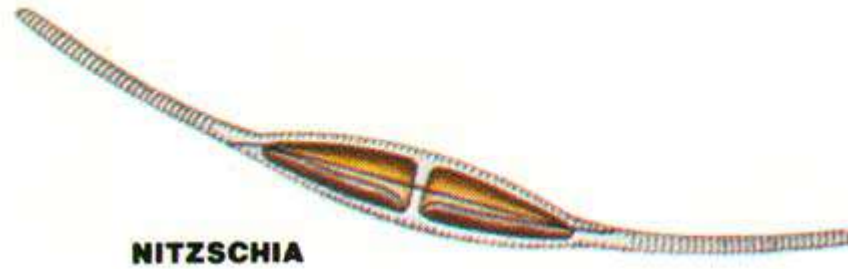
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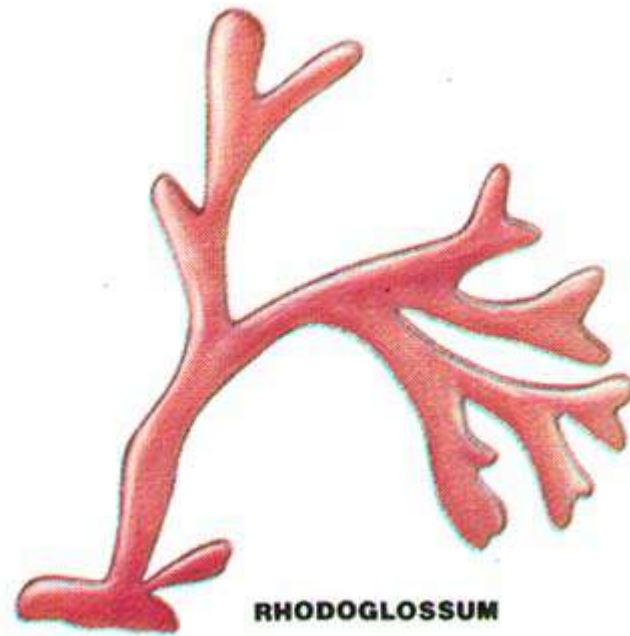
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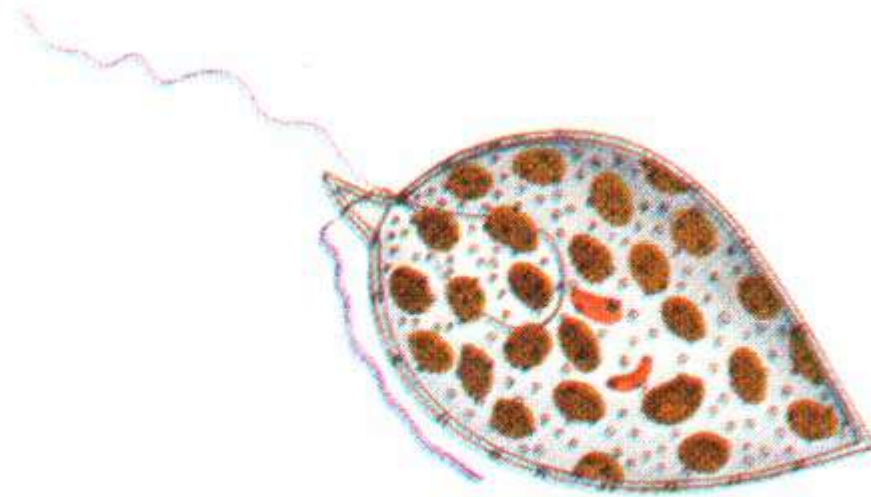
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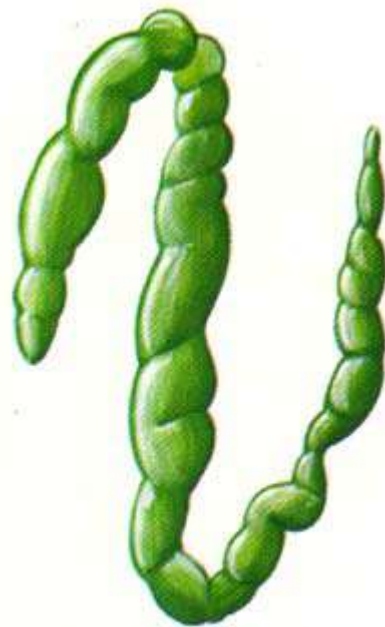
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Preparation of Common Types of Desk Reagents Specified in This Book

Acid Solutions

Prepare the following reagents by cautiously adding required amount of concentrated acid, with mixing, to designated volume of proper type of distilled water. Dilute to 1000 mL and mix thoroughly.

See Table A for preparation of HCl, H₂SO₄, and HNO₃ solutions.

Alkaline Solutions

a. *Stock sodium hydroxide*, NaOH, 15*N* (for preparing 6*N*, 1*N*, and 0.1*N* solutions):
Cautiously dissolve 625 g solid NaOH in 800 mL distilled water to form 1 L of solution.

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Remove sodium carbonate precipitate by keeping solution at the boiling point for a few hours in a hot water bath or by letting particles settle for at least 48 h in an alkali-resistant container (wax-lined or polyethylene) protected from atmospheric CO₂ with a soda lime tube. Use the supernate for preparing dilute solutions listed in Table B.

Alternatively prepare dilute solutions by dissolving the weight of solid NaOH indicated in Table B in CO₂-free distilled water and diluting to 1000 mL.

Store NaOH solutions in polyethylene (rigid, heavy-type) bottles with polyethylene screw caps, paraffin-coated bottles with rubber or neoprene stoppers, or borosilicate-glass bottles with rubber or neoprene stoppers. Check solutions periodically. Protect them by attaching a tube of CO₂-absorbing granular material such as soda lime or a commercially available CO₂-removing agent. (1) Use at least 70 cm of rubber tubing to minimize vapor diffusion from bottle. Replace absorption tube before it becomes exhausted. Withdraw solution by a siphon to avoid opening bottle.

b. *Ammonium hydroxide solutions*, NH₄OH: Prepare 5N, 3N, and 0.2N NH₄OH solutions by diluting 333 mL, 200 mL, and 13 mL, respectively, of the concentrated reagent (sp gr 0.90, 29.0%, 15N) to 1000 mL with distilled water.

Indicator Solutions

a. *Phenolphthalein indicator solution*: Use either the aqueous (1) or alcoholic (2) solution.

1) Dissolve 5 g phenolphthalein disodium salt in distilled water and dilute to 1 L.

2) Dissolve 5 g phenolphthalein in 500 mL 95% ethyl or isopropyl alcohol and add 500 mL distilled water.

If necessary, add 0.02N NaOH dropwise until a faint pink color appears in solution 1) or 2).

b. *Methyl orange indicator solution*: Dissolve 500 mg methyl orange powder in distilled water and dilute to 1 L.

TABLES

Table A: PREPARATION OF UNIFORM ACID SOLUTIONS*

Desired Component	Hydrochloric Acid (HCl)	Sulfuric Acid (H ₂ SO ₄)
Specific gravity (20/4°C) of ACS-grade conc acid	1.174–1.189	1.834–1.84
Percent of active ingredient in conc reagent	36–37	96–98
Normality of conc reagent	11–12	36

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Desired Component	Hydrochloric Acid (HCl)	Sulfuric Acid (H ₂ SO ₄)
Volume (mL) of conc reagent to prepare 1 L of:		
18 <i>N</i> solution	—	500 (1 + 1)
6 <i>N</i> solution	500 (1 + 1)†	167 (1 + 5)
1 <i>N</i> solution	83 (1 + 11)†	28
0.1 <i>N</i> solution	8.3	2.8
Volume (mL) of 6 <i>N</i> reagent to prepare 1 L of 0.1 <i>N</i> solution	17	17
Volume (mL) of 1 <i>N</i> reagent to prepare 1 L of 0.02 <i>N</i> solution	20	20

*All values approximate.

†The $a + b$ system of specifying preparatory volumes appears frequently throughout this manual and means that a volumes of the concentrated reagent are diluted with b volumes of distilled water to form the required solution.

Table B: PREPARATION OF UNIFORM SODIUM HYDROXIDE SOLUTIONS

Normality of NaOH Solution	Required Weight of NaOH to Prepare 1000 mL of Solution <i>g</i>	Required Volume of 15 <i>N</i> NaOH to Prepare 1000 mL of Solution <i>mL</i>
6.0	240	400
1.0	40	67
0.1	4	6.7

Standard Atomic Weights, 1995[Scaled to $A_r(^{12}\text{C}) = 12$]

The atomic weights of many elements are not invariant but depend on the origin and treatment of the material. The standard values of $A_r(\text{E})$ and the uncertainties (in parentheses, following the

Standard Methods for the Examination of Water and Wastewater

last significant figure to which they are attributed) apply to elements of natural terrestrial origin. The footnotes to this Table elaborate the types of variation which may occur for individual elements and which may be larger than the listed uncertainties of values of $A_r(E)$. Names of elements with atomic number 104 to 111 are temporary.

Name	Symbol	Atomic Number	Atomic Weight	Footnotes
Actinium*	Ac	89		
Aluminium	Al	13	26.981538(2)	
Americium*	Am	95		
Antimony	Sb	51	121.760(1)	g
Argon	Ar	18	39.948(1)	g, r
Arsenic	As	33	74.92160(2)	
Astatine*	At	85		
Barium	Ba	56	137.327(7)	
Berkelium*	Bk	97		
Beryllium	Be	4	9.012182(3)	
Bismuth	Bi	83	208.98038(2)	
Boron	B	5	10.811(7)	g, m, r
Bromine	Br	35	79.904(1)	
Cadmium	Cd	48	112.411(8)	g
Calcium	Ca	20	40.078(4)	g
Californium*	Cf	98		
Carbon	C	6	12.0107(8)	g, r
Cerium	Ce	58	140.116(1)	g
Cesium	Cs	55	132.90545(2)	
Chlorine	Cl	17	35.4527(9)	m
Chromium	Cr	24	51.9961(6)	
Cobalt	Co	27	58.933200(9)	
Copper	Cu	29	63.546(3)	r
Curium*	Cm	96		
Dysprosium	Dy	66	162.50(3)	g
Einsteinium*	Es	99		
Erbium	Er	68	167.26(3)	g
Europium	Eu	63	151.964(1)	g
Fermium*	Fm	100		

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Name	Symbol	Atomic Number	Atomic Weight	Footnotes
Fluorine	F	9	18.9984032(5)	
Francium*	Fr	87		
Gadolinium	Gd	64	157.25(3)	g
Gallium	Ga	31	69.723(1)	
Germanium	Ge	32	72.61(2)	
Gold	Au	79	196.96655(2)	
Hafnium	Hf	72	178.49(2)	
Helium	He	2	4.002602(2)	g, r
Holmium	Ho	67	164.93032(3)	
Hydrogen	H	1	1.00794(7)	g, m, r
Indium	In	49	114.818(3)	
Iodine	I	53	126.90447(3)	
Iridium	Ir	77	192.217(3)	
Iron	Fe	26	55.845(2)	
Krypton	Kr	36	83.80(1)	g, m
Lanthanum	La	57	138.9055(2)	g
Lawrencium*	Lr	103		
Lead	Pb	82	207.2(1)	g, r
Lithium	Li	3	[6.941(2)]†	g, m, r
Lutetium	Lu	71	174.967(1)	g
Magnesium	Mg	12	24.3050(6)	
Manganese	Mn	25	54.938049(9)	
Mendelevium*	Md	101		
Mercury	Hg	80	200.59(2)	
Molybdenum	Mo	42	95.94(1)	g
Neodymium	Nd	60	144.24(3)	g
Neon	Ne	10	20.1797(6)	g, m
Neptunium*	Np	93		
Nickel	Ni	28	58.6934(2)	
Niobium	Nb	41	92.90638(2)	
Nitrogen	N	7	14.00674(7)	g, r
Nobelium*	No	102		

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Name	Symbol	Atomic Number	Atomic Weight	Footnotes
Osmium	Os	76	190.23(3)	g
Oxygen	O	8	15.9994(3)	g, r
Palladium	Pd	46	106.42(1)	g
Phosphorus	P	15	30.973762(4)	
Platinum	Pt	78	195.078(2)	
Plutonium*	Pu	94		
Polonium	Po	84		
Potassium	K	19	39.0983(1)	g
Praseodymium	Pr	59	140.90765(3)	
Promethium*	Pm	61		
Protactinium*	Pa	91	231.03588(2)	
Radium*	Ra	88		
Radon*	Rn	86		
Rhenium	Re	75	186.207(1)	
Rhodium	Rh	45	102.90550(2)	
Rubidium	Rb	37	85.4678(3)	g
Ruthenium	Ru	44	101.07(2)	g
Samarium	Sm	62	150.36(3)	g
Scandium	Sc	21	44.955910(8)	
Selenium	Se	34	78.96(3)	
Silicon	Si	14	28.0855(3)	r
Silver	Ag	47	107.8682(2)	g
Sodium	Na	11	22.989770(2)	
Strontium	Sr	38	87.62(1)	g, r
Sulfur	S	16	32.066(6)	g, r
Tantalum	Ta	73	180.9479(1)	
Technetium*	Tc	43		
Tellurium	Te	52	127.60(3)	g
Terbium	Tb	65	158.92534(3)	
Thallium	Tl	81	204.3833(2)	
Thorium*	Th	90	232.0381(1)	g
Thulium	Tm	69	168.93421(2)	

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Name	Symbol	Atomic Number	Atomic Weight	Footnotes
Tin	Sn	50	118.710(7)	g
Titanium	Ti	22	47.867(1)	
Tungsten	W	74	183.84(1)	
Unnilennium*	Une	109		
Unnilhexium*	Unh	106		
Unniloctium*	Uno	108		
Unnilpentium*	Unp	105		
Unnilquadium*	Unq	104		
Unnilseptium*	Uns	107		
Ununnilium	Uun	110		
Unununium	Uuu	111		
Uranium*	U	92	238.0289(1)	g, m
Vanadium	V	23	50.9415(1)	
Xenon	Xe	54	131.29(2)	g, m
Ytterbium	Yb	70	173.04(3)	g
Yttrium	Y	39	88.90585(2)	
Zinc	Zn	30	65.39(2)	
Zirconium	Zr	40	91.224(2)	g

* Element has no stable nuclides.

† Commercially available Li materials have atomic weights that range between 6.94 and 6.99; if a more accurate value is required, it must be determined for the specific material.

g geological specimens are known in which the element has an isotopic composition outside the limits for normal material. The difference between the atomic weight of the element in such specimens and that given in the Table may exceed the stated uncertainty.

m modified isotopic compositions may be found in commercially available material because it has been subjected to an undisclosed or inadvertent isotopic fractionation. Substantial deviations in atomic weight of the element from that given in the Table can occur.

r range in isotopic composition of normal terrestrial material prevents a more precise $A_r(E)$ being given; the tabulated $A_r(E)$ value should be applicable to any normal material.

Source: INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY. 1996. Atomic weights of the elements, 1996, *Pure Appl. Chem.* 68:2339.

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Abbreviations

The following symbols and abbreviations are used throughout this book:

Abbreviation	Referent
AA	atomic absorption
A or amp	ampere(s)
AC	alternating current
ACS	American Chemical Society
amu	atomic mass units
APHA	American Public Health Association
ASTM	American Society for Testing and Materials
AWWA	American Water Works Association
BOD	biochemical oxygen demand
°C	degree(s) Celsius
c	count(s)
Ci	curie(s)
cm, cm ² , cm ³	centimeter(s), square centimeter(s), cubic centimeter(s)
COD	chemical oxygen demand
conc	concentrated
cpm	counts per minute
cps	counts per second
d	day
DC	direct current
diam	diameter
DO	dissolved oxygen
DOX	dissolved organic halogen
dpm	disintegrations per minute
g	gram(s)

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<i>Abbreviation</i>	<i>Referent</i>
<i>g</i>	gravity, unit acceleration of
GC	gas chromatograph
GC/MS	gas chromatograph/mass spectrometer
<i>h</i>	hour
HPLC	high-performance liquid chromatography
IC	ion chromatograph
ICP	inductively coupled plasma
ID	inside diameter
IU	international unit(s)
KeV	kiloelectron volt(s)
kg	kilogram(s)
kPa	kilopascal
L	liter(s)
<i>M</i>	mole or molar
m, m ² , m ³	meter(s), square meter(s), cubic meter(s)
MCL	maximum contaminant level
MDL	method detection level
me	milliequivalent(s)
meV	megaelectron volt(s)
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
mm, mm ² , mm ³	millimeter(s), square millimeter(s), cubic millimeter(s)
mol wt	molecular weight
MPN	most probable number
MS	mass spectrometer
mV	millivolt(s)
μA	microampere(s)

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Abbreviation	Referent
μCi	microcurie(s)
μg	microgram(s)
μL	microliter(s)
μm	micrometer(s)
 N	 normal
nCi	nanocurie(s)
ng	nanogram(s)
NIST	National Institute of Standards and Technology
No.	number
NTU	nephelometric turbidity unit(s)
 OD	 outside diameter
 Pa	 pascal
pCi	picocurie(s)
pg	picogram(s)
PTFE	polytetrafluoroethylene
PVC	polyvinyl chloride
 rpm	 revolution(s) per minute
rps	revolution(s) per second
 SD	 standard deviation
SDI	sludge density index
s	second(s)
sp., spp.	species
sp gr	specific gravity
ST	standard taper
SVI	sludge volume index
 TFE	 tetrafluoroethylene
THM	trihalomethane(s)

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Abbreviation	Referent
TOC	total organic carbon
TON	threshold odor number
TOX	total organic halogen
Toxicity terms	see Section 8010B
U	unit(s)
USGS	U.S. Geological Survey
USP	United States Pharmacopoeia
UV	ultraviolet
V	volt(s)
v/v	volume ratio
W	watt(s)
WEF	Water Environment Federation
WPCF	see WEF

Abbreviations of periodical titles in reference lists and bibliographies are based on those given in *Biosis. List of Serials with Title Abbreviations*, 1970. Biosciences Information Service of Biological Abstracts, Philadelphia, Pa.

General Information

Table A: UNIT PREFIXES

Symbol	Prefix	Multiples and Submultiples
M	mega-	10^6
k	kilo-	10^3
m	milli-	10^{-3}
μ	micro-	10^{-6}

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Symbol	Prefix	Multiples and Submultiples
n	nano-	10^{-9}
p	pico-	10^{-12}

Table B: METRIC-ENGLISH EQUIVALENTS

Metric Unit	Multiplied by	= English Unit
m	3.279	ft
lux	0.0929	ft-c
L	0.2642	gal
cm	0.394	in.
kg	2.203	lb
g	0.0353	oz
kPa	0.145	psi

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Endnotes

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Part 4000

INORGANIC NONMETALLIC CONSTITUENTS

4010 INTRODUCTION

The analytical methods included in this part make use of classical wet chemical techniques and their automated variations and such modern instrumental techniques as ion chromatography. Methods that measure various forms of chlorine, nitrogen, and phosphorus are presented. The procedures are intended for use in the assessment and control of receiving water quality, the treatment and supply of potable water, and the measurement of operation and process efficiency in wastewater treatment. The methods also are appropriate and applicable in evaluation of environmental water-quality concerns. The introduction to each procedure contains reference to special field sampling conditions, appropriate sample containers, proper procedures for sampling and storage, and the applicability of the method.

4020 QUALITY ASSURANCE/QUALITY CONTROL

4020 A. Introduction

Without quality control results there is no confidence in analytical results reported from tests. As described in Part 1000 and Section 3020, essential quality control measurements include: method calibration, standardization of reagents, assessment of individual capability to perform the analysis, performance of blind check samples, determination of the sensitivity of the test procedure (method detection level), and daily evaluation of bias, precision, and the presence of laboratory contamination or other analytical interference. Details of these procedures, expected ranges of results, and frequency of performance should be formalized in a written Quality Assurance Manual and Standard Operating Procedures.

For a number of the procedures contained in Part 4000, the traditional determination of bias using a known addition to either a sample or a blank, is not possible. Examples of these procedures include pH, dissolved oxygen, residual chlorine, and carbon dioxide. The inability to perform a reliable known addition does not relieve the analyst of the responsibility for evaluating test bias. Analysts are encouraged to purchase certified ready-made solutions of known levels of these constituents as a means of measuring bias. In any situation, evaluate precision through analysis of sample duplicates.

Participate in a regular program (at a minimum, annually, and preferably semi-annually) of proficiency testing (PT)/performance evaluation (PE) studies. The information and analytical confidence gained in the routine performance of the studies more than offset any costs associated

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with these studies. An unacceptable result on a PT study sample is often the first indication that a test protocol is not being followed successfully. Investigate circumstances fully to find the cause. Within many jurisdictions, participation in PT studies is a required part of laboratory certification.

Many of the methods contained in Part 4000 include specific quality-control procedures. These are considered to be the minimum quality controls necessary to successful performance of the method. Additional quality control procedures can and should be used. Section 4020B describes a number of QC procedures that are applicable to many of the methods.

4020 B. Quality Control Practices

1. Initial Quality Control

See Section 3020B.1.

2. Calibration

See Section 3020B.2. Most methods for inorganic nonmetals do not have wide dynamic ranges. Standards for initial calibration therefore should be spaced more closely than one order of magnitude under these circumstances. Verify calibration by analyzing a midpoint or lower calibration standard and blank as directed. Alternatively, verify calibration with two standards, one near the low end and one near the high end, if the blank is used to zero the instrument.

3. Batch Quality Control

See Section 3020B.3a through d.

4110 DETERMINATION OF ANIONS BY ION CHROMATOGRAPHY*#(1)

4110 A. Introduction

Because of rapid changes in technology, this section is currently undergoing substantial revision.

Determination of the common anions such as bromide, chloride, fluoride, nitrate, nitrite, phosphate, and sulfate often is desirable to characterize a water and/or to assess the need for specific treatment. Although conventional colorimetric, electrometric, or titrimetric methods are available for determining individual anions, only ion chromatography provides a single instrumental technique that may be used for their rapid, sequential measurement. Ion chromatography eliminates the need to use hazardous reagents and it effectively distinguishes among the halides (Br^- , Cl^- , and F^-) and the oxy-ions (SO_3^{2-} , SO_4^{2-} or NO_2^- , NO_3^-).

This method is applicable, after filtration to remove particles larger than $0.2\ \mu\text{m}$, to surface,

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ground, and wastewaters as well as drinking water. Some industrial process waters, such as boiler water and cooling water, also may be analyzed by this method.

4110 B. Ion Chromatography with Chemical Suppression of Eluent Conductivity

1. General Discussion

a. Principle: A water sample is injected into a stream of carbonate-bicarbonate eluent and passed through a series of ion exchangers. The anions of interest are separated on the basis of their relative affinities for a low capacity, strongly basic anion exchanger (guard and separator columns). The separated anions are directed through a hollow fiber cation exchanger membrane (fiber suppressor) or micromembrane suppressor bathed in continuously flowing strongly acid solution (regenerant solution). In the suppressor the separated anions are converted to their highly conductive acid forms and the carbonate-bicarbonate eluent is converted to weakly conductive carbonic acid. The separated anions in their acid forms are measured by conductivity. They are identified on the basis of retention time as compared to standards. Quantitation is by measurement of peak area or peak height.

b. Interferences: Any substance that has a retention time coinciding with that of any anion to be determined and produces a detector response will interfere. For example, relatively high concentrations of low-molecular-weight organic acids interfere with the determination of chloride and fluoride by isocratic analyses. A high concentration of any one ion also interferes with the resolution, and sometimes retention, of others. Sample dilution or gradient elution overcomes many interferences. To resolve uncertainties of identification or quantitation use the method of known additions. Spurious peaks may result from contaminants in reagent water, glassware, or sample processing apparatus. Because small sample volumes are used, scrupulously avoid contamination. Modifications such as preconcentration of samples, gradient elution, or reinjection of portions of the eluted sample may alleviate some interferences but require individual validation for precision and bias.

c. Minimum detectable concentration: The minimum detectable concentration of an anion is a function of sample size and conductivity scale used. Generally, minimum detectable concentrations are near 0.1 mg/L for Br^- , Cl^- , NO_3^- , NO_2^- , PO_4^{3-} , and SO_4^{2-} with a 100- μL sample loop and a 10- $\mu\text{S}/\text{cm}$ full-scale setting on the conductivity detector. Lower values may be achieved by using a higher scale setting, an electronic integrator, or a larger sample size.

d. Limitations: This method is not recommended for the determination of F^- in unknown matrices. Equivalency studies have indicated positive or negative bias and poor precision in some samples. Recent interlaboratory studies show acceptable results. Two effects are common: first, F^- is difficult to quantitate at low concentrations because of the major negative contribution of the “water dip” (corresponding to the elution of water); second, the simple organic acids (formic, carbonic, etc.) elute close to fluoride and will interfere. Determine precision and bias

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before analyzing samples. F^- can be determined accurately by ion chromatography using special techniques such as dilute eluent or gradient elution using an NaOH eluent or alternative columns.

2. Apparatus

a. *Ion chromatograph*, including an injection valve, a sample loop, guard column, separator column, and fiber or membrane suppressors, a temperature-compensated small-volume conductivity cell and detector (6 μL or less), and a strip-chart recorder capable of full-scale response of 2 s or less. An electronic peak integrator is optional. Use an ion chromatograph capable of delivering 2 to 5 mL eluent/min at a pressure of 1400 to 6900 kPa.

b. *Anion separator column*, with styrene divinylbenzene-based low-capacity pellicular anion-exchange resin capable of resolving Br^- , Cl^- , NO_3^- , NO_2^- , PO_4^{3-} , and SO_4^{2-} .^{*(2)}

c. *Guard column*, identical to separator column^{†(3)} to protect separator column from fouling by particulates or organics.

d. *Fiber suppressor or membrane suppressor*:^{‡(4)} Cation-exchange membrane capable of continuously converting eluent and separated anions to their acid forms. Alternatively, use continuously regenerated suppression systems.

3. Reagents

a. *Deionized or distilled water* free from interferences at the minimum detection limit of each constituent, filtered through a 0.2- μm membrane filter to avoid plugging columns, and having a conductance of $< 0.1 \mu\text{S}/\text{cm}$.

b. *Eluent solution*, sodium bicarbonate-sodium carbonate, 0.0017M NaHCO_3 -0.0018M Na_2CO_3 : Dissolve 0.5712 g NaHCO_3 and 0.7632 g Na_2CO_3 in water and dilute to 4 L.

c. *Regenerant solution*, H_2SO_4 , 0.025N: Dilute 2.8 mL conc H_2SO_4 to 4 L.

d. *Standard anion solutions*, 1000 mg/L: Prepare a series of standard anion solutions by weighing the indicated amount of salt, dried to a constant weight at 105°C , to 1000 mL. Store in plastic bottles in a refrigerator; these solutions are stable for at least 1 month. Verify stability.

Anion ^s	Salt	Amount g/L
Cl^-	NaCl	1.6485
Br^-	NaBr	1.2876
NO_3^-	NaNO_3	1.3707 (226 mg NO_3^- -N/L)
NO_2^-	NaNO_2	1.4998 (304 mg NO_2^- -N/L)
PO_4^{3-}	KH_2PO_4	1.4330 (326 mg PO_4^{3-} -P/L)

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Anion [§]	Salt	Amount g/L
SO ₄ ²⁻	K ₂ SO ₄	1.8141

[§] Expressed as compound.

|| Do not oven-dry, but dry to constant weight in a desiccator.

e. Combined working standard solution, high range: Combine 12 mL of standard anion solutions, 1000 mg/L (¶ 3d) of NO₂⁻, NO₃⁻, HPO₄²⁻, and Br⁻, 20 mL of Cl⁻, and 80 mL of SO₄²⁻. Dilute to 1000 mL and store in a plastic bottle protected from light. Solution contains 12 mg/L each of NO₂⁻, NO₃⁻, HPO₄²⁻, and Br⁻, 20 mg/L of Cl⁻, and 80 mg/L of SO₄²⁻. Prepare fresh daily.

f. Combined working standard solution, low range: Dilute 25 mL of the high-range mixture (¶ 3e) to 100 mL and store in a plastic bottle protected from light. Solution contains 3 mg/L each of NO₂⁻, NO₃⁻, HPO₄²⁻, and Br⁻, 5 mg/L Cl⁻, and 20 mg/L of SO₄²⁻. Prepare fresh daily.

g. Alternative combined working standard solutions: Prepare appropriate combinations according to anion concentration to be determined. If NO₂⁻ and PO₄³⁻ are not included, the combined working standard is stable for 1 month. Dilute solutions containing NO₂⁻ and PO₄³⁻ must be made daily.

4. Procedure

a. System equilibration: Turn on ion chromatograph and adjust eluent flow rate to approximate the separation achieved in Figure 4110:1 (about 2 mL/min). Adjust detector to desired setting (usually 10 to 30 µS) and let system come to equilibrium (15 to 20 min). A stable base line indicates equilibrium conditions. Adjust detector offset to zero out eluent conductivity; with the fiber or membrane suppressor adjust the regeneration flow rate to maintain stability, usually 2.5 to 3 mL/min.

b. Calibration: Inject standards containing a single anion or a mixture and determine approximate retention times. Observed times vary with conditions but if standard eluent and anion separator column are used, retention always is in the order F⁻, Cl⁻, NO₂⁻, Br⁻, NO₃⁻, HPO₄²⁻, and SO₄²⁻. Inject at least three different concentrations (one near the minimum reporting limit) for each anion to be measured and construct a calibration curve by plotting peak height or area against concentration on linear graph paper. Recalibrate whenever the detector setting, eluent, or regenerant is changed. To minimize the effect of the “water dip”##(5) on F⁻ analysis, analyze standards that bracket the expected result or eliminate the water dip by diluting the sample with eluent or by adding concentrated eluent to the sample to give the same

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$\text{HCO}_3^-/\text{CO}_3^{2-}$ concentration as in the eluent. If sample adjustments are made, adjust standards and blanks identically.

If linearity is established for a given detector setting, single standard calibration is acceptable. Record peak height or area and retention time for calculation of the calibration factor, F . However, a calibration curve will result in better precision and bias. HPO_4^{2-} is nonlinear below 1.0 mg/L.

c. Sample analysis: Remove sample particulates, if necessary, by filtering through a prewashed 0.2- μm -pore-diam membrane filter. Using a prewashed syringe of 1 to 10 mL capacity equipped with a male luer fitting inject sample or standard. Inject enough sample to flush sample loop several times: for 0.1 mL sample loop inject at least 1 mL. Switch ion chromatograph from load to inject mode and record peak heights and retention times on strip chart recorder. After the last peak (SO_4^{2-}) has appeared and the conductivity signal has returned to base line, another sample can be injected.

5. Calculations

Calculate concentration of each anion, in milligrams per liter, by referring to the appropriate calibration curve. Alternatively, when the response is shown to be linear, use the following equation:

$$C = H \times F \times D$$

where:

C = mg anion/L,

H = peak height or area,

F = response factor = concentration of standard/height (or area) of standard, and

D = dilution factor for those samples requiring dilution.

6. Quality Control

See Section 4020 for minimum QC guidelines.

7. Precision and Bias

The data in Table 4110:I, Table 4110:II, Table 4110:III, Table 4110:IV, Table 4110:V, Table 4110:VI, and Table 4110:VII were produced in a joint validation study with EPA and ASTM participation. Nineteen laboratories participated and used known additions of six prepared concentrates in three waters (reagent, waste, and drinking) of their choice.

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4110 C. Single-Column Ion Chromatography with Electronic Suppression of Eluent Conductivity and Conductimetric Detection

1. General Discussion

a. Principle: A small portion of a filtered, homogeneous, aqueous sample or a sample containing no particles larger than 0.45 μm is injected into an ion chromatograph. The sample merges with the eluent stream and is pumped through the ion chromatographic system. Anions are separated on the basis of their affinity for the active sites of the column packing material. Conductivity detector readings (either peak area or peak height) are used to compute concentrations.

b. Interferences: Any two species that have similar retention times can be considered to interfere with each other. This method has potential coelution interference between short-chain acids and fluoride and chloride. Solid-phase extraction cartridges can be used to retain organic acids and pass inorganic anions. The interference-free solution then can be introduced into the ion chromatograph for separation.

This method is usable but not recommended for fluoride. Acetate, formate, and carbonate interfere in determining fluoride under the conditions listed in Table 4110:VIII. Filtering devices may be used to remove organic materials for fluoride measurements; simultaneously, use a lower eluent flow rate.

Chlorate and bromide coelute under the specified conditions. Determine whether other anions in the sample coelute with the anions of interest.

Additional interference occurs when anions of high concentrations overlap neighboring anionic species. Minimize this by sample dilution with reagent water.

Best separation is achieved with sample pH between 5 and 9. When samples are injected the eluent pH will seldom change unless the sample pH is very low. Raise sample pH by adding a small amount of a hydroxide salt to enable the eluent to control pH.

Because method sensitivity is high, avoid contamination by reagent water and equipment. Determine any background or interference due to the matrix when adding the QC sample into any matrix other than reagent water.

c. Minimum detectable concentration: The minimum detectable concentration of an anion is a function of sample volume and the signal-to-noise ratio of the detector-recorder combination. Generally, minimum detectable concentrations are about 0.1 mg/L for the anions with an

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injection volume of 100 μL . Preconcentrators or using larger injection volumes can reduce detection limits to nanogram-per-liter levels for the common anions. However, coelution is a possible problem with large injection volumes. Determine method detection limit for each anion of interest.

d. Prefiltration: If particularly contaminated samples are run, prefilter before or during injection. If the guard column becomes contaminated, follow manufacturer's suggestions for cleanup.

2. Apparatus

a. Ion chromatograph, complete with all required accessories including syringes, analytical columns, gases, detector, and a data system. Required accessories are listed below.

b. Filter device, 0.45 μm , placed before separator column to protect it from fouling by particulates or organic constituents.*#(6)

c. Anion separator column, packed with low-capacity anion-exchange resin capable of resolving fluoride, chloride, nitrite, bromide, nitrate, orthophosphate, and sulfate.†#(7)

d. Conductivity detector, flow-through, with integral heat-exchange unit allowing automatic temperature control and with separate working and reference electrodes.

e. Pump, constant flow rate controlled, high-pressure liquid chromatographic type, to deliver 1.5 mL/min.

f. Data system, including one or more computer, integrator, or strip chart recorder compatible with detector output voltage.

g. Sample injector: Either an automatic sample processor or a manual injector. If manual injector is used, provide several glass syringes of > 200 μL capacity. The automatic device must be compatible and able to inject a minimum sample volume of 100 μL .

3. Reagents

a. Reagent water: Distilled or deionized water of 18 megohm-cm resistivity containing no particles larger than 0.20 μm .

b. Borate/gluconate concentrate: Combine 16.00 g sodium gluconate, 18.00 g boric acid, 25.00 g sodium tetraborate decahydrate, and 125 mL glycerin in 600 mL reagent water. Mix and dilute to 1 L with reagent water.

c. Eluent solution, 0.0110M borate, 0.0015M gluconate, 12% (v/v) acetonitrile: Combine 20 mL borate/gluconate concentrate, 120 mL HPLC-grade acetonitrile, and 20 mL HPLC-grade *n*-butanol, and dilute to 1 L with reagent water. Use an in-line filter before the separator column to assure freedom from particulates. If the base line drifts, degas eluent with an inert gas such as helium or argon.

d. Stock standard solutions: See Section 4110B.3e.

e. Combined working standard solutions, high-range: See Section 4110B.3e.

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f. Combined working standard solutions, low-range: See Section 4110B.3 *f*.

4. Procedure

a. System equilibration: Set up ion chromatograph in accordance with the manufacturer's directions. Install guard and separator columns and begin pumping eluent until a stable base line is achieved. The background conductivity of the eluent solution is $278 \mu\text{S} \pm 10\%$.

b. Calibration: Determine retention time for each anion by injecting a standard solution containing only the anion of interest and noting the time required for a peak to appear. Retention times vary with operating conditions and with anion concentration. Late eluters show the greatest variation. The shift in retention time is inversely proportional to concentration. The order of elution is shown in Figure 4110:2.

Construct a calibration curve by injecting prepared standards including each anion of interest. Use at least three concentrations plus a blank. Cover the range of concentrations expected for samples. Use one concentration near but above the method detection limit established for each anion to be measured. Unless the detector's attenuation range settings have been proven to be linear, calibrate each setting individually. Construct calibration curve by plotting either peak height or peak area versus concentration. If a data system is being used, make a hard copy of the calibration curve available.

Verify that the working calibration curve is within $\pm 10\%$ of the previous value on each working day; if not, reconstruct it. Also, verify when the eluent is changed and after every 20 samples. If response or retention time for any anion varies from the previous value by more than $\pm 10\%$, reconstruct the curve using fresh calibration standards.

c. Sample analysis: Inject enough sample (about two to three times the loop volume) to insure that sample loop is properly flushed. Inject sample into chromatograph and let all peaks elute before injecting another sample (usually this occurs in about 20 min). Compare response in peak height or peak area and retention time to values obtained in calibration.

5. Calculation

Determine the concentration of the anions of interest from the appropriate standard curve. If sample dilutions were made, calculate concentration:

$$C = A \times F$$

where:

C = anion concentration, mg/L,

A = mg/L from calibration curve, and

F = dilution factor.

6. Quality Control

a. If columns other than those listed in Section 4110C.2c are used, demonstrate that the resolution of all peaks is similar to that shown in Figure 4110:2.

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b. Generate accuracy and precision data with this method by using a reference standard of known concentration prepared independently of the laboratory making the analysis. Compare with data in Precision and Bias, below.

c. Analyze a quality control sample at least every 10 samples. Follow general guidelines from Section 4020.

7. Precision and Bias

Precision and bias data are given in Table 4110:IX.

8. Reference

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4120 SEGMENTED CONTINUOUS FLOW ANALYSIS*#(8)

4120 A. Introduction

1. Background and Applications

Air-segmented flow analysis (SFA) is a method that automates a large number of wet chemical analyses. An SFA analyzer can be thought of as a “conveyor belt” system for wet chemical analysis, in which reagents are added in a “production-line” manner. Applications have been developed to duplicate manual procedures precisely. SFA was first applied to analysis of sodium and potassium in human serum, with a flame photometer as the detection device, by removing protein interferences with a selectively porous membrane (dialyzer).

The advantages of segmented flow, compared to the manual method, include reduced sample and reagent consumption, improved repeatability, and minimal operator contact with hazardous materials. A typical SFA system can analyze 30 to 120 samples/ h. Reproducibility is enhanced by the precise timing and repeatability of the system. Because of this, the chemical reactions do not need to go to 100% completion. Decreasing the number of manual sample/solution manipulations reduces labor costs, improves workplace safety, and improves analytical precision. Complex chemistries using dangerous chemicals can be carried out in sealed systems. Unstable reagents can be made up in situ. An SFA analyzer uses smaller volumes of reagents and samples than manual methods, producing less chemical waste needing disposal.

SFA is not limited to single-phase colorimetric determinations. Segmented-flow techniques often include analytical procedures such as mixing, dilution, distillation, digestion, dialysis, solvent extractions, and/or catalytic conversion. In-line distillation methods are used for the determinations of ammonia, fluoride, cyanide, phenols, and other volatile compounds. In-line digestion can be used for the determination of total phosphorous, total cyanide, and total nitrogen

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(kjeldahl + NO₂ + NO₃). Dialysis membranes are used to eliminate interferences such as proteins and color, and other types of membranes are available for various analytical needs. SFA also is well-suited for automated liquid/liquid extractions, such as in the determination of MBAS. Packed-bed ion exchange columns can be used to remove interferences and enhance sensitivity and selectivity of the detection.

Specific automated SFA methods are described in the sections for the analytes of interest.

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4120 B. Segmented Flow Analysis Method

1. General Discussion

a. Principle: A rudimentary system (Figure 4120:1) contains four basic components: a sampling device, a liquid transport device such as a peristaltic pump, the analytical cartridge where the chemistry takes place, and the detector to quantify the analyte.

In a generalized system, samples are loaded onto an automatic sampler. The sampler arm moves the sample pickup needle between the sample cup and a wash reservoir containing a solution closely matching the sample matrix and free of the analyte. The wash solution is pumped continuously through the reservoir to eliminate cross-contamination. The sample is pumped to the analytical cartridge as a discrete portion separated from the wash by an air-bubble created during the sampler arm's travel from wash reservoir to sample cup and back.

In the analytical cartridge, the system adds the sample to the reagent(s) and introduces proportionately identical air-bubbles to reagent or sample stream. Alternatively, another gas or immiscible fluid can be substituted for air. The analyzer then proportions the analyte sample into a number of analytical segments depending on sample time, wash time, and segmentation frequency. Relative flow and initial reagent concentration determine the amount and concentration of each reagent added. The micro-circulation pattern enhances mixing, as do mixing coils, which swirl the analytical system to utilize gravitational forces. Chemical reactions, solvent separation, catalytic reaction, dilution, distillation, heating, and/or special applications take place in their appropriate sections of the analytical cartridge as the segmented stream flows toward the detector.

A typical SFA detector is a spectrophotometer that measures the color development at a

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specific wavelength. Other detectors, such as flame photometers and ion-selective electrodes, can be used. SFA detectors utilize flow-through cells, and typically send their output to a computerized data-collection system and/or a chart-recorder. The baseline is the reading when only the reagents and wash water are flowing through the system. Because gas bubbles are compressible, highly reflective, and electrically nonconductive, they severely distort the signal in the detector; therefore, many systems remove the bubbles before the optical light path. However, if the system removes the bubbles at any point within the system, the segregated liquids will be able to interact and pool. This interaction can cause cross-contamination or loss of wash, and decreases the rate at which samples can be processed. Real-time analog or digital data reconstruction techniques known as curve regeneration can remove the effect of pooling at the flow-cell debubbler and/or any other unsegmented zones of the system. “Bubble-gating” is a technique that does not remove the bubbles, but instead uses analog or digital processing to remove the distortion caused by the bubbles. Bubble-gating requires a sufficiently fast detector response time and requires that the volume of the measurement cell be smaller than the volume of the individual liquid segment.

b. Sample dispersion and interferences: Theoretically, the output of the detector is square-wave. Several carryover processes can deform the output exponentially. The first process, longitudinal dispersion, occurs as a result of laminar flow. Segmentation of the flow with air bubbles minimizes the dispersion and mixing between segments. The second process is axial or lag-phase dispersion. It arises from stagnant liquid film that wets the inner surfaces of the transmission tubing. Segmented streams depend on wet surfaces for hydraulic stability. The back-pressure within non-wet tubing increases in direct proportion to the number of bubbles it contains and causes surging and bubble breakup. Corrective measures include adding specific wetting agents (surfactants) to reagents and minimizing the length of transmission tubing.

Loose or leaking connections are another cause of carryover and can cause poor reproducibility. Wrap TFE tape around leaking screw fittings. When necessary, slightly flange the ends of types of tubing that require it for a tight connection. For other connections, sleeve one size of tubing over another size. Use a noninterfering lubricant for other tubing connections. Blockages in the tubing can cause back-pressure and leaks. Clean out or replace any blocked tubing or connection. A good indicator for problems is the bubble pattern; visually inspect the system for any abnormal bubble pattern that may indicate problems with flow.

For each analysis, check individual method for compounds that can interfere with color development and/or color reading. Other possible interferences include turbidity, color, and salinity. Turbid and/or colored samples may require filtration. In another interference-elimination technique, known as matrix correction, the solution is measured at two separate wavelengths, and the result at the interference wavelength is subtracted from that at the analytical wavelength.

2. Apparatus

a. Tubing and connections: Use mini- or micro-bore tubing on analytical cartridges. Replace flexible tubing that becomes discolored, develops a “sticky” texture, or loses ability to spring

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back into shape immediately after compression. Also see manufacturer's manual and specific methods.

b. Electrical equipment and connections: Make electrical connections with screw terminals or plug-and-socket connections. Use shielded electrical cables. Use conditioned power or a universal power supply if electrical current is subject to fluctuations. See manufacturer's manual for additional information.

c. Automated analytical equipment: Dedicate a chemistry manifold and tubing to each specific chemistry. See specific methods and manufacturer's manual for additional information.

d. Water baths: When necessary, use a thermostatically controlled heating/cooling bath to decrease analysis time and/or improve sensitivity. Several types of baths are available; the most common are coils heated or cooled by water or oil. Temperature-controlled laboratories reduce drift in temperature-sensitive chemistries if water baths are not used.

3. Reagents

Prepare reagents according to specific methods and manufacturer's instructions. If required, filter or degas a reagent. Use reagent water (see Section 1080) if available; if not, use a grade of water that is free of the analyte and interfering substances. Run blanks to demonstrate purity of the water used to prepare reagents and wash SFA system. Minimize exposure of reagents to air, and refrigerate if necessary. If reagents are made in large quantities, preferably decant a volume sufficient for one analytical run into a smaller container. If using a wetting agent, add it to the reagent just before the start of the run. Reagents and wetting agents have a limited shelf-life. Old reagents or wetting agents can produce poor reproducibility and distorted peaks. Do not change reagent solutions or add reagent to any reagent reservoirs during analysis. Always start with a sufficient quantity to last through the analytical run.

4. Procedure

For specific operating instructions, consult manufacturer's directions and methods for analytes of interest. At startup of a system, pump reagents and wash water through system until system has reached equilibrium (bubble pattern smooth and consistent) and base line is stable. Meanwhile, load samples and standards into sample cups or tubes and type corresponding tags into computer table. When ready, command computer to begin run. Most systems will run the highest standard to trigger the beginning of the run, followed by a blank to check return to base line, and then a set of standards covering the analytical range (sampling from lowest to highest concentration). Construct a curve plotting concentration against absorbance or detector reading and extrapolate results (many systems will do this automatically). Run a new curve daily immediately before use. Calculation and interpretation of results depend on individual chemistry and are analogous to the manual method. Insert blanks and standards periodically to check and correct for any drift of base line and/or sensitivity. Some systems will run a specific standard periodically as a "drift," and automatically will adjust sample results. At end of a run, let system flush according to manufacturer's recommendations.

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5. Quality Control

See Section 4020 and individual methods for quality control methods and precision and bias data.

4130 INORGANIC NONMETALS BY FLOW INJECTION ANALYSIS*(9)

4130 A. Introduction

1. Principle

Flow injection analysis (FIA) is an automated method of introducing a precisely measured portion of liquid sample into a continuously flowing carrier stream. The sample portion usually is injected into the carrier stream by either an injection valve with a fixed-volume sample loop or an injection valve in which a fixed time period determines injected sample volume. As the sample portion leaves the injection valve, it disperses into the carrier stream and forms an asymmetric Gaussian gradient in analyte concentration. This concentration gradient is detected continuously by either a color reaction or another analyte-specific detector through which the carrier and gradient flow.

When a color reaction is used as the detector, the color reaction reagents also flow continuously into the carrier stream. Each color reagent merges with the carrier stream and is added to the analyte gradient in the carrier in a proportion equal to the relative flow rates of the carrier stream and merging color reagent. The color reagent becomes part of the carrier after it is injected and has the effect of modifying or derivatizing the analyte in the gradient. Each subsequent color reagent has a similar effect, finally resulting in a color gradient proportional to the analyte gradient. When the color gradient passes through a flow cell placed in a flow-through absorbance detector, an absorbance peak is formed. The area of this peak is proportional to the analyte concentration in the injected sample. A series of calibration standards is injected to generate detector response data used to produce a calibration curve. It is important that the FIA flow rates, injected sample portion volume, temperature, and time the sample is flowing through the system ("residence time") be the same for calibration standards and unknowns. Careful selection of flow rate, injected sample volume, frequency of sample injection, reagent flow rates, and residence time determines the precise dilution of the sample's original analyte concentration into the useful concentration range of the color reaction. All of these parameters ultimately determine the sample throughput, dynamic range of the method, reaction time of the color reaction discrimination against slow interference reactions, signal-to-noise ratio, and method detection level (MDL).

2. Applications

FIA enjoys the advantages of all continuous-flow methods: There is a constantly measured

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reagent blank, the “base line” against which all samples are measured; high sample throughput encourages frequent use of quality control samples; large numbers of samples can be analyzed in batches; sample volume measurement, reagent addition, reaction time, and detection occur reproducibly without the need for discrete measurement and transfer vessels such as cuvettes, pipets, and volumetric flasks; and all samples share a single reaction manifold or vessel consisting of inert flow tubing.

Specific FIA methods are presented as Section 4500-Br⁻.D, Section 4500-Cl⁻.G, Section 4500-CN⁻.N and Section 4500-CN⁻.O, Section 4500-F⁻.G, Section 4500-NH₃.H, Section 4500-NO₃⁻.I, Section 4500-N.B, Section 4500-N_{org}.D, Section 4500-P.G, Section 4500-P.H, Section 4500-P.I, Section 4500-SiO₂.F, Section 4500-SO₄²⁻.G, and Section 4500-S²⁻.I.

4130 B. Quality Control

When FIA methods are used, follow a formal laboratory quality control program. The minimum requirements consist of an initial demonstration of laboratory capability and periodic analysis of laboratory reagent blanks, fortified blanks, and other laboratory solutions as a continuing check on performance. Maintain performance records that define the quality of the data generated.

See Section 1020, Quality Assurance, and Section 4020 for the elements of such a quality control program.

4140 INORGANIC ANIONS BY CAPILLARY ION ELECTROPHORESIS (PROPOSED)*#(10)

4140 A. Introduction

Determination of common inorganic anions such as fluoride, chloride, bromide, nitrite, nitrate, orthophosphate, and sulfate is a significant component of water quality analysis. Instrumental techniques that can determine multiple analytes in a single analysis, i.e., ion chromatography (Section 4110) and capillary ion electrophoresis, offer significant time and operating cost savings over traditional single-analyte wet chemical analysis.

Capillary ion electrophoresis is rapid (complete analysis in less than 5 min) and provides additional anion information, i.e., organic acids, not available with isocratic ion chromatography (IC). Operating costs are significantly less than those of ion chromatography. Capillary ion electrophoresis can detect all anions present in the sample matrix, providing an anionic “fingerprint.”

Anion selectivity of capillary ion electrophoresis is different from that of IC and eliminates many of the difficulties present in the early portion of an IC chromatogram. For example, sample

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matrix neutral organics, water, and cations do not interfere with anion analysis, and fluoride is well resolved from monovalent organic acids. Sample preparation typically is dilution with reagent water and removal of suspended solids by filtration. If necessary, hydrophobic sample components such as oil and grease can be removed with the use of HPLC solid-phase extraction cartridges without biasing anion concentrations.

4140 B. Capillary Ion Electrophoresis with Indirect UV Detection

1. General Discussion

a. Principle: A buffered aqueous electrolyte solution containing a UV-absorbing anion salt (sodium chromate) and an electroosmotic flow modifier (OFM) is used to fill a 75- μm -ID silica capillary. An electric field is generated by applying 15 kV of applied voltage using a negative power supply; this defines the detector end of the capillary as the anode. Sample is introduced at the cathodic end of the capillary and anions are separated on the basis of their differences in mobility in the electric field as they migrate through the capillary. Cations migrate in the opposite direction and are not detected. Water and neutral organics are not attracted towards the anode; they migrate after the anions and thus do not interfere with anion analysis. Anions are detected as they displace charge-for-charge the UV-absorbing electrolyte anion (chromate), causing a net decrease in UV absorbance in the analyte anion zone compared to the background electrolyte. Detector polarity is reversed to provide positive mv response to the data system (Figure 4140:1). As in chromatography, the analytes are identified by their migration time and quantitated by using time-corrected peak area relative to standards. After the analytes of interest are detected, the capillary is purged with fresh electrolyte, eliminating the remainder of the sample matrix before the next analysis.

b. Interferences: Any anion that has a migration time similar to the analytes of interest can be considered an interference. This method has been designed to minimize potential interference typically found in environmental waters, groundwater, drinking water, and wastewater.

Formate is a common potential interference with fluoride; it is a common impurity in reagent water, has a migration time similar to that of fluoride, and is an indicator of loss of water purification system performance and TOC greater than 0.1 mg/L. The addition of 5 mg formate/L in the mixed working anion standard, and to sample where identification of fluoride is in question, aids in the correct identification of fluoride.

Generally, a high concentration of any one ion may interfere with resolution of analyte anions in close proximity. Dilution in reagent water usually is helpful. Modifications in the electrolyte formulation can overcome resolution problems but require individual validation for precision and bias. This method is capable of interference-free resolution of a 1:100 differential of Br^- to Cl^- , and NO_2^- and NO_3^- to SO_4^{2-} , and 1:1000 differential of Cl^- and SO_4^{2-} .

Dissolved ferric iron in the mg/L range gives a low bias for PO_4 . However, transition metals do not precipitate with chromate because of the alkaline electrolyte pH.

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c. *Minimum detectable concentrations:* The minimum detectable concentration for an anion is a function of sample size. Generally, for a 30-s sampling time, the minimum detectable concentrations are 0.1 mg/L (Figure 4140:2). According to the method for calculating MDL given in Section 1030, the calculated detection limits are below 0.1 mg/L. These detection limits can be compromised by analyte impurities in the electrolyte.

d. *Limitations:* Samples with high ionic strength may show a decrease in analyte migration time. This variable is addressed by using normalized migration time with respect to a reference peak, chloride, for identification, and using time-corrected area for quantitation. With electrophoresis, published data indicate that analyte peak area is a function of migration time. At high analyte anion concentrations, peak shape becomes asymmetrical; this phenomenon is typical and is different from that observed in ion chromatography.

2. Apparatus

a. *Capillary ion electrophoresis (CIE) system:**(11) Various commercial instruments are available that integrate a negative high-voltage power supply, electrolyte reservoirs, covered sample carousel, hydrostatic sampling mechanism, capillary purge mechanism, self-aligning capillary holder, and UV detector capable of 254-nm detection in a single temperature-controlled compartment at 25°C. Optimal detection limits are attained with a fixed-wavelength UV detector with Hg lamp and 254-nm filter.

b. *Capillary:* 75- μ m-ID $\times$ 375- μ m-OD $\times$ 60-cm-long fused silica capillary with a portion of its outer coating removed to act as the UV detector window. Capillaries can be purchased premade* or on a spool and prepared as needed.

c. *Data system:**(12) HPLC-based integrator or computer. Optimum performance is attained with a computer data system and electrophoresis-specific data processing including data acquisition at 20 points/s, migration times determined at midpoint of peak width, identification based on normalized migration times with respect to a reference peak, and time-corrected peak area.

3. Reagents

a. *Reagent water:* See Section 1080. Ensure that water is analyte-free. The concentration of dissolved organic material will influence overall performance; preferably use reagent water with <50 μ g TOC/L.

b. *Chromate electrolyte solution:* Prepare as directed from individual reagents, or purchase electrolyte preformulated.

1) *Sodium chromate concentrate, 100 mM:* In a 1-L volumetric flask dissolve 23.41 g sodium chromate tetrahydrate, $\text{Na}_2\text{CrO}_4 \cdot 4\text{H}_2\text{O}$, in 500 mL water and dilute to 1 L with water. Store in a capped glass or plastic container at ambient temperature; this reagent is stable for 1 year.

2) *Electroosmotic flow modifier concentrate, 100 mM:* In a 100-mL volumetric flask

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dissolve 3.365 g tetradecyltrimethyl ammonium bromide (TTAB), mol wt 336.4, in 50 mL water and dilute to 100 mL. Store in a capped glass or plastic container at ambient temperature; this reagent is stable for 1 year.

3) *Buffer concentrate*, 100 mM: In a 1-L volumetric flask dissolve 20.73 g 2-[N-cyclohexylamino]-ethane sulfonate (CHES), mol wt 207.29, in 500 mL water and dilute to 1 L. Store in a capped glass or plastic container at ambient temperature; this reagent is stable for 1 year.

4) *Calcium gluconate concentrate*, 1 mM: In a 1-L volumetric flask dissolve 0.43 g calcium gluconate, mol wt 430.38, in 500 mL water and dilute to 1 L. Store in a capped glass or plastic container at ambient temperature; this reagent is stable for 1 year.

5) *Sodium hydroxide solution*, NaOH, 100 mM: In a 1-L plastic volumetric flask dissolve 4 g sodium hydroxide, NaOH, in 500 mL water and dilute to 1 L. Store in a capped plastic container at ambient temperature; this reagent is stable for 1 month.

6) *Chromate electrolyte solution*: Prerinse an anion exchange cartridge in the hydroxide form with 10 mL 100-mM NaOH followed by 10 mL water; discard the washings. Slowly pass 4 mL 100-mM TTAB concentrate through the cartridge into a 100-mL volumetric flask. Rinse cartridge with 10 mL water and add to flask. (NOTE: This step is needed to convert the TTAB from the bromide form into the hydroxide form TTAOH. The step can be eliminated if commercially available 100 mM TTAOH is used.)

To the 100-mL volumetric flask containing the TTAOH add 4.7 mL sodium chromate concentrate, 10 mL CHES buffer concentrate, and 10 mL calcium gluconate concentrate. Mix and dilute to 100 mL with water. The pH should be 9 ± 0.1 ; final solution is 4.7 mM sodium chromate, 4 mM TTAOH, 10 mM CHES, and 0.1 mM calcium gluconate. Filter and degas through a 0.45- μ m aqueous membrane, using a vacuum apparatus. Store any remaining electrolyte in a capped plastic container at ambient temperature for up to 1 month.

c. *Standard anion solution*, 1000mg/L: Prepare a series of individual standard anion solutions by adding the indicated amount of salt, dried to constant weight at 105°C, to 100mL with water. Store in plastic bottles; these solutions are stable for 3 months. (Alternatively, purchase individual certified 1000-mg/L anion standards and store following manufacturer's directions.)

Anion	Salt	Amount g/100mL
Chloride	NaCl	0.1649
Bromide	NaBr	0.1288
Formate	NaCO ₂ H	0.1510
Fluoride	NaF	0.2210
Nitrite	NaNO ₂	0.1499* (1000 mg NO ₂ ⁻ /L = 304.3 mg NO ₂ ⁻ -N/L)

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Anion	Salt	Amount g/100mL
Nitrate	NaNO ₃	0.1371 (1000 mg NO ₃ ⁻ /L = 225.8 mg NO ₃ ⁻ -N/L)
Phosphate	Na ₂ HPO ₄ †	0.1500 (1000 mg PO ₄ ³⁻ /L = 326.1 mg PO ₄ ³⁻ -P/L)
Sulfate	Na ₂ SO ₄ †	0.1480 (1000 mg SO ₄ ²⁻ /L = 676.3 mg SO ₄ ²⁻ -S/L)

* Do not oven-dry, but dry to constant weight in a desiccator over phosphorous pentoxide.

† Potassium salts can be used, but with corresponding modification of salt amounts.

d. Mixed working anion standard solutions: Prepare at least three different working anion standard solutions that bracket the expected sample range, from 0.1 to 50 mg/L. Add 5 mg formate/ L to all standards. Use 0.1 mL standard anion solution/100 mL working anion solution (equal to 1 mg anion/L). (Above 50 mg/ L each anion, chloride, bromide, nitrite, sulfate, and nitrate are no longer baseline-resolved. Analytes that are not baseline-resolved may give a low bias. If the analytes are baseline-resolved, quantitation is linear to 100 mg/L.) Store in plastic containers in the refrigerator; prepare fresh standards weekly. Figure 4140:3 shows representative electropherograms of anion standards and Table 4140:I gives the composition of the standards.

e. Calibration verification sample: Use a certified performance evaluation standard, or equivalent, within the range of the mixed working anion standard solutions analyzed as an unknown. Refer to Section 4020.

f. Analyte known-addition sample: To each sample matrix add a known amount of analyte, and use to evaluate analyte recovery.

4. Procedure

a. Capillary conditioning: Set up CIE system according to manufacturer's instructions. Rinse capillary with 100 mM NaOH for 5 min. Place fresh degassed electrolyte into both reservoirs and purge capillary with electrolyte for 3 min to remove all previous solutions and air bubbles. Apply voltage of 15 kV and note the current; if the expected 14 ± 1 μ A is observed, the CIE system is ready for use. Zero UV detector to 0.000 absorbance.

b. Analysis conditions: Program CE system to apply constant current of 14 μ A for the run time. Use 30 s hydrostatic sampling time for all standard and sample introduction. Analysis time is 5 min.

c. Analyte migration time calibration: Determine migration time of each analyte daily using the midrange mixed working anion standard. Perform duplicate analysis to insure migration time stability. Use the midpoint of peak width, defined as midpoint between the start and stop integration marks, as the migration time for each analyte; this accounts for the observed non-symmetrical peak shapes. (Use of peak apex may result in analyte misidentification.) The

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migration order is always Cl^- , Br^- , NO_2^- , SO_4^{2-} , NO_3^- , F^- , and PO_4^{3-} . Dissolved HCO_3^- is the last peak in the standard (see Figure 4140:1). Set analyte migration time window as 2% of the migration time determined above, except for Cl^- , which is set at 10%. Chloride is always the first peak and is used as the reference peak for analyte qualitative identification; identify anions on the basis of normalized migration times with respect to the reference peak, or migration time ratio. (See Figure 4140:1 and Table 4140:II.)

d. Analyte response calibration: Analyze all three mixed working anion standards in duplicate. Plot time-corrected peak area for each analyte versus concentration using a linear regression through zero. (In capillary electrophoresis peak area is a function of analyte migration time, which may change during analyses. Time-corrected peak area is a well-documented CE normalization routine, i.e., peak area divided by migration time. (NOTE: *Do not use analyte peak height.*) Calibration is accepted as linear if regression coefficient of variation, R^2 , is greater than 0.995. Linearity calibration curves for anions are shown in Figure 4140:4, Figure 4140:5 and Figure 4140:6.

e. Sample analysis: After initial calibration run samples in the following order: calibration verification sample, reagent blank, 10 unknown samples, calibration verification sample, reagent blank, etc. Filter samples containing high concentrations of suspended solids. If peaks are not baseline-resolved, dilute sample 1:5 with water and repeat analysis for unresolved analyte quantitation. Resolved analytes in the undiluted sample are considered correct quantitation. Electropherograms of typical samples are shown in Figure 4140:7, Figure 4140:8, and Figure 4140:9.

5. Calculation

Relate the time-corrected peak area for each sample analyte with the calibration curve to determine concentration of analyte. If the sample was diluted, multiply anion concentration by the dilution factor to obtain original sample concentration, as follows:

$$C = A \times F$$

where:

C = analyte concentration in original sample, mg/L,

A = analyte concentration from calibration curve, mg/L, and

F = scale factor or dilution factor. (For a 1:5 sample dilution, $F = 5$.)

6. Quality Control

a. Analytical performance check: Unless analyst has already demonstrated ability to generate data with acceptable precision and bias by this method, proceed as follows: Analyze seven replicates of a certified performance evaluation standard containing the analytes of interest. Calculate mean and standard deviation of these data. The mean must be within the performance evaluation standard's 95% confidence interval. Calculate percent relative standard deviation (RSD) for these data as $(\text{SD} \times 100) / \text{mean}$; % RSD should conform to acceptance limit

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given in Section 1020B.

b. Calibration verification: Analyze an independent, certified performance evaluation standard at the beginning and end of the analyses, or if many samples are analyzed, after every 10 samples. The determined analyte concentration should be within $\pm 10\%$ of the true value, and the migration time of the Cl^- reference peak should be within 5% of the calibrated migration time. If the Cl^- reference peak differs by more than 5% of the calibrated migration time, repeat capillary conditioning and recalibrate before proceeding.

c. Water blank analysis: At the beginning of every set of analyses run a water blank to demonstrate that the water is free of analyte anions. Dissolved bicarbonate will always be observed as a positive or negative peak having a migration time greater than PO_4^{3-} and does not interfere with the analysis. Any negative peak indicates the presence of an anion impurity in the electrolyte; a positive peak indicates the presence of an impurity in the reagent water. If this is noted, discard electrolyte and prepare electrolyte and sample dilutions again with water from a different source.

d. Analyte recovery verification: For each sample matrix analyzed, e.g., drinking water, surface water, groundwater, or wastewater, analyze duplicate known-addition samples (§ 3 f). Analyte recoveries should conform to acceptance limits given in Section 1020B.

e. Blind check sample: Analyze an unknown certified performance evaluation check sample at least once every 6 months to verify method accuracy.

f. Sample duplicates: Analyze one or more sample duplicates every 10 samples.

7. Precision and Bias

Table 4140:III compares results of capillary ion electrophoresis with those of other approved methods. Precision and bias data are given in Table 4140:IV and Table 4140:V. Comparison of other methods and capillary ion electrophoresis for wastewater effluent, drinking water, and landfill leachates are given in Table 4140:VI.

8. Bibliography

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4500-B BORON*#(13)

4500-B A. Introduction

1. Occurrence and Significance

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Boron (B) is the first element in Group IIIA of the periodic table; it has an atomic number of 5, an atomic weight of 10.81, and a valence of 3. The average abundance of B in the earth's crust is 9 ppm; in soils it is 18 to 63 ppm; in streams it is 10 µg/L; and in groundwaters it is 0.01 to 10 mg/L. The most important mineral is borax, which is used in the preparation of heat-resistant glasses, detergents, porcelain enamels, fertilizers, and fiberglass.

The most common form of boron in natural waters is H_3BO_3 . Although boron is an element essential for plant growth, in excess of 2.0 mg/L in irrigation water, it is deleterious to certain plants and some plants may be affected adversely by concentrations as low as 1.0 mg/L (or even less in commercial greenhouses). Drinking waters rarely contain more than 1 mg B/L and generally less than 0.1 mg/L, concentrations considered innocuous for human consumption. Seawater contains approximately 5 mg B/L and this element is found in saline estuaries in association with other seawater salts.

The ingestion of large amounts of boron can affect the central nervous system. Protracted ingestion may result in a clinical syndrome known as borism.

2. Selection of Method

Preferably, perform analyses by the inductively coupled plasma method (Section 3120). The inductively coupled plasma/mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though boron is not specifically listed as an analyte in the method.

The curcumin method (B) is applicable in the 0.10- to 1.0-mg/L range, while the carmine method (C) is suitable for the determination of boron concentration in the 1- to 10-mg/L range. The range of these methods can be extended by dilution or concentration of the sample.

3. Sampling and Storage

Store samples in polyethylene bottles or alkali-resistant, boron-free glassware.

4500-B B. Curcumin Method

1. General Discussion

a. Principle: When a sample of water containing boron is acidified and evaporated in the presence of curcumin, a red-colored product called rosocyanine is formed. The rosocyanine is taken up in a suitable solvent and the red color is compared with standards visually or photometrically.

b. Interference: NO_3^- -N concentrations above 20 mg/L interfere. Significantly high results are possible when the total of calcium and magnesium hardness exceeds 100 mg/L as calcium carbonate (CaCO_3). Moderate hardness levels also can cause a considerable percentage error in the low boron range. This interference springs from the insolubility of the hardness salts in 95% ethanol and consequent turbidity in the final solution. Filter the final solution or pass the original

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sample through a column of strongly acidic cation-exchange resin in the hydrogen form to remove interfering cations. The latter procedure permits application of the method to samples of high hardness or solids content. Phosphate does not interfere.

c. Minimum detectable quantity: 0.2 µg B.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

- 1) *Spectrophotometer*, for use at 540 nm, with a minimum light path of 1 cm.
- 2) *Filter photometer*, equipped with a green filter having a maximum transmittance near 540 nm, with a minimum light path of 1 cm.

b. Evaporating dishes, 100- to 150-mL capacity, of high-silica glass,*(14) platinum, or other suitable material.

c. Water bath, set at $55 \pm 2^{\circ}\text{C}$.

d. Glass-stoppered volumetric flasks, 25- and 50-mL capacity.

e. Ion-exchange column, 50 cm long by 1.3 cm in diameter.

3. Reagents

Store all reagents in polyethylene or boron-free containers.

a. Stock boron solution: Dissolve 571.6 mg anhydrous boric acid, H_3BO_3 , in distilled water and dilute to 1000 mL; 1.00 mL = 100 µg B. Because H_3BO_3 loses weight on drying at 105°C , use a reagent meeting ACS specifications and keep the bottle tightly stoppered to prevent entrance of atmospheric moisture.

b. Standard boron solution: Dilute 10.00 mL stock boron solution to 1000 mL with distilled water; 1.00 mL = 1.00 µg B.

c. Curcumin reagent: Dissolve 40 mg finely ground curcumin†(15) and 5.0 g oxalic acid in 80 mL 95% ethyl alcohol. Add 4.2 mL conc HCl, make up to 100 mL with ethyl alcohol in a 100-mL volumetric flask, and filter if reagent is turbid (isopropyl alcohol, 95%, may be used in place of ethyl alcohol). This reagent is stable for several days if stored in a refrigerator.

d. Ethyl or isopropyl alcohol, 95%.

e. Reagents for removal of high hardness and cation interference:

- 1) *Strongly acidic cation-exchange resin*.
- 2) *Hydrochloric acid*, HCl, 1 + 5.

4. Procedure

a. Precautions: Closely control such variables as volumes and concentrations of reagents, as well as time and temperature of drying. Use evaporating dishes identical in shape, size, and composition to insure equal evaporation time because increasing the time increases intensity of the resulting color.

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b. Preparation of calibration curve: Pipet 0 (blank), 0.25, 0.50, 0.75, and 1.00 μg boron into evaporating dishes of the same type, shape, and size. Add distilled water to each standard to bring total volume to 1.0 mL. Add 4.0 mL curcumin reagent to each and swirl gently to mix contents thoroughly. Float dishes on a water bath set at $55 \pm 2^\circ\text{C}$ and let them remain for 80 min, which is usually sufficient for complete drying and removal of HCl. Keep drying time constant for standards and samples. After dishes cool to room temperature, add 10 mL 95% ethyl alcohol to each dish and stir gently with a polyethylene rod to insure complete dissolution of the red-colored product.

Wash contents of dish into a 25-mL volumetric flask, using 95% ethyl alcohol. Make up to mark with 95% ethyl alcohol and mix thoroughly by inverting. Read absorbance of standards and samples at a wavelength of 540 nm after setting reagent blank at zero absorbance. The calibration curve is linear from 0 to 1.00 μg boron. Make photometric readings within 1 h of drying samples.

c. Sample treatment: For waters containing 0.10 to 1.00 mg B/L, use 1.00 mL sample. For waters containing more than 1.00 mg B/L, make an appropriate dilution with boron-free distilled water, so that a 1.00-mL portion contains approximately 0.50 μg boron.

Pipet 1.00 mL sample or dilution into an evaporating dish. Unless the calibration curve is being determined at the same time, prepare a blank and a standard containing 0.50 μg boron and run in conjunction with the sample. Proceed as in ¶ 4b, beginning with “Add 4.0 mL curcumin reagent. . . .” If the final solution is turbid, filter through filter paper #16 before reading absorbance. Calculate boron content from calibration curve.

d. Visual comparison: The photometric method may be adapted to visual estimation of low boron concentrations, from 50 to 200 $\mu\text{g/L}$, as follows: Dilute the standard boron solution 1 + 3 with distilled water; 1.00 mL = 0.20 μg B. Pipet 0, 0.05, 0.10, 0.15, and 0.20 μg boron into evaporating dishes as indicated in ¶ 4b. At the same time add an appropriate volume of sample (1.00 mL or portion diluted to 1.00 mL) to an identical evaporating dish. The total boron should be between 0.05 and 0.20 μg . Proceed as in ¶ 4b, beginning with “Add 4.0 mL curcumin reagent. . . .” Compare color of samples with standards within 1 h of drying samples.

e. Removal of high hardness and cation interference: Prepare an ion-exchange column of approximately 20 cm $\times$ 1.3 cm diam. Charge column with a strongly acidic cation-exchange resin. Backwash column with distilled water to remove entrained air bubbles. Keep the resin covered with liquid at all times. Pass 50 mL 1 + 5 HCl through column at a rate of 0.2 mL acid/mL resin in column/min and wash column free of acid with distilled water.

Pipet 25 mL sample, or a smaller sample of known high boron content diluted to 25 mL, onto the resin column. Adjust rate of flow to about 2 drops/s and collect effluent in a 50-mL volumetric flask. Wash column with small portions of distilled water until flask is filled to mark. Mix and transfer 2.00 mL into evaporating dish. Add 4.0 mL curcumin reagent and complete the analysis as described in ¶ 4b preceding.

5. Calculation

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Use the following equation to calculate boron concentration from absorbance readings:

$$\text{mg B/L} = \frac{A_2 \times C}{A_1 \times S}$$

where:

A_1 = absorbance of standard,

A_2 = absorbance of sample,

C = $\mu\text{g B}$ in standard taken, and

S = mL sample.

6. Precision and Bias

A synthetic sample containing 240 $\mu\text{g B/L}$, 40 $\mu\text{g As/L}$, 250 $\mu\text{g Be/L}$, 20 $\mu\text{g Se/L}$, and 6 $\mu\text{g V/L}$ in distilled water was analyzed in 30 laboratories by the curcumin method with a relative standard deviation of 22.8% and a relative error of 0%.

7. Bibliography

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4500-B C. Carmine Method

1. General Discussion

a. Principle: In the presence of boron, a solution of carmine or carminic acid in concentrated sulfuric acid changes from a bright red to a bluish red or blue, depending on the concentration of boron present.

b. Interference: The ions commonly found in water and wastewater do not interfere.

c. Minimum detectable quantity: 2 $\mu\text{g B}$.

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2. Apparatus

Colorimetric equipment: One of the following is required:

- a. *Spectrophotometer*, for use at 585 nm, with a minimum light path of 1 cm.
- b. *Filter photometer*, equipped with an orange filter having a maximum transmittance near 585 nm, with a minimum light path of 1 cm.

3. Reagents

Store all reagents in polyethylene or boron-free containers.

- a. *Standard boron solution:* Prepare as directed in Method B, ¶ 3b.
- b. *Hydrochloric acid*, HCl, conc and 1 + 11.
- c. *Sulfuric acid*, H₂SO₄, conc.
- d. *Carmine reagent:* Dissolve 920 mg carmine N.F. 40, or carminic acid, in 1 L conc H₂SO₄. (If unable to zero spectrophotometer, dilute carmine 1 + 1 with conc H₂SO₄ to replace above reagent.)

4. Procedure

a. *Preliminary sample treatment:* If sample contains less than 1 mg B/L, pipet a portion containing 2 to 20 µg B into a platinum dish, make alkaline with 1N NaOH plus a slight excess, and evaporate to dryness on a steam or hot water bath. If necessary, destroy any organic material by ignition at 500 to 550°C. Acidify cooled residue (ignited or not) with 2.5 mL 1 + 11 HCl and triturate with a rubber policeman to dissolve. Centrifuge if necessary to obtain a clear solution. Pipet 2.00 mL clear concentrate into a small flask or 30-mL test tube. Treat reagent blank identically.

b. *Color development:* Prepare a series of boron standard solutions (100, 250, 500, 750, and 1000 µg) in 100 mL with distilled water. Pipet 2.00 mL of each standard solution into a small flask or 30-mL test tube.

Treat blank and calibration standards exactly as the sample. Add 2 drops (0.1 mL) conc HCl, carefully introduce 10.0 mL conc H₂SO₄, mix, and let cool to room temperature. Add 10.0 mL carmine reagent, mix well, and after 45 to 60 min measure absorbance at 585 nm in a cell of 1-cm or longer light path, using the blank as reference.

To avoid error, make sure that no bubbles are present in the optical cell while photometric readings are being made. Bubbles may appear as a result of incomplete mixing of reagents. Because carmine reagent deteriorates, check calibration curve daily.

5. Calculation

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$$\text{mg B/L} = \frac{\mu\text{g B (in approx. 22 mL final volume)}}{\text{mL sample}} \times 1.25$$

6. Precision and Bias

A synthetic sample containing 180 µg B/L, 50 µg As/L, 400 µg Be/L, and 50 µg Se/L in distilled water was analyzed in nine laboratories by the carmine method with a relative standard deviation of 35.5% and a relative error of 0.6%.

7. Bibliography

HATCHER, J.T. & L.V. WILCOX. 1950. Colorimetric determination of boron using carmine. *Anal. Chem.* 22:567.

4500-Br⁻ BROMIDE*(17)

4500-Br⁻ A. Introduction

1. Occurrence

Bromide occurs in varying amounts in ground and surface waters in coastal areas as a result of seawater intrusion and sea-spray-affected precipitation. The bromide content of ground waters and stream baseflows also can be affected by connate water. Industrial and oil-field brine discharges can contribute to the bromide in water sources. Under normal circumstances, the bromide content of most drinking waters is small, seldom exceeding 1 mg/L. Even levels of <100 µg/L can lead to formation of bromate or brominated by-products in disinfected waters.

2. Selection of Method

Described here are a colorimetric procedure suitable for the determination of bromide in most drinking waters and a flow injection analysis method. Bromide preferably is determined by the ion chromatography method (Section 4110) or by capillary ion electrophoresis (Section 4140).

4500-Br⁻ B. Phenol Red Colorimetric Method

1. General Discussion

a. Principle: When a sample containing bromide ions (Br⁻) is treated with a dilute solution of chloramine-T in the presence of phenol red, the oxidation of bromide and subsequent

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bromination of the phenol red occur readily. If the reaction is buffered to pH 4.5 to 4.7, the color of the brominated compound will range from reddish to violet, depending on the bromide concentration. Thus, a sharp differentiation can be made among various concentrations of bromide. The concentration of chloramine-T and timing of the reaction before dechlorination are critical.

b. Interference: Most materials present in ordinary tap water do not interfere, but oxidizing and reducing agents and higher concentrations of chloride and bicarbonate can interfere. Free chlorine in samples should be destroyed as directed in Section 5210B.4e2); analyze bromide in a portion of dechlorinated sample. Addition of substantial chloride to the pH buffer solution (see ¶ 3a below) can eliminate chloride interference for waters with very low bromide/chloride ratios, such as those affected by dissolved road salt. Small amounts of dissolved iodide do not interfere, but small concentrations of ammonium ion interfere substantially. Sample dilution may reduce interferences to acceptable levels for some saline and waste waters. However, if two dilutions differing by a factor of at least five do not give comparable values, the method is inapplicable. Bromide concentration in diluted samples must be within the range of the method (0.1 to 1 mg/L).

c. Minimum detectable concentration: 0.1 mg Br⁻/L.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

- 1) *Spectrophotometer*, for use at 590 nm, providing a light path of at least 2 cm.
- 2) *Filter photometer*, providing a light path of at least 2 cm and equipped with an orange filter having a maximum transmittance near 590 nm.
- 3) *Nessler tubes*, matched, 100-mL, tall form.

b. Acid-washed glassware: Wash all glassware with 1 + 6 HNO₃ and rinse with distilled water to remove all trace of adsorbed bromide.

3. Reagents

a. Acetate buffer solution: Dissolve 90 g NaCl and 68 g sodium acetate trihydrate, NaC₂H₃O₂·3H₂O, in distilled water. Add 30 mL conc (glacial) acetic acid and make up to 1 L. The pH should be 4.6 to 4.7.

b. Phenol red indicator solution: Dissolve 21 mg phenolsulfonephthalein sodium salt and dilute to 100 mL with distilled water.

c. Chloramine-T solution: Dissolve 500 mg chloramine-T, sodium p-toluenesulfonchloramide, and dilute to 100 mL with distilled water. Store in a dark bottle and refrigerate.

d. Sodium thiosulfate, 2M: Dissolve 49.6 g Na₂S₂O₃·5H₂O or 31.6 g Na₂S₂O₃ and dilute to 100 mL with distilled water.

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e. Stock bromide solution: Dissolve 744.6 mg anhydrous KBr in distilled water and make up to 1000 mL; 1.00 mL = 500 $\mu\text{g Br}^-$.

f. Standard bromide solution: Dilute 10.00 mL stock bromide solution to 1000 mL with distilled water; 1.00 mL = 5.00 $\mu\text{g Br}^-$.

4. Procedure

a. Preparation of bromide standards: Prepare at least six standards, 0, 0.20, 0.40, 0.60, 0.80 and 1.00 mg Br^-/L , by diluting 0.0, 2.00, 4.00, 6.00, 8.00, and 10.00 mL standard bromide solution to 50.00 mL with distilled water. Treat standards the same as samples in ¶ 4b.

b. Treatment of sample: Add 2 mL buffer solution, 2 mL phenol red solution, and 0.5 mL chloramine-T solution to 50.0 mL sample or two separate sample dilutions (see 1b above) such that the final bromide concentration is in the range of 0.1 to 1.0 mg Br^-/L . Mix thoroughly immediately after each addition. Exactly 20 min after adding chloramine-T, dechlorinate by adding, with mixing, 0.5 mL $\text{Na}_2\text{S}_2\text{O}_3$ solution. Compare visually in nessler tubes against bromide standards prepared simultaneously, or preferably read in a photometer at 590 nm against a reagent blank. Determine the bromide values from a calibration curve of mg Br^-/L (in 55 mL final volume) against absorbance. A 2.54-cm light path yields an absorbance value of approximately 0.36 for 1 mg Br^-/L .

5. Calculation

$\text{mg Br}^-/\text{L} = \text{mg Br}^-/\text{L (from calibration curve)} \times \text{dilution factor (if any)}$. Results are based on 55 mL final volume for samples and standards.

6. Bibliography

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4500-Br⁻ C. (Reserved)

4500-Br⁻ D. Flow Injection Analysis (PROPOSED)

1. General Discussion

a. Principle: Bromide is oxidized to bromine by chloramine-T, followed by substitution of bromine on phenol red to produce bromphenol blue. The absorbance measured at 590 nm is proportional to the concentration of bromide in the sample. Sodium thiosulfate is added to reduce interference from chloride.

This method is suitable for the determination of bromide in waters containing up to 20 000 mg Cl⁻/L, including drinking, ground, and surface waters, and domestic and industrial wastes. The method determines total bromide, or, if the sample is filtered through a 0.45-μm-pore-size filter, the result is called “dissolved bromide.” The difference between total bromide and dissolved bromide is called “insoluble bromide.”

Also see Section 4500-Br⁻.A and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Chloride interference is reduced by the addition of sodium thiosulfate. Chloramine-T dissociates in aqueous solution to form hypochlorous acid, which can then react with chloride, causing substitution of chloride at positions ortho to the hydroxy groups on phenol red, just as in bromination. Sodium thiosulfate reacts with chlorine to reduce this interferent to a selectivity (ratio of analyte to interferent concentration) of >28 000.

2. Apparatus

Flow injection analysis equipment consisting of:

- a. FIA injection valve* with sample loop or equivalent.
- b. Multichannel proportioning pump.*
- c. FIA manifold with flow cell* (Figure 4500-Br⁻:1). Relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*#(18)
- d. Absorbance detector*, 590 nm, 10-nm bandpass.
- e. Valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. As an alternative to

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preparing reagents by weight/weight, use weight/volume.

a. *Chloramine-T*: To a tared 1-L container add 0.40 g chloramine-T hydrate (mol wt 227.65) and 999 g water. Cap and invert container to dissolve. Discard after 1 week.

b. *Phenol red*: To a tared 1-L container add 929 g water and 30.0 g glacial acetic acid. Swirl contents of container. Add 41.0 g sodium acetate and swirl container until it is dissolved. Add 0.040 g phenol red. Mix with a magnetic stirrer. Discard after 1 week.

c. *Thiosulfate*: To a tared 1-L container, add 724 g water and 500 g sodium thiosulfate pentahydrate, $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$. Dissolve by adding the solid slowly while stirring. The solid should be completely dissolved within 30 min. Gentle heating may be required. Discard after 1 week.

d. *Stock bromide standard, 100.0 mg Br^-/L* : To a 1-L volumetric flask add 0.129 g sodium bromide, NaBr. Dissolve in sufficient water, dilute to mark, and invert to mix.

e. *Stock bromide standard, 10.0 mg Br^-/L* : To a 500-mL volumetric flask add 50 mL stock standard (¶ 3d). Dilute to mark and invert to mix. Prepare fresh monthly.

f. *Standard bromide solutions*: Prepare bromide standards for the calibration curve in the desired concentration range, using the stock standard (¶ e), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500- Br^- :1 and follow method supplied by manufacturer, or laboratory standard operating procedure for this method. Follow quality control guidelines outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold vs. bromide concentration. The calibration curve gives a good fit to a second-order polynomial.

6. Precision and Bias

a. *Precision*: With a 300- μL sample loop, ten replicates of a 5.0-mg Br^-/L standard gave a mean of 5.10 mg Br^-/L and a relative standard deviation of 0.73%.

b. *Bias*: With a 300- μL sample loop, solutions of sodium chloride were fortified in triplicate with bromide and mean blanks and recoveries were measured. From a 10 000-mg Cl^-/L solution, a blank gave 0.13 mg Br^-/L . Corrected for this blank, a 1.0-mg Br^-/L known addition gave 98% recovery and a 5.0-mg Br^-/L known addition gave 102 % recovery. From a 20 000 mg Cl^-/L solution, a blank gave 0.27 mg Br^-/L . Corrected for this blank, a 1.0-mg Br^-/L known addition gave 100% recovery and a 5.0-mg Br^-/L known addition gave 101% recovery.

c. *MDL*: Using a published MDL method¹ and a 300- μL sample loop, analysts ran 21 replicates of a 0.5-mg Br^-/L standard. These gave a mean of 0.468 mg Br^-/L , a standard deviation of 0.030 mg Br^-/L , and an MDL of 0.07 mg Br^-/L . A lower MDL may be obtained by

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increasing the sample loop volume and increasing the ratio of carrier flow rate to reagents flow rate.

7. Reference

1. U.S. Environmental Protection Agency. 1989. Definition and procedure for the determination of method detection limits. Appendix B to CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-CO₂ CARBON DIOXIDE*#(19)

4500-CO₂ A. Introduction

1. Occurrence and Significance

Surface waters normally contain less than 10 mg free carbon dioxide (CO₂) per liter while some groundwaters may easily exceed that concentration. The CO₂ content of a water may contribute significantly to corrosion. Recarbonation of a supply during the last stages of water softening is a recognized treatment process. The subject of saturation with respect to calcium carbonate is discussed in Section 2330.

2. Selection of Method

A nomographic and a titrimetric method are described for the estimation of free CO₂ in drinking water. The titration may be performed potentiometrically or with phenolphthalein indicator. Properly conducted, the more rapid, simple indicator method is satisfactory for field tests and for control and routine applications if it is understood that the method gives, at best, only an approximation.

The nomographic method (B) usually gives a closer estimation of the total free CO₂ when the pH and alkalinity determinations are made immediately and correctly at the time of sampling. The pH measurement preferably should be made with an electrometric pH meter, properly calibrated with standard buffer solutions in the pH range of 7 to 8. The error resulting from inaccurate pH measurements grows with an increase in total alkalinity. For example, an inaccuracy of 0.1 in the pH determination causes a CO₂ error of 2 to 4 mg/L in the pH range of 7.0 to 7.3 and a total alkalinity of 100 mg CaCO₃/L. In the same pH range, the error approaches 10 to 15 mg/L when the total alkalinity is 400 mg as CaCO₃/L.

Under favorable conditions, agreement between the titrimetric and nomographic methods is reasonably good. When agreement is not precise and the CO₂ determination is of particular importance, state the method used.

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The calculation of the total CO_2 , free and combined, is given in Method D.

4500- CO_2 B. Nomographic Determination of Free Carbon Dioxide and the Three Forms of Alkalinity*(20)

1. General Discussion

Diagrams and nomographs enable the rapid calculation of the CO_2 , bicarbonate, carbonate, and hydroxide content of natural and treated waters. These graphical presentations are based on equations relating the ionization equilibria of the carbonates and water. If pH, total alkalinity, temperature, and total mineral content are known, any or all of the alkalinity forms and CO_2 can be determined nomographically.

A set of charts, Figure 4500- CO_2 :1, Figure 4500- CO_2 :2, Figure 4500- CO_2 :3, and Figure 4500- CO_2 :4 †(21) is presented for use where their accuracy for the individual water supply is confirmed. The nomographs and the equations on which they are based are valid only when the salts of weak acids other than carbonic acid are absent or present in extremely small amounts.

Some treatment processes, such as superchlorination and coagulation, can affect significantly pH and total-alkalinity values of a poorly buffered water of low alkalinity and low total-dissolved-mineral content. In such instances the nomographs may not be applicable.

2. Precision and Bias

The precision possible with the nomographs depends on the size and range of the scales. With practice, the recommended nomographs can be read with a precision of 1%. However, the overall bias of the results depends on the bias of the analytical data applied to the nomographs and the validity of the theoretical equations and the numerical constants on which the nomographs are based. An approximate check of the bias of the calculations can be made by summing the three forms of alkalinity. Their sum should equal the total alkalinity.

3. Bibliography

MOORE, E.W. 1939. Graphic determination of carbon dioxide and the three forms of alkalinity. *J. Amer. Water Works Assoc.* 31:51.

4500- CO_2 C. Titrimetric Method for Free Carbon Dioxide

1. General Discussion

a. *Principle:* Free CO_2 reacts with sodium carbonate or sodium hydroxide to form sodium bicarbonate. Completion of the reaction is indicated potentiometrically or by the development of the pink color characteristic of phenolphthalein indicator at the equivalence pH of 8.3. A 0.01N

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sodium bicarbonate (NaHCO_3) solution containing the recommended volume of phenolphthalein indicator is a suitable color standard until familiarity is obtained with the color at the end point.

b. Interference: Cations and anions that quantitatively disturb the normal CO_2 -carbonate equilibrium interfere with the determination. Metal ions that precipitate in alkaline solution, such as aluminum, chromium, copper, and iron, contribute to high results. Ferrous ion should not exceed 1.0 mg/L. Positive errors also are caused by weak bases, such as ammonia or amines, and by salts of weak acids and strong bases, such as borate, nitrite, phosphate, silicate, and sulfide. Such substances should not exceed 5% of the CO_2 concentration. The titrimetric method for CO_2 is inapplicable to samples containing acid mine wastes and effluent from acid-regenerated cation exchangers. Negative errors may be introduced by high total dissolved solids, such as those encountered in seawater, or by addition of excess indicator.

c. Sampling and storage: Even with a careful collection technique, some loss in free CO_2 can be expected in storage and transit. This occurs more frequently when the gas is present in large amounts. Occasionally a sample may show an increase in free CO_2 content on standing. Consequently, determine free CO_2 immediately at the point of sampling. Where a field determination is impractical, fill completely a bottle for laboratory examination. Keep the sample, until tested, at a temperature lower than that at which the water was collected. Make the laboratory examination as soon as possible to minimize the effect of CO_2 changes.

2. Apparatus

See Section 2310B.2.

3. Reagents

See Section 2310B.3.

4. Procedure

Follow the procedure given in Section 2310B.4*b*, phenolphthalein, or Section 2310B.4*d*, using end-point pH 8.3.

5. Calculation

$$\text{mg CO}_2/\text{L} = \frac{A \times N \times 44\,000}{\text{mL sample}}$$

where:

A = mL titrant and

N = normality of NaOH.

6. Precision and Bias

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Precision and bias of the titrimetric method are on the order of $\pm 10\%$ of the known CO_2 concentration.

4500- CO_2 D. Carbon Dioxide and Forms of Alkalinity by Calculation

1. General Discussion

When the total alkalinity of a water (Section 2320) is due almost entirely to hydroxides, carbonates, or bicarbonates, and the total dissolved solids (Section 2540) is not greater than 500 mg/L, the alkalinity forms and free CO_2 can be calculated from the sample pH and total alkalinity. The calculation is subject to the same limitations as the nomographic procedure given above and the additional restriction of using a single temperature, 25°C . The calculations are based on the ionization constants:

$$K_1 = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3^*]} \quad (K_1 = 10^{-6.36})$$

and

$$K_2 = \frac{[\text{H}^+][\text{CO}_3^{2-}]}{[\text{HCO}_3^-]} \quad (K_2 = 10^{-10.33})$$

where:

$$[\text{H}_2\text{CO}_3^*] = [\text{H}_2\text{CO}_3] + [\text{CO}_2(\text{aq})]$$

Activity coefficients are assumed equal to unity.

2. Calculation

Compute the forms of alkalinity and sample pH and total alkalinity using the following equations:

a. Bicarbonate alkalinity:

$$\text{HCO}_3^- \text{ as mg CaCO}_3/\text{L} = \frac{T - 5.0 \times 10^{(\text{pH} - 10)}}{1 + 0.94 \times 10^{(\text{pH} - 10)}}$$

where:

T = total alkalinity, mg CaCO_3/L

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b. Carbonate alkalinity:

$$\text{CO}_3^{2-} \text{ as mg CaCO}_3/\text{L} = 0.94 \times B \times 10^{(\text{pH}-10)}$$

where:

B = bicarbonate alkalinity, from *a*.

c. Hydroxide alkalinity:

$$\text{OH}^- \text{ as mg CaCO}_3/\text{L} = 5.0 \times 10^{(\text{pH}-10)}$$

d. Free carbon dioxide:

$$\text{mg CO}_2/\text{L} = 2.0 \times B \times 10^{(6-\text{pH})}$$

where:

B = bicarbonate alkalinity, from *a*.

e. Total carbon dioxide:

$$\text{mg total CO}_2/\text{L} = A + 0.44 (2B + C)$$

where:

A = mg free CO_2/L ,

B = bicarbonate alkalinity from *a*, and

C = carbonate alkalinity from *b*.

3. Bibliography

DYE, J.F. 1958. Correlation of the two principal methods of calculating the three kinds of alkalinity. *J. Amer. Water Works Assoc.* 50:812.

4500-CN⁻ CYANIDE*#(22)

4500-CN⁻ A. Introduction

1. General Discussion

“Cyanide” refers to all of the CN groups in cyanide compounds that can be determined as the cyanide ion, CN^- , by the methods used. The cyanide compounds in which cyanide can be

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obtained as CN^- are classed as simple and complex cyanides.

Simple cyanides are represented by the formula $\text{A}(\text{CN})_x$, where A is an alkali (sodium, potassium, ammonium) or a metal, and x, the valence of A, is the number of CN groups. In aqueous solutions of simple alkali cyanides, the CN group is present as CN^- and molecular HCN, the ratio depending on pH and the dissociation constant for molecular HCN ($\text{pK}_a \sim 9.2$). In most natural waters HCN greatly predominates.¹ In solutions of simple metal cyanides, the CN group may occur also in the form of complex metal-cyanide anions of varying stability. Many simple metal cyanides are sparingly soluble or almost insoluble [CuCN , AgCN , $\text{Zn}(\text{CN})_2$], but they form a variety of highly soluble, complex metal cyanides in the presence of alkali cyanides.

Complex cyanides have a variety of formulae, but the alkali-metallic cyanides normally can be represented by $\text{A}_y\text{M}(\text{CN})_x$. In this formula, A represents the alkali present y times, M the heavy metal (ferrous and ferric iron, cadmium, copper, nickel, silver, zinc, or others), and x the number of CN groups; x is equal to the valence of A taken y times plus that of the heavy metal. Initial dissociation of each of these soluble, alkali-metallic, complex cyanides yields an anion that is the radical $\text{M}(\text{CN})_x^{y-}$. This may dissociate further, depending on several factors, with the liberation of CN^- and consequent formation of HCN.

The great toxicity to aquatic life of molecular HCN is well known;²⁻⁵ it is formed in solutions of cyanide by hydrolytic reaction of CN^- with water. The toxicity of CN^- is less than that of HCN; it usually is unimportant because most of the free cyanide (CN group present as CN^- or as HCN) exists as HCN,²⁻⁵ as the pH of most natural waters is substantially lower than the pK_a for molecular HCN. The toxicity to fish of most tested solutions of complex cyanides is attributable mainly to the HCN resulting from dissociation of the complexes.^{2,4,5} Analytical distinction between HCN and other cyanide species in solutions of complex cyanides is possible.^{2,5-9,10}

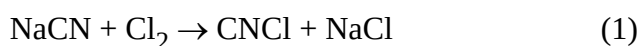
The degree of dissociation of the various metalcyanide complexes at equilibrium, which may not be attained for a long time, increases with decreased concentration and decreased pH, and is inversely related to the highly variable stability of the complexes.^{2,4,5} The zinc- and cadmium-cyanide complexes are dissociated almost totally in very dilute solutions; thus these complexes can result in acute toxicity to fish at any ordinary pH. In equally dilute solutions there is much less dissociation for the nickel-cyanide complex and the more stable cyanide complexes formed with copper (I) and silver. Acute toxicity to fish from dilute solutions containing copper-cyanide or silver-cyanide complex anions can be due to the toxicity of the undissociated ions, although the complex ions are much less toxic than HCN.^{2,5}

The iron-cyanide complex ions are very stable and not materially toxic; in the dark, acutely toxic levels of HCN are attained only in solutions that are not very dilute and have been aged for a long time. However, these complexes are subject to extensive and rapid photolysis, yielding

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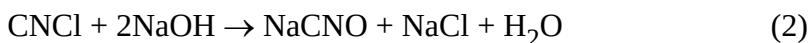
toxic HCN, on exposure of dilute solutions to direct sunlight.^{2,11} The photodecomposition depends on exposure to ultraviolet radiation, and therefore is slow in deep, turbid, or shaded receiving waters. Loss of HCN to the atmosphere and its bacterial and chemical destruction concurrent with its production tend to prevent increases of HCN concentrations to harmful levels. Regulatory distinction between cyanide complexed with iron and that bound in less stable complexes, as well as between the complexed cyanide and free cyanide or HCN, can, therefore, be justified.

Historically, the generally accepted physicochemical technique for industrial waste treatment of cyanide compounds is alkaline chlorination:

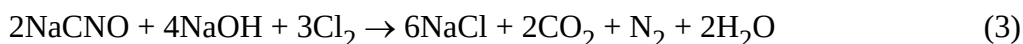


The first reaction product on chlorination is cyanogen chloride (CNCl), a highly toxic gas of limited solubility. The toxicity of CNCl may exceed that of equal concentrations of cyanide.^{2,3,12} At an alkaline pH, CNCl hydrolyzes to the cyanate ion (CNO^-), which has only limited toxicity.

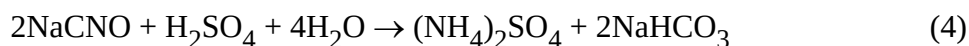
There is no known natural reduction reaction that may convert CNO^- to CN^- .¹³ On the other hand, breakdown of toxic CNCl is pH- and time-dependent. At pH 9, with no excess chlorine present, CNCl may persist for 24 h.^{14,15}



CNO^- can be oxidized further with chlorine at a nearly neutral pH to CO_2 and N_2 :



CNO^- also will be converted on acidification to NH_4^+ :



The alkaline chlorination of cyanide compounds is relatively fast, but depends equally on the dissociation constant, which also governs toxicity. Metal cyanide complexes, such as nickel, cobalt, silver, and gold, do not dissociate readily. The chlorination reaction therefore requires more time and a significant chlorine excess.¹⁶ Iron cyanides, because they do not dissociate to any degree, are not oxidized by chlorination. There is correlation between the refractory properties of the noted complexes, in their resistance to chlorination and lack of toxicity.

Thus, it is advantageous to differentiate between *total cyanide* and *cyanides amenable to chlorination*. When total cyanide is determined, the almost nondissociable cyanides, as well as cyanide bound in complexes that are readily dissociable and complexes of intermediate stability, are measured. Cyanide compounds that are amenable to chlorination include free cyanide as well as those complex cyanides that are potentially dissociable, almost wholly or in large degree, and

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therefore, potentially toxic at low concentrations, even in the dark. The chlorination test procedure is carried out under rigorous conditions appropriate for measurement of the more dissociable forms of cyanide.

The free and potentially dissociable cyanides also may be estimated when using the *weak acid dissociable* procedure. These methods depend on a rigorous distillation, but the solution is only slightly acidified, and elimination of iron cyanides is insured by the earlier addition of precipitation chemicals to the distillation flask and by the avoidance of ultraviolet irradiation.

The *cyanogen chloride* procedure is common with the colorimetric test for cyanides amenable to chlorination. This test is based on the addition of chloramine-T and subsequent color complex formation with pyridine-barbituric acid solution. Without the addition of chloramine-T, only existing CNCl is measured. CNCl is a gas that hydrolyzes to CNO^- ; sample preservation is not possible. Because of this, spot testing of CNCl levels may be best. This procedure can be adapted and used when the sample is collected.

There may be analytical requirements for the determination of CNO^- , even though the reported toxicity level is low. On acidification, CNO^- decomposes to ammonia (NH_3).³ Molecular ammonia and metal-ammonia complexes are toxic to aquatic life.¹⁷

Thiocyanate (SCN^-) is not very toxic to aquatic life.^{2,18} However, upon chlorination, toxic CNCl is formed, as discussed above.^{2,3,12} At least where subsequent chlorination is anticipated, the determination of SCN^- is desirable. Thiocyanate is biodegradable; ammonium is released in this reaction. Although the typical detoxifying agents used in cyanide poisoning induce thiocyanate formation, biochemical cyclic reactions with cyanide are possible, resulting in detectable levels of cyanide from exposure to thiocyanate.¹⁸ Thiocyanate may be analyzed in samples properly preserved for determination of cyanide; however, thiocyanate also can be preserved in samples by acidification with H_2SO_4 to $\text{pH} \leq 2$.

2. Cyanide in Solid Waste

a. Soluble cyanide: Determination of soluble cyanide requires sample leaching with distilled water until solubility equilibrium is established. One hour of stirring in distilled water should be satisfactory. Cyanide analysis is then performed on the leachate. Low cyanide concentration in the leachate may indicate presence of sparingly soluble metal cyanides. The cyanide content of the leachate is indicative of residual solubility of insoluble metal cyanides in the waste.

High levels of cyanide in the leachate indicate soluble cyanide in the solid waste. When 500 mL distilled water are stirred into a 500-mg solid waste sample, the cyanide concentration (mg/L) of the leachate multiplied by 1000 will give the solubility level of the cyanide in the solid waste in milligrams per kilogram. The leachate may be analyzed for total cyanide and/or cyanide amenable to chlorination.

b. Insoluble cyanide: The insoluble cyanide of the solid waste can be determined with the total cyanide method by placing a 500-mg sample with 500 mL distilled water in the distillation

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flask and in general following the distillation procedure (Section 4500-CN⁻.C). In calculating, multiply by 1000 to give the cyanide content of the solid sample in milligrams per kilogram. Insoluble iron cyanides in the solid can be leached out earlier by stirring a weighed sample for 12 to 16 h in a 10% NaOH solution. The leached and wash waters of the solid waste will give the iron cyanide content with the distillation procedure. Prechlorination will have eliminated all cyanide amenable to chlorination. Do not expose sample to sunlight.

3. Selection of Method

a. Total cyanide after distillation: After removal of interfering substances, the metal cyanide is converted to HCN gas, which is distilled and absorbed in sodium hydroxide (NaOH) solution.¹⁹ Because of the catalytic decomposition of cyanide in the presence of cobalt at high temperature in a strong acid solution,^{20,21} cobalticyanide is not recovered completely. Indications are that cyanide complexes of the noble metals, i.e., gold, platinum, and palladium, are not recovered fully by this procedure either. Distillation also separates cyanide from other color-producing and possibly interfering organic or inorganic contaminants. Subsequent analysis is for the simple salt, sodium cyanide (NaCN). Some organic cyanide compounds, such as cyanohydrins, are decomposed by the distillation. Aldehydes convert cyanide to cyanohydrins.

The absorption liquid is analyzed by a titrimetric, colorimetric, or cyanide-ion-selective electrode procedure:

- 1) The titration method (D) is suitable for cyanide concentrations above 1 mg/L.
- 2) The colorimetric methods (E, N, and O) are suitable for cyanide concentrations as low as 1 to 5 µg/L under ideal conditions. Method N uses flow injection analysis of the distillate. Method O uses flow injection analysis following transfer through a semipermeable membrane for separating gaseous cyanide, and colorimetric analysis. Method E uses conventional colorimetric analysis of the distillate from Method C.
- 3) The ion-selective electrode method (F) using the cyanide ion electrode is applicable in the concentration range of 0.05 to 10 mg/L.

b. Cyanide amenable to chlorination:

- 1) Distillation of two samples is required, one that has been chlorinated to destroy all amenable cyanide present and the other unchlorinated. Analyze absorption liquids from both tests for total cyanide. The observed difference equals cyanides amenable to chlorination.
- 2) The colorimetric methods, by conversion of amenable cyanide and SCN⁻ to CNCl and developing the color complex with pyridine-barbituric acid solution, are used for the determination of the total of these cyanides (H, N, and O). Repeating the test with the cyanide masked by the addition of formaldehyde provides a measure of the SCN⁻ content. When subtracted from the earlier results this provides an estimate of the amenable CN⁻ content. This method is useful for natural and ground waters, clean metal finishing, and heat treating effluents. Sanitary wastes may exhibit interference.

- 3) The *weak acid dissociable cyanides* procedure also measures the cyanide amenable to

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chlorination by freeing HCN from the dissociable cyanide. After being collected in a NaOH absorption solution, CN^- may be determined by one of the finishing procedures given for the total cyanide determination. An automated procedure (O) also is presented.

It should be noted that although cyanide amenable to chlorination and weak acid dissociable cyanide appear to be identical, certain industrial effluents (e.g., pulp and paper, petroleum refining industry effluents) contain some poorly understood substances that may produce interference. Application of the procedure for cyanide amenable to chlorination yields negative values. For natural waters and metal-finishing effluents, the direct colorimetric determination appears to be the simplest and most economical.

c. *Cyanogen chloride*: The colorimetric method for measuring cyanide amenable to chlorination may be used, but omit the chloramine-T addition. The spot test also may be used.

d. *Spot test for sample screening*: This procedure allows a quick sample screening to establish whether more than 50 $\mu\text{g/L}$ cyanide amenable to chlorination is present. The test also may be used to estimate the CNCl content at the time of sampling.

e. *Cyanate*: CNO^- is converted to ammonium carbonate, $(\text{NH}_4)_2\text{CO}_3$, by acid hydrolysis at elevated temperature. Ammonia (NH_3) is determined before the conversion of the CNO^- and again afterwards. The CNO^- is estimated from the difference in NH_3 found in the two tests.²²⁻²⁴ Measure NH_3 by either:

- 1) The selective electrode method, using the NH_3 gas electrode (Section 4500- NH_3 .D); or
- 2) The colorimetric method, using the phenate method for NH_3 (Section 4500- NH_3 .F or Section 4500- NH_3 .G).

f. *Thiocyanate*: Use the colorimetric determination with ferric nitrate as a color-producing compound.

4. References

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4500-CN⁻ B. Preliminary Treatment of Samples

CAUTION—Use care in manipulating cyanide-containing samples because of toxicity. Process in a hood or other well-ventilated area. Avoid contact, inhalation, or ingestion.

1. General Discussion

The nature of the preliminary treatment will vary according to the interfering substance present. Sulfides, fatty acids, and oxidizing agents are removed by special procedures. Most other interfering substances are removed by distillation. The importance of the distillation procedure cannot be overemphasized.

2. Preservation of Samples

Oxidizing agents, such as chlorine, decompose most cyanides. Test by placing a drop of sample on a strip of potassium iodide (KI)-starch paper previously moistened with acetate buffer solution, pH 4 (Section 4500-Cl.C.3e). If a bluish discoloration is noted, add 0.1 g sodium arsenite (NaAsO₂)/L sample and retest. Repeat addition if necessary. Sodium thiosulfate or ascorbic acid also may be used, but avoid an excess greater than 0.1 g Na₂S₂O₃/ L. Manganese dioxide, nitrosyl chloride, etc., if present, also may cause discoloration of the test paper. If possible, carry out this procedure before preserving sample as described below. If the following test indicates presence of sulfide, oxidizing compounds would not be expected.

Oxidized products of sulfide convert CN⁻ to SCN⁻ rapidly, especially at high pH.¹ Test for S²⁻ by placing a drop of sample on lead acetate test paper previously moistened with acetic acid buffer solution, pH 4 (Section 4500-Cl.C.3e). Darkening of the paper indicates presence of S²⁻. Add lead acetate, or if the S²⁻ concentration is too high, add powdered lead carbonate [Pb(CO₃)₂] to avoid significantly reducing pH. Repeat test until a drop of treated sample no longer darkens the acidified lead acetate test paper. Filter sample before raising pH for stabilization. When particulate, metal cyanide complexes are suspected, filter solution before removing S²⁻. Reconstitute sample by returning filtered particulates to the sample bottle after S²⁻ removal. Homogenize particulates before analyses.

Aldehydes convert cyanide to cyanohydrin. Longer contact times between cyanide and the aldehyde and the higher ratios of aldehyde to cyanide both result in increasing losses of cyanide that are not reversible during analysis. If the presence of aldehydes is suspected, stabilize with NaOH at time of collection and add 2 mL 3.5% ethylenediamine solution per 100 mL of sample.

Because most cyanides are very reactive and unstable, analyze samples as soon as possible. If sample cannot be analyzed immediately, add NaOH pellets or a strong NaOH solution to raise sample pH to 12 to 12.5, add dechlorinating agent if sample is disinfected, and store in a closed, dark bottle in a cool place.

To analyze for CNCl collect a separate sample and omit NaOH addition because CNCl is

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converted rapidly to CNO^- at high pH. Make colorimetric estimation immediately after sampling.

3. Interferences

a. *Oxidizing agents* may destroy most of the cyanide during storage and manipulation. Add NaAsO_2 or $\text{Na}_2\text{S}_2\text{O}_3$ as directed above; avoid excess $\text{Na}_2\text{S}_2\text{O}_3$.

b. *Sulfide* will distill over with cyanide and, therefore, adversely affect colorimetric, titrimetric, and electrode procedures. Test for and remove S^{2-} as directed above. Treat 25 mL more than required for the distillation to provide sufficient filtrate volume.

c. *Fatty acids* that distill and form soaps under alkaline titration conditions make the end point almost impossible to detect. Remove fatty acids by extraction.² Acidify sample with acetic acid (1 + 9) to pH 6.0 to 7.0. (CAUTION—*Perform this operation in a hood as quickly as possible.*) Immediately extract with iso-octane, hexane, or CHCl_3 (preference in order named). Use a solvent volume equal to 20% of sample volume. One extraction usually is adequate to reduce fatty acid concentration below the interference level. Avoid multiple extractions or a long contact time at low pH to minimize loss of HCN . When extraction is completed, immediately raise pH to >12 with NaOH solution.

d. *Carbonate* in high concentration may affect the distillation procedure by causing the violent release of carbon dioxide with excessive foaming when acid is added before distillation and by reducing pH of the absorption solution. Use calcium hydroxide to preserve such samples.³ Add calcium hydroxide slowly, with stirring, to pH 12 to 12.5. After precipitate settles, decant supernatant liquid for determining cyanide.

Insoluble complex cyanide compounds will not be determined. If such compounds are present, filter a measured amount of well-mixed treated sample through a glass fiber or membrane filter (47-mm diam or less). Rinse filter with dilute (1 to 9) acetic acid until effervescence ceases. Treat entire filter with insoluble material as insoluble cyanide (Section 4500- CN^- .A.2b) or add to filtrate before distillation.

e. *Other possible interferences* include substances that might contribute color or turbidity. In most cases, distillation will remove these.

Note, however, that the strong acid distillation procedure requires using sulfuric acid with various reagents. With certain wastes, these conditions may result in reactions that otherwise would not occur in the aqueous sample. As a quality control measure, periodically conduct addition and recovery tests with industrial waste samples.

f. *Aldehydes* convert cyanide to cyanohydrin, which forms nitrile under the distillation conditions. Only direct titration without distillation can be used, which reveals only non-complex cyanides. Formaldehyde interference is noticeable in concentrations exceeding 0.5 mg/L. Use the following spot test to establish absence or presence of aldehydes (detection limit 0.05 mg/L):⁴⁻⁶

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1) Reagents

a) *MBTH indicator solution*: Dissolve 0.05 g 3-methyl, 2-benzothiazolone hydrazone hydrochloride in 100 mL water. Filter if turbid.

b) *Ferric chloride oxidizing solution*: Dissolve 1.6 g sulfamic acid and 1 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 100 mL water.

c) *Ethylenediamine solution, 3.5%*: Dilute 3.5 mL pharmaceutical-grade anhydrous $\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ to 100 mL with water.

2) Procedure—If the sample is alkaline, add 1 + 1 H_2SO_4 to 10 mL sample to adjust pH to less than 8. Place 1 drop of sample and 1 drop distilled water for a blank in separate cavities of a white spot plate. Add 1 drop MBTH solution and then 1 drop FeCl_3 oxidizing solution to each spot. Allow 10 min for color development. The color change will be from a faint green-yellow to a deeper green with blue-green to blue at higher concentrations of aldehyde. The blank should remain yellow.

To minimize aldehyde interference, add 2 mL of 3.5% ethylenediamine solution/100 mL sample. This quantity overcomes the interference caused by up to 50 mg/L formaldehyde.

When using a known addition in testing, 100% recovery of the CN^- is not necessarily to be expected. Recovery depends on the aldehyde excess, time of contact, and sample temperature.

g. Glucose and other sugars, especially at the pH of preservation, lead to cyanohydrin formation by reaction of cyanide with aldose.⁷ Reduce cyanohydrin to cyanide with ethylenediamine (see above). MBTH is not applicable.

h. Nitrite may form HCN during distillation in Methods C, G, and L, by reacting with organic compounds.^{8,9} Also, NO_3^- may reduce to NO_2^- , which interferes. To avoid NO_2^- interference, add 2 g sulfamic acid to the sample before distillation. *Nitrate* also may interfere by reacting with SCN^- .¹⁰

i. Some sulfur compounds may decompose during distillation, releasing S, H_2S , or SO_2 . Sulfur compounds may convert cyanide to thiocyanate and also may interfere with the analytical procedures for CN^- . To avoid this potential interference, add 50 mg PbCO_3 to the absorption solution before distillation. Filter sample before proceeding with the colorimetric or titrimetric determination.

Absorbed SO_2 forms Na_2SO_3 which consumes chloramine-T added in the colorimetric determination. The volume of chloramine-T added is sufficient to overcome 100 to 200 mg SO_3^{2-} /L. Test for presence of chloramine-T after adding it by placing a drop of sample on KI-starch test paper; add more chloramine-T if the test paper remains blank, or use Method F.

Some wastewaters, such as those from coal gasification or chemical extraction mining, contain high concentrations of sulfites. Pretreat sample to avoid overloading the absorption solution with SO_3^{2-} . Titrate a suitable sample iodometrically (Section 4500-O) with dropwise

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addition of 30% H_2O_2 solution to determine volume of H_2O_2 needed for the 500 mL distillation sample. Subsequently, add H_2O_2 dropwise while stirring, but in only such volume that not more than 300 to 400 mg $\text{SO}_3^{2-}/\text{L}$ will remain. Adding a lesser quantity than calculated is required to avoid oxidizing any CN^- that may be present.

j. Alternate procedure: The strong acid distillation procedure uses concentrated acid with magnesium chloride to dissociate metal-cyanide complexes. In some instances, particularly with industrial wastes, it may be susceptible to interferences such as those from conversion of thiocyanate to cyanide in the presence of an oxidant, e.g., nitrate. If such interferences are present use a ligand displacement procedure with a mildly acidic medium with EDTA to dissociate metal-cyanide complexes.¹⁰ Under such conditions thiocyanate is relatively stable and many oxidants, including nitrate, are weaker.

If any cyanide procedure is revised to meet specific requirements, obtain recovery data by the addition of known amounts of cyanide.

4. References

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thiocyanate-containing waste water. *Anal. Chem.* 55:1677.

4500-CN⁻ C. Total Cyanide after Distillation

1. General Discussion

Hydrogen cyanide (HCN) is liberated from an acidified sample by distillation and purging with air. The HCN gas is collected by passing it through an NaOH scrubbing solution. Cyanide concentration in the scrubbing solution is determined by titrimetric, colorimetric, or potentiometric procedures.

2. Apparatus

The apparatus is shown in Figure 4500-CN⁻:1. It includes:

- Boiling flask*, 1 L, with inlet tube and provision for water-cooled condenser.
- Gas absorber*, with gas dispersion tube equipped with medium-porosity fritted outlet.
- Heating element*, adjustable.
- Ground glass ST joints*, TFE-sleeved or with an appropriate lubricant for the boiling flask and condenser. Neoprene stopper and plastic threaded joints also may be used.

3. Reagents

- Sodium hydroxide solution*: Dissolve 40 g NaOH in water and dilute to 1 L.
- Magnesium chloride reagent*: Dissolve 510 g MgCl₂·6H₂O in water and dilute to 1 L.
- Sulfuric acid*, H₂SO₄, 1 + 1.
- Lead carbonate*, PbCO₃, powdered.
- Sulfamic acid*, NH₂SO₃H.

4. Procedure

- Add 500 mL sample, containing not more than 10 mg CN⁻/ L (diluted if necessary with distilled water) to the boiling flask. If a higher CN⁻ content is anticipated, use the spot test (4500-CN⁻.K) to approximate the required dilution. Add 10 mL NaOH solution to the gas scrubber and dilute, if necessary, with distilled water to obtain an adequate liquid depth in the absorber. Do not use more than 225 mL total volume of absorber solution. When S²⁻ generation from the distilling flask is anticipated add 50 or more mg powdered PbCO₃ to the absorber solution to precipitate S²⁻. Connect the train, consisting of boiling flask air inlet, flask, condenser, gas washer, suction flask trap, and aspirator. Adjust suction so that approximately 1 air bubble/s enters the boiling flask. This air rate will carry HCN gas from flask to absorber and usually will prevent a reverse flow of HCN through the air inlet. If this air rate does not prevent

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sample backup in the delivery tube, increase air-flow rate to 2 air bubbles/s. Observe air purge rate in the absorber where the liquid level should be raised not more than 6.5 to 10 mm. Maintain air flow throughout the reaction.

b. Add 2 g sulfamic acid through the air inlet tube and wash down with distilled water.

c. Add 50 mL 11 M H_2SO_4 through the air inlet tube. Rinse tube with distilled water and let air mix flask contents for 3 min. Add 20 mL MgCl_2 reagent through air inlet and wash down with stream of water. A precipitate that may form redissolves on heating.

d. Heat with rapid boiling, but do not flood condenser inlet or permit vapors to rise more than halfway into condenser. Adequate refluxing is indicated by a reflux rate of 40 to 50 drops/min from the condenser lip. Reflux for at least 1 h. Discontinue heating but continue air flow for 15 min. Cool and quantitatively transfer absorption solution to a 250-mL volumetric flask. Rinse absorber and its connecting tubing sparingly with distilled water and add to flask. Dilute to volume with distilled water and mix thoroughly.

e. Determine cyanide concentration in the absorption solution by procedure of Section 4500-CN⁻.D, Section 4500-CN⁻.E, or Section 4500-CN⁻.F.

f. Distillation gives quantitative recovery of even refractory cyanides such as iron complexes. To obtain complete recovery of cobalticyanide use ultraviolet radiation pretreatment.^{1,2} If incomplete recovery is suspected, distill again by refilling the gas washer with a fresh charge of NaOH solution and refluxing 1 h more. The cyanide from the second reflux, if any, will indicate completeness of recovery.

g. As a quality control measure, periodically test apparatus, reagents, and other potential variables in the concentration range of interest. As an example at least $100 \pm 4\%$ recovery from 1 mg CN⁻/L standard should be obtained.

5. References

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4500-CN⁻ D. Titrimetric Method

1. General Discussion

a. *Principle:* CN⁻ in the alkaline distillate from the preliminary treatment procedure is titrated with standard silver nitrate (AgNO_3) to form the soluble cyanide complex, $\text{Ag}(\text{CN})_2^-$. As soon as all CN⁻ has been complexed and a small excess of Ag^+ has been added, the excess Ag^+

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is detected by the silver-sensitive indicator, *p*-dimethylaminobenzalrhodanine, which immediately turns from a yellow to a salmon color.¹ The distillation has provided a 2:1 concentration. The indicator is sensitive to about 0.1 mg Ag/L. If titration shows that CN⁻ is below 1 mg/L, examine another portion colorimetrically or potentiometrically.

2. Apparatus

Koch microburet, 10-mL capacity.

3. Reagents

a. Indicator solution: Dissolve 20 mg *p*-dimethylaminobenzalrhodanine in 100 mL acetone.

b. Standard silver nitrate titrant: Dissolve 3.27 g AgNO₃ in 1 L distilled water. Standardize against standard NaCl solution, using the argentometric method with K₂CrO₄ indicator, as directed in Chloride, Section 4500-Cl⁻.B.

Dilute 500 mL AgNO₃ solution according to the titer found so that 1.00 mL is equivalent to 1.00 mg CN⁻.

c. Sodium hydroxide dilution solution: Dissolve 1.6 g NaOH in 1 L distilled water.

4. Procedure

a. From the absorption solution take a measured volume of sample so that the titration will require approximately 1 to 10 mL AgNO₃ titrant. Dilute to 100 mL using the NaOH dilution solution or to some other convenient volume to be used for all titrations. For samples with low cyanide concentration (≤5 mg/L) do not dilute. Add 0.5 mL indicator solution.

b. Titrate with standard AgNO₃ titrant to the first change in color from a canary yellow to a salmon hue. Titrate a blank containing the same amount of alkali and water, i.e., 100 mL NaOH dilution solution (or volume used for sample). As the analyst becomes accustomed to the end point, blank titrations decrease from the high values usually experienced in the first few trials to 1 drop or less, with a corresponding improvement in precision.

5. Calculation

$$\text{mg CN}^{-}/\text{L} = \frac{(A - B) \times 1000}{\text{mL original sample}} \times \frac{250}{\text{mL portion used}}$$

where:

A = mL standard AgNO₃ for sample and

B = mL standard AgNO₃ for blank.

6. Precision and Bias²

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Based on the results of six operators in three laboratories, the overall and single-operator precision of this method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.04x + 0.038$$

$$S_o = 0.01x + 0.018$$

$$\text{Selected water matrices: } S_T = 0.06x + 0.711$$

$$S_o = 0.04x + 0.027$$

where:

S_T = overall precision, mg/L,

S_o = single-operator precision, mg/L, and

x = cyanide concentration, mg/L.

Recoveries of known amounts of cyanide from reagent water and selected water matrices are:

Medium	Added mg/L	Recovered mg/L	<i>n</i>	S_T	Bias	% Bias
Reagent water	2.00	2.10	18	0.1267	0.10	5
	5.00	4.65	18	0.2199	-0.35	-7
	5.00	5.18	18	0.2612	0.18	4
Selected water matrices	2.00	2.80	18	0.8695	0.80	40
	5.00	5.29	18	1.1160	0.29	6
	5.00	5.75	18	0.9970	0.75	15

7. References

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4500-CN⁻ E. Colorimetric Method

1. General Discussion

a. Principle: CN⁻ in the alkaline distillate from preliminary treatment is converted to CNCl

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by reaction with chloramine-T at pH <8 without hydrolyzing to CNO^- .¹ (CAUTION— CNCl is a toxic gas; avoid inhalation.) After the reaction is complete, CNCl forms a red-blue color on addition of a pyridine-barbituric acid reagent. Maximum color absorbance in aqueous solution is between 575 and 582 nm. To obtain colors of comparable intensity, have the same salt content in sample and standards.

b. Interference: All known interferences are eliminated or reduced to a minimum by distillation.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 578 nm, providing a light path of 10 mm or longer.

b. Filter photometer, providing a light path of at least 10 mm and equipped with a red filter having maximum transmittance at 570 to 580 nm.

3. Reagents

a. Chloramine-T solution: Dissolve 1.0 g white, water-soluble powder in 100 mL water. Prepare weekly and store in refrigerator.

b. Stock cyanide solution: Dissolve approximately 1.6 g NaOH and 2.51 g KCN in 1 L distilled water. (CAUTION— KCN is highly toxic; avoid contact or inhalation.) Standardize against standard silver nitrate (AgNO_3) titrant as described in Section 4500- CN^- -D.4, using 25 mL KCN solution. Check titer weekly because the solution gradually loses strength; 1 mL = 1 mg CN^- .

c. Standard cyanide solution: Based on the concentration determined for the KCN stock solution (§ 3b) calculate volume required (approximately 10 mL) to prepare 1 L of a 10 $\mu\text{g CN}^-/\text{mL}$ solution. Dilute with the NaOH dilution solution. Dilute 10 mL of the 10 $\mu\text{g CN}^-/\text{mL}$ solution to 100 mL with the NaOH dilution solution; 1.0 mL = 1.0 $\mu\text{g CN}^-$. Prepare fresh daily and keep in a glass-stoppered bottle. (CAUTION—Toxic; take care to avoid ingestion.)

d. Pyridine-barbituric acid reagent: Place 15 g barbituric acid in a 250-mL volumetric flask and add just enough water to wash sides of flask and wet barbituric acid. Add 75 mL pyridine and mix. Add 15 mL conc hydrochloric acid (HCl), mix, and cool to room temperature. Dilute to volume and mix until barbituric acid is dissolved. The solution is stable for approximately 6 months if stored in an amber bottle under refrigeration; discard if precipitate develops.

e. Acetate buffer: Dissolve 410 g sodium acetate trihydrate, $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$, in 500 mL of water. Add glacial acetic acid to adjust to pH 4.5, approximately 500 mL.

f. Sodium hydroxide dilution solution: Dissolve 1.6 g NaOH in 1 L distilled water.

4. Procedure

a. Preparation of standard curve: Pipet a series of standards containing 1 to 10 $\mu\text{g CN}^-$ into

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50-mL volumetric flasks (0.02 to 0.2 $\mu\text{g CN}^-/\text{mL}$). Dilute to 40 mL with NaOH dilution solution. Use 40 mL of NaOH dilution solution as blank. Develop and measure absorbance in 10-mm cells as described in ¶ b for both standards and blank. For concentrations lower than 0.02 $\mu\text{g CN}^-/\text{mL}$ use 100-mm cells.

Recheck calibration curve periodically and each time a new reagent is prepared.

b. Color development: Pipet a portion of absorption solution into a 50-mL volumetric flask and dilute to 40 mL with NaOH dilution solution. Add 1 mL acetate buffer and 2 mL chloramine-T solution, stopper, and mix by inversion twice. Let stand exactly 2 min.

Add 5 mL pyridine-barbituric acid reagent, dilute to volume with distilled water, mix thoroughly, and let stand exactly 8 min. Measure absorbance against distilled water at 578 nm.

Measure absorbance of blank (0.0 mg CN^-/L) using 40 mL NaOH dilution solution and procedures for color development.

5. Calculation

Use the linear regression feature available on most scientific calculators, or compute slope and intercept of standard curve as follows:

$$m = \frac{n \sum ca - \sum c \sum a}{n \sum a^2 - (\sum a)^2}$$
$$b = \frac{\sum a^2 \sum c - \sum a \sum ac}{n \sum a^2 - (\sum a)^2}$$

where:

- a = absorbance of standard solution,
- c = concentration of CN^- in standard, mg/L,
- n = number of standard solutions,
- m = slope of standard curve, and
- b = intercept on c axis.

Include the blank concentration, 0.0 mg CN^-/L and blank absorbance in the calculations above.

$$\text{CN}^-, \text{ mg/L} = (ma_1 + b) \times \frac{50}{X} \times \frac{250}{Y}$$

where:

X = absorption solution, mL,

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Y = original sample, mL, and
 a_1 = absorbance of sample solution.

6. Precision and Bias²

Based on the results of nine operators in nine laboratories, the overall and single-operator precision of this method within its designated ranges may be expressed as follows:

$$\text{Reagent water: } S_T = 0.06x + 0.003$$

$$S_o = 0.11x + 0.010$$

$$\text{Selected water matrices: } S_T = 0.04x + 0.018$$

$$S_o = 0.04x + 0.008$$

where:

S_T = overall precision, mg/L,

S_o = single-operator precision, mg/L, and

x = cyanide concentration, mg/L.

Recoveries of known amounts of cyanide from reagent water and selected water matrices (coke plant and refinery wastes, sewage, and surface water) are:

Medium	Added mg/L	Recovered mg/L	n	S_T	Bias	% Bias
Reagent water	0.060	0.060	26	0.0101	0.000	0
	0.500	0.480	23	0.0258	-0.020	-4
	0.900	0.996	27	0.0669	0.096	11
Selected water matrices	0.060	0.060	25	0.0145	0.000	0
	0.500	0.489	26	0.0501	-0.011	-3
	0.900	0.959	24	0.0509	0.059	7

7. References

1. AMUS, E. & H. GARSCHAGEN. 1953. Über die Verwendung der Barbitsäure für die photometrische Bestimmung von Cyanid und Rhodanid. *Z. Anal. Chem.* 138:414.
2. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1987. Research Rep. D2036:19-1131. American Soc. Testing & Materials, Philadelphia, Pa.

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4500-CN⁻ F. Cyanide-Selective Electrode Method

1. General Discussion

CN⁻ in the alkaline distillate from the preliminary treatment procedures can be determined potentiometrically by using a CN⁻-selective electrode in combination with a double-junction reference electrode and a pH meter having an expanded millivolt scale, or a specific ion meter. This method can be used to determine CN⁻ concentration in place of either the colorimetric or titrimetric procedures in the concentration range of 0.05 to 10 mg CN⁻/L.¹⁻³ If the CN⁻-selective electrode method is used, the previously described titration screening step can be omitted.

2. Apparatus

- a. *Expanded-scale pH meter or specific-ion meter.*
- b. *Cyanide-ion-selective electrode.*#(23)*
- c. *Reference electrode, double-junction.*
- d. *Magnetic mixer with TFE-coated stirring bar.*
- e. *Koch microburet, 10-mL capacity.*

3. Reagents

- a. *Stock standard cyanide solution:* See Section 4500-CN⁻.E.3b.
- b. *Sodium hydroxide dilution solution:* Dissolve 1.6 g NaOH in water and dilute to 1 L.
- c. *Standard cyanide solution:* Dilute a calculated volume (approximately 25 mL) of stock KCN solution, based on the determined concentration, to 1000 mL with NaOH diluent. Mix thoroughly; 1 mL 25 µg CN⁻.
- d. *Dilute standard cyanide solution:* Dilute 100.0 mL standard CN⁻ solution to 1000 mL with NaOH diluent; 1.00 mL = 2.5 µg CN⁻. Prepare daily and keep in a dark, glass-stoppered bottle.
- e. *Potassium nitrate solution:* Dissolve 100 g KNO₃ in water and dilute to 1 L. Adjust to pH 12 with KOH. This is the outer filling solution for the double-junction reference electrode.

4. Procedure

a. *Calibration:* Using Koch microburet and standard CN⁻ solution, prepare four (or more) additional solutions containing 2.5, 0.25, 0.125, and 0.025 µg CN⁻/mL in NaOH dilution solution. Transfer approximately 100 mL of each of these standard solutions into a 250-mL beaker prerinsed with a small portion of standard being tested. Immerse CN⁻ and double-junction reference electrodes. Mix well on a magnetic stirrer at 25°C, maintaining as closely as possible the same stirring rate for all solutions.

Always progress from the lowest to the highest concentration of standard because otherwise

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equilibrium is reached only slowly. The electrode membrane dissolves in solutions of high CN^- concentration; do not use with a concentration above $25 \mu\text{g CN}^-/\text{mL}$. After making measurements remove electrode and soak in water.

After equilibrium is reached (at least 5 min and not more than 10 min), record potential (millivolt) readings. Plot CN^- concentration on logarithmic axis of semilogarithmic paper versus potential developed in solution on linear axis. A straight line with a slope of approximately 59 mV per decade indicates that the instrument and electrodes are operating properly. Record slope of line obtained (millivolts/decade of concentration). The slope may vary somewhat from the theoretical value of 59.2 mV per decade because of manufacturing variation and reference electrode (liquid-junction) potentials. The slope should be a straight line and is the basis for calculating sample concentration. Follow manufacturer's instructions for direct-reading ion meters.

b. Measurement of sample: Place 100 mL of absorption liquid obtained in Section 4500- CN^- .C.4d (or an accurately measured portion diluted to 100 mL with NaOH dilution solution) into a 250-mL beaker. When measuring low CN^- concentrations, first rinse beaker and electrodes with a small volume of sample. Immerse CN^- and double-junction reference electrodes and mix on a magnetic stirrer at the same stirring rate used for calibration. After equilibrium is reached (at least 5 min and not more than 10 min), record values indicated on ion meter or found from graph prepared as above. Calculate concentration as directed below.

5. Calculations

$$\text{mg CN}^-/\text{L} = \mu\text{g CN}^-/\text{mL from graph or meter} \times \frac{100}{x} \times \frac{250}{y}$$

where:

x = volume of absorption solution, mL, and

y = volume of original sample, mL.

6. Precision and Bias⁴

The precision of the CN^- -ion-selective electrode method using the absorption solution from total cyanide distillation has been found in collaborative testing to be linear within its designated range.

Based on the results of six operators in five laboratories, the overall and single-operator precision of this method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.06x + 0.003$$

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$$S_o = 0.03x + 0.008$$

Selected water matrices: $S_T = 0.05x + 0.008$

$$S_o = 0.03x + 0.012$$

where:

S_T = overall precision, mg/L,

S_o = single-operator precision, mg/L, and

x = cyanide concentration, mg/L.

Recoveries of known amounts of cyanide from reagent water and selected water matrices are:

Medium	Added mg/L	Recovered mg/L	<i>n</i>	S_T	Bias	% Bias
Reagent water	0.060	0.059	18	0.0086	-0.001	2
	0.500	0.459	18	0.0281	-0.041	-8
	0.900	0.911	18	0.0552	0.011	1
	5.00	5.07	18	0.297	0.07	1
Selected water matrices	0.060	0.058	14	0.0071	-0.002	-3
	0.500	0.468	21	0.0414	-0.032	-6
	0.900	0.922	19	0.0532	0.022	2
	5.00	5.13	20	0.2839	0.13	3

7. References

1. ORION RESEARCH, INC. 1975. Cyanide Ion Electrode Instruction Manual. Cambridge, Mass.
2. FRANT, M.S., J.W. ROSS & J.H. RISEMAN. 1972. An electrode indicator technique for measuring low levels of cyanide. *Anal. Chem.* 44:2227.
3. SEKERKA, J. & J.F. LECHNER. 1976. Potentiometric determination of low levels of simple and total cyanides. *Water Res.* 10:479.
4. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1987. Research Rep. D2036:19-1131. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN⁻ G. Cyanides Amenable to Chlorination after Distillation

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1. General Discussion

This method is applicable to the determination of cyanides amenable to chlorination.

After part of the sample is chlorinated to decompose the cyanides, both the chlorinated and the untreated sample are subjected to distillation as described in Section 4500-CN⁻.C. The difference between the CN⁻ concentrations found in the two samples is expressed as cyanides amenable to chlorination.

Some unidentified organic chemicals may oxidize or form breakdown products during chlorination, giving higher results for cyanide after chlorination than before chlorination. This may lead to a negative value for cyanides amenable to chlorination after distillation for wastes from, for example, the steel industry, petroleum refining, and pulp and paper processing. Where such interferences are encountered use Method Section 4500-CN⁻.I for determining dissociable cyanide.

Protect sample from exposure to ultraviolet radiation, and perform manipulations under incandescent light, to prevent photodecomposition of some metal-cyanide complexes by ultraviolet light.

2. Apparatus

a. *Distillation apparatus:* See Section 4500-CN⁻.C.2.

b. *Apparatus for determining cyanide* by either the titrimetric method, Section 4500-CN⁻.D.2, the colorimetric method, Section 4500-CN⁻.E.2, or the electrode method, Section 4500-CN⁻.F.2.

3. Reagents

a. *All reagents listed in Section 4500-CN⁻.C.3.*

b. *All reagents listed in Section 4500-CN⁻.D.3, Section 4500-CN⁻.E.3, or Section 4500-CN⁻.F.3, depending on method of estimation.*

c. *Calcium hypochlorite solution:* Dissolve 5 g Ca(OCl)₂ in 100 mL distilled water. Store in an amber-colored glass bottle in the dark. Prepare monthly.

d. *Potassium iodide(KI)-starch test paper.*

4. Procedure

a. Divide sample into two equal portions of 500 mL (or equal portions diluted to 500 mL) and chlorinate one as in ¶ b below. Analyze both portions for CN⁻. The difference in determined concentrations is the cyanide amenable to chlorination.

b. Place one portion in a 1-L beaker covered with aluminum foil or black paper. Keep beaker covered with a wrapped watch glass during chlorination. Add Ca(OCl)₂ solution dropwise to sample while agitating and maintaining pH between 11 and 12 by adding NaOH solution. Test

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for chlorine by placing a drop of treated sample on a strip of KI-starch paper. A distinct blue color indicates sufficient chlorine (approximately 50 to 100 mg Cl₂/L). Maintain excess residual chlorine for 1 h while agitating. If necessary, add more Ca(OCl)₂ and/or NaOH.

c. After 1 h remove any residual chlorine by dropwise addition of NaAsO₂ solution (2 g/100 mL) or by addition of 8 drops H₂O₂ (3%) followed by 4 drops Na₂S₂O₃ solution (500 g/L). Test with KI-starch paper until there is no color change.

d. Distill both chlorinated and unchlorinated samples as in Section 4500-CN⁻.C. Test according to Methods D, E, or F.

5. Calculation

$$\text{mg CN}^- \text{ amenable to chlorination/L} = G - H$$

where:

G = mg CN⁻/L found in unchlorinated portion of sample and

H = mg CN⁻/L found in chlorinated portion of sample.

For samples containing significant quantities of iron cyanides, it is possible that the second distillation will give a higher value for CN⁻ than the test for total cyanide, leading to a negative result. When the difference is within the precision limits of the method, report, “no determinable quantities of cyanide amenable to chlorination.” If the difference is greater than the precision limit, ascertain the cause such as presence of interferences, manipulation of the procedure, etc., or use Method I.

6. Precision and Bias¹

The precision and bias information given in this section may not apply to waters of untested matrices.

a. Precision:

1) Colorimetric—Based on the results of eight operators in seven laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.18x + 0.005$$

$$S_o = 0.06x + 0.003$$

$$\text{Selected water matrices: } S_T = 0.20x + 0.009$$

$$S_o = 0.05x + 0.005$$

2) Titrimetric—Based on the results of six operators in three laboratories, the overall and

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single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.01x + 0.439$$

$$S_o = 0.241 - 0.03x$$

$$\text{Selected water matrices: } S_T = 0.12x + 0.378$$

$$S_o = 0.209 - 0.01x$$

where:

S_T = overall precision, mg/L,

S_o = single-operator precision, mg/L, and

x = cyanide concentration, mg CN⁻/L

b. Bias: Recoveries of known amount of cyanide amenable to chlorination from reagent water and selected water matrices are shown below:

Medium	Technique	Added mg/L	Recovered mg/L	<i>n</i>	S_T	Bias	% Bias
Reagent water	Colorimetric	0.008	0.009	21	0.0033	0.001	13
		0.019	0.023	20	0.0070	0.004	21
		0.080	0.103	20	0.0304	0.018	23
		0.191	0.228	21	0.0428	0.037	19
	Titrimetric	1.00	0.73	18	0.350	-0.27	-27
		1.00	0.81	18	0.551	-0.19	-19
		4.00	3.29	18	0.477	-0.71	-18
Selected water matrices	Colorimetric	0.008	0.013	17	0.0077	0.005	63
		0.019	0.025	18	0.0121	0.006	32
		0.080	0.100	18	0.0372	0.020	25
		0.191	0.229	18	0.0503	0.038	20
	Titrimetric	1.00	1.20	18	0.703	0.20	20
		1.00	1.10	18	0.328	0.10	10
		4.00	3.83	18	0.818	-0.17	-4

7. Reference

Standard Methods for the Examination of Water and Wastewater

1. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1987. Research Rep. D2036:19-1131. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN⁻ H. Cyanides Amenable to Chlorination without Distillation (Short-Cut Method)

1. General Discussion

This method covers the determination of HCN and of CN complexes that are amenable to chlorination and also thiocyanates (SCN⁻). The procedure does not measure cyanates (CNO⁻) or iron cyanide complexes, but does determine cyanogen chloride (CNCl). It may be modified for use in presence of thiocyanates. The method requires neither lengthy distillation nor the chlorination of one sample before distillation. The recovery of CN⁻ from metal cyanide complexes will be comparable to that in Methods G and I.

The cyanides are converted to CNCl by chloramine-T after the sample has been heated. In the absence of nickel, copper, silver, and gold cyanide complexes or SCN⁻, the CNCl may be developed at room temperature. The pyridine-barbituric acid reagent produces a red-blue color in the sample. The color can be estimated visually against standards or photometrically at 578 nm. The dissolved salt content in the standards used for the development of the calibration curve should be near the salt content of the sample, including the added NaOH and phosphate buffer.

The method's usefulness is limited by thiocyanate interference. Although the procedure allows the specific determination of CN⁻ amenable to chlorination (see Section 4500-CN⁻.H.2 and Section 4500-CN⁻.H.5) by masking the CN⁻ content and thereby establishing a correction for the thiocyanide content, the ratio of SCN⁻ to CN⁻ should not exceed 3 to be applicable. In working with unknown samples, screen the sample for SCN⁻ by the spot test (Section 4500-CN⁻.K).

2. Interferences

a. Remove interfering agents as described in Section 4500-CN⁻.B with the exception of NO₂⁻ and NO₃⁻ (Section 4500-CN⁻.B.3h).

b. The SCN⁻ ion reacts with chloramine-T to give a positive error equivalent to its concentration. The procedure allows the separate determination of SCN⁻ and subtraction of this value from the results for the total. Use the spot test (Section 4500-CN⁻.K) for SCN⁻ when its presence is suspected. If the SCN⁻ content is more than three times the CN⁻ content, use Method G or I.

c. Reducing chemical compounds, such as SO₃²⁻, may interfere by consuming chlorine in the chloramine-T addition. A significant excess of chlorine is provided, but the procedure prescribes

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a test (Section 4500-CN⁻.H.5d) to avoid this interference.

d. Color and turbidity may interfere with the colorimetric determination. Overcome this interference by extraction with chloroform (Section 4500-CN⁻.B.3c) but omit reduction of the pH. Otherwise, use Method G or I.

Compensation for color and turbidity may be made by determining absorbance of a second sample solution to which all reagents except chloramine-T have been added.

e. Color intensity and absorption are affected by wide variations in total dissolved solids content of the sample.

For samples containing high concentrations of dissolved solids (3000 to 10 000 mg/L), add 6 g NaCl/L NaOH solution (1.6 g/L) used to prepare standards. For samples containing dissolved solids concentrations greater than 10 000 mg/L, add sufficient NaCl to the NaOH solution to approximate the dissolved solids content.

3. Apparatus

a. Apparatus listed in Section 4500-CN⁻.E.2.

b. Hot water bath.

4. Reagents

a. Reagents listed in Section 4500-CN⁻.B and Section 4500-CN⁻.E.3.

b. Sodium chloride, NaCl, crystals.

c. Sodium carbonate, Na₂CO₃, crystals.

d. Sulfuric acid solution, H₂SO₄, 1N.

e. EDTA solution, 0.05M: Dissolve 18.5 g disodium salt of ethylenediamine tetraacetic acid in water and dilute to 1 L.

f. Formaldehyde solution, 10%: Dilute 27 mL formaldehyde (37% pharmaceutical grade) to 100 mL.

g. Phosphate buffer: Dissolve 138 g sodium dihydrogen phosphate monohydrate, NaH₂PO₄·H₂O, in water and dilute to 1 L. Refrigerate.

5. Procedure

a. Calibrate as directed in Section 4500-CN⁻.E.1a and Section 4500-CN⁻.E.4a. For samples with more than 3000 mg total dissolved solids/L, prepare a calibration curve from standards and blank NaOH solutions containing 6 g NaCl/L. Samples containing total dissolved solids exceeding 10 000 mg/L require appropriate standards and a new calibration curve.

b. Adjust pH of 50 mL sample to between 11.4 and 11.8. If acid is needed, add a small amount (0.2 to 0.4 g) of sodium carbonate and stir to dissolve. Then add HCl solution (1+9) dropwise while stirring. For raising the pH, use NaOH solution (40 g/L).

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c. Pipet 20.0 mL of adjusted sample into a 50-mL volumetric flask. If the cyanide concentration is greater than 0.3 mg/L, use a smaller portion and dilute to 20 mL with NaOH solution. Do not exceed the concentration limit of 0.3 mg/L.

d. To insure uniform color development, both in calibration and testing, maintain a uniform temperature. Immerse flasks in a water bath held at $27 \pm 1^\circ\text{C}$ for 10 min before adding reagents and keep samples in water bath until all reagents have been added.

Add 4 mL phosphate buffer and swirl to mix. Add one drop of EDTA solution, and mix.

e. Add 2 mL chloramine-T solution and swirl to mix. Place 1 drop of sample on potassium iodide-starch test paper that has been moistened previously with acetate buffer solution. Repeat the chloramine-T addition if required. After exactly 3 min, add 5 mL pyridine-barbituric acid reagent and swirl to mix.

f. Remove samples from water bath, dilute to volume, and mix. Allow 8 min from the addition of the pyridine-barbituric acid reagent for color development.

Determine absorbance at 578 nm in a 1.0-cm cell versus distilled water.

Calculate concentration of cyanide, mg/L in the original sample following instructions given in 4500-CN⁻ E.

g. If the presence of thiocyanate is suspected, pipet a second 20-mL portion of pH-adjusted sample into a 50-mL volumetric flask. Add 3 drops 10% formaldehyde solution. Mix and let stand 10 min. Place in a water bath at $27 \pm 1^\circ\text{C}$ for an additional 10 min before the addition of the reagent chemicals and hold in the bath until all reagents have been added.

Continue with *b* above.

Calculate the concentration of cyanide, as milligrams per liter, in the original sample following instructions given in Section 4500-CN⁻.E.

h. In the presence of thiocyanate, cyanide amenable to chlorination is equal to the difference between the concentrations of cyanide obtained in *d* and *g*.

6. Calculation

See Section 4500-CN⁻.E.5.

Deduct SCN⁻ value from the results found when the CN⁻ has not been masked by formaldehyde addition (total) for cyanide content.

7. Precision and Bias¹

This precision and bias information may not apply to waters of untested matrices.

a. *Precision:* Based on the results of 14 operators in nine laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.10x + 0.006$$

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$$S_o = 0.07x + 0.005$$

Selected water matrices: $S_T = 0.11x + 0.007$

$$S_o = 0.02x + 0.005$$

where:

S_T = overall precision, mg/L,

S_o = single-operator precision, mg/L, and

x = cyanide concentration, mg/L.

b. Bias: Recoveries of known amounts of cyanide from reagent water and selected water matrices including creek waters, diluted sewage (1 to 20), and industrial wastewater are shown below.

Medium	Added mg/L		Recovered		S_T	Bias	% Bias
	CN ⁻	SCN ⁻	mg/L	<i>n</i>			
Reagent water	0.005		0.007	42	0.0049	0.002	40
	0.027		0.036	41	0.0109	0.009	25
	0.090		0.100	42	0.0167	0.010	11
	0.090	0.080	0.080	39	0.0121	-0.010	11
	0.270		0.276	42	0.0320	0.006	2
Selected water matrices	0.005		0.003	40	0.0042	-0.002	40
	0.027		0.026	42	0.0093	-0.001	4
	0.090		0.087	42	0.0202	-0.003	3
	0.090	0.080	0.068	37	0.0146	-0.022	24
	0.270		0.245	41	0.0319	-0.025	9

8. Reference

1. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1987. Research Rep. D2036:19-1074. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN⁻ I. Weak Acid Dissociable Cyanide

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1. General Discussion

Hydrogen cyanide (HCN) is liberated from a slightly acidified (pH 4.5 to 6.0) sample under the prescribed distillation conditions. The method does not recover CN^- from tight complexes that would not be amenable to oxidation by chlorine. The acetate buffer used contains zinc salts to precipitate iron cyanide as a further assurance of the selectivity of the method. In other respects the method is similar to 4500- CN^- .C.

2. Interferences

See Section 4500- CN^- .B.3.

Protect sample and apparatus from ultraviolet light to prevent photodecomposition of some metal-cyanide complexes and an increase in concentration of weak acid dissociable cyanide.

If procedure is used to determine low concentrations of cyanide in samples of ferri- and ferrocyanide, add more, e.g., fivefold excess, zinc acetate solution before adding acid and distilling.

3. Apparatus

See Section 4500- CN^- .C.2 and Figure 4500- CN^- :1, and also Section 4500- CN^- .D.2, Section 4500- CN^- .E.2, or Section 4500- CN^- .F.2, depending on method of estimation.

4. Reagents

a. Reagents listed in Section 4500- CN^- .C.3.

b. Reagents listed in Section 4500- CN^- .D.3, Section 4500- CN^- .E.3, or Section 4500- CN^- .F.3, depending on method of estimation.

c. *Acetic acid*, 1 + 9: Mix 1 volume of glacial acetic acid with 9 volumes of water.

d. *Acetate buffer*: Dissolve 410 g sodium acetate trihydrate ($\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$) in 500 mL water. Add glacial acetic acid to yield a solution pH of 4.5 (approximately 500 mL).

e. *Zinc acetate solution*, 100 g/L: Dissolve 120 g $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$ in 500 mL water. Dilute to 1 L.

f. *Methyl red indicator*.

5. Procedure

Follow procedure described in Section 4500- CN^- .C.4, but with the following modifications:

a. Do not add sulfamic acid, because NO_2^- and NO_3^- do not interfere.

b. Instead of H_2SO_4 and MgCl_2 reagents, add 20 mL each of the acetate buffer and zinc acetate solutions through air inlet tube. Also add 2 to 3 drops methyl red indicator. Rinse air inlet tube with water and let air mix contents. If the solution is not pink, add acetic acid (1 + 9) dropwise through air inlet tube until a pink color persists.

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c. Follow instructions beginning with Section 4500-CN⁻.C.4d.

d. For determining CN⁻ in the absorption solution, use the preferred finish method (Section 4500-CN⁻.D, Section 4500-CN⁻.E, or Section 4500-CN⁻.F).

6. Precision and Bias¹

The precision and bias information given in this section may not apply to waters of untested matrices.

a. Precision:

1) Colorimetric—Based on the results of nine operators in nine laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.09x + 0.010$$

$$S_o = 0.08x + 0.005$$

$$\text{Selected water matrices: } S_T = 0.08x + 0.012$$

$$S_o = 0.05x + 0.008$$

2) Electrode—Based on the results of six operators in five laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.09x + 0.004$$

$$S_o = 0.02x - 0.009$$

$$\text{Selected water matrices: } S_T = 0.08x + 0.005$$

$$S_o = 0.02x + 0.004$$

3) Titrimetric—Based on the results of six operators in three laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.532 - 0.10x$$

$$S_o = 0.151 - 0.01x$$

$$\text{Selected water matrices: } S_T = 0.604 - 0.06x$$

$$S_o = 0.092 + 0.02x$$

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where:

S_T = overall precision,

S_o = single-operator precision, and

x = cyanide concentration, mg/L.

b. Bias: Recoveries of known amounts of cyanide from reagent water and selected water matrices are shown below.

Medium	Technique	Added mg/L	Recovered mg/L	<i>n</i>	S_T	Bias	% Bias
Reagent water	Colorimetric	0.030	0.030	25	0.0089	0.000	0
		0.100	0.117	27	0.0251	0.017	17
		0.400	0.361	27	0.0400	-0.039	-10
	Electrode	0.030	0.030	21	0.0059	0.000	0
		0.100	0.095	21	0.0163	-0.005	-5
		0.400	0.365	21	0.0316	-0.035	-9
		1.000	0.940	21	0.0903	-0.060	-6
	Titrimetric	1.00	1.35	18	0.4348	0.35	35
		1.00	1.38	18	0.3688	0.38	38
		4.00	3.67	18	0.1830	-0.33	-8
Selected water matrices	Colorimetric	0.030	0.029	15	0.0062	0.001	3
		0.100	0.118	24	0.0312	0.018	18
		0.400	0.381	23	0.0389	-0.019	-5
	Electrode	0.030	0.029	20	0.0048	-0.001	-3
		0.100	0.104	21	0.0125	0.004	4
		0.400	0.357	21	0.0372	-0.043	-11
		1.000	0.935	21	0.0739	-0.065	-7
	Titrimetric	1.00	1.55	18	0.5466	0.55	55
		1.00	1.53	18	0.4625	0.53	53
		4.00	3.90	18	0.3513	-0.10	-3

7. Reference

1. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1987. Research Rep. D2036:19-1131. American Soc. Testing & Materials, Philadelphia, Pa.

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4500-CN⁻ J. Cyanogen Chloride

1. General Discussion

Cyanogen chloride (CNCl) is the first reaction product when cyanide compounds are chlorinated. It is a volatile gas, only slightly soluble in water, but highly toxic even in low concentrations. (CAUTION: *Avoid inhalation or contact.*) A mixed pyridine-barbituric acid reagent produces a red-blue color with CNCl.

Because CNCl hydrolyzes to cyanate (CNO⁻) at a pH of 12 or more, collect a separate sample for CNCl analysis (See Section 4500-CN⁻.B.2) in a closed container without sodium hydroxide (NaOH). A quick test with a spot plate or comparator as soon as the sample is collected may be the only procedure for avoiding hydrolysis of CNCl due to time lapse between sampling and analysis.

If starch-iodide (KI) test paper indicates presence of chlorine or other oxidizing agents, add sodium thiosulfate (Na₂S₂O₃) immediately as directed in Section 4500-CN⁻.B.2.

2. Apparatus

See Section 4500-CN⁻.E.2.

3. Reagents

a. *Reagents listed in Section 4500-CN⁻.E.3 and Section 4500-CN⁻.H.4.*

b. *Phosphate buffer:* Dissolve 138 g sodium dihydrogen phosphate monohydrate, NaH₂PO₄·H₂O, in water and dilute to 1 L. Refrigerate.

4. Procedure

a. *Preparation of standard curve:* Pipet a series of standards containing 1 to 10 µg CN⁻ into 50-mL volumetric flasks (0.02 to 0.2 µg CN⁻/mL). Dilute to 20 mL with NaOH dilution solution. Use 20 mL of NaOH dilution solution for the blank. Add 2 mL chloramine-T solution and 4 mL phosphate buffer; stopper and mix by inversion two or three times. Add 5 mL pyridine-barbituric acid reagent, dilute to volume with water, mix thoroughly, and let stand exactly 8 min for color development. Measure absorbance at 578 nm in a 10-mm cell using distilled water as a reference. Calculate slope and intercept of the curve.

b. If sample pH is above 8, reduce it to 8.0 to 8.5 by careful addition of phosphate buffer. Measure 20 mL sample portion into 50-mL volumetric flask. If more than 0.20 mg CNCl-CN⁻/L is present use a smaller portion diluted to 20 mL with water. Add 1 mL phosphate buffer, stopper and mix by inversion *one* time. Let stand 2 min. Add 5 mL pyridine-barbituric acid reagent, stopper and mix by inversion *one* time. Let color develop 3 min, dilute to volume with water,

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mix thoroughly, and let stand an additional 5 min. Measure absorbance at 578 nm in 10-mm cell using distilled water as a reference.

5. Calculation

Compute slope (m) and intercept (b) of standard curve as directed in 4500-CN⁻.E.5.

$$\text{Cyanogen chloride as CN}^{-}, \text{ mg/L} = (ma_1 + b) \times \frac{50}{\text{mL sample}}$$

where:

a_1 = absorbance of sample solution.

6. Precision¹

Cyanogen chloride is unstable and round-robin testing is not possible. Single-operator precision is as follows:

Six operators made 70 duplicate analyses on samples of different concentrations within the applicable range of the method. The overall single-operator precision within its designated range may be expressed as follows:

$$\log S_o = (0.5308 \log c) - 1.9842$$

$$\log R = (0.5292 \log c) - 1.8436$$

where:

c = mg CNCl-CN⁻/L,

S_o = single-operator precision in the range of the method (precision is dependent on concentration), and

R = range between duplicate determinations.

The collaborative test data were obtained on reagent-grade water. For other matrices, these data may not apply.

7. Reference

1. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1989. Research Rep. D4165:19-1100. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN⁻ K. Spot Test for Sample Screening

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1. General Discussion

The spot test procedure permits quick screening to establish whether more than 50 µg/L of cyanide amenable to chlorination is present. The test also establishes the presence or absence of cyanogen chloride (CNCl). With practice and dilution, the test reveals the approximate concentration range of these compounds by the color development compared with that of similarly treated standards.

When chloramine-T is added to cyanides amenable to chlorination, CNCl is formed. CNCl forms a red-blue color with the mixed reagent pyridine-barbituric acid. When testing for CNCl omit the chloramine-T addition. (CAUTION: *CNCl is a toxic gas; avoid inhalation.*)

The presence of formaldehyde in excess of 0.5 mg/L interferes with the test. A spot test for the presence of aldehydes and a method for removal of this interference are given in Section 4500-CN⁻.B.3.

Thiocyanate (SCN⁻) reacts with chloramine-T, thereby creating a positive interference. The CN⁻ can be masked with formaldehyde and the sample retested. This makes the spot test specific for SCN⁻. In this manner it is possible to determine whether the spot discoloration is due to the presence of CN⁻, SCN⁻, or both.

2. Apparatus

- a. Porcelain spot plate with 6 to 12 cavities.
- b. Dropping pipets.
- c. Glass stirring rods.

3. Reagents

- a. Chloramine-T solution: See Section 4500-CN⁻.E.3a.
- b. Stock cyanide solution: See Section 4500-CN⁻.E.3b.
- c. Pyridine-barbituric acid reagent: See Section 4500-CN⁻.E.3d.
- d. Hydrochloric acid, HCl, 1 + 9.
- e. Phenolphthalein indicator aqueous solution.
- f. Sodium carbonate, Na₂CO₃, anhydrous.
- g. Formaldehyde, 37%, pharmaceutical grade.

4. Procedure

If the solution to be tested has a pH greater than 10, neutralize a 20- to 25-mL portion. Add about 250 mg Na₂CO₃ and swirl to dissolve. Add 1 drop phenolphthalein indicator. Add 1 + 9 HCl dropwise with constant swirling until the solution becomes colorless. Place 3 drops sample and 3 drops distilled water (for blanks) in separate cavities of the spot plate. To each cavity, add 1 drop chloramine-T solution and mix with a clean stirring rod. Add 1 drop pyridine-barbituric

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acid solution to each cavity and again mix. After 1 min, the sample spot will turn pink to red if 50 µg/L or more of CN⁻ are present. The blank spot will be faint yellow because of the color of the reagents. Until familiarity with the spot test is gained, use, in place of the water blank, a standard solution containing 50 µg CN⁻/L for color comparison. This standard can be made by diluting the stock cyanide solution (§ 3b).

If SCN⁻ is suspected, test a second sample pretreated as follows: Heat a 20- to 25-mL sample in a water bath at 50°C; add 0.1 mL formaldehyde and hold for 10 min. This treatment will mask up to 5 mg CN⁻/L, if present. Repeat spot testing procedure. Color development indicates presence of SCN⁻. Comparing color intensity in the two spot tests is useful in judging relative concentration of CN⁻ and SCN⁻. If deep coloration is produced, serial dilution of sample and additional testing may allow closer approximation of the concentrations.

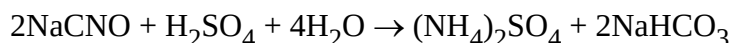
4500-CN⁻ L. Cyanates

1. General Discussion

Cyanate (CNO⁻) may be of interest in analysis of industrial waste samples because the alkaline chlorination process used for the oxidation of cyanide yields cyanate in the second reaction.

Cyanate is unstable at neutral or low pH; therefore, stabilize the sample as soon as collected by adding sodium hydroxide (NaOH) to pH >12. Remove residual chlorine by adding sodium thiosulfate (Na₂S₂O₃) (see Section 4500-CN⁻.B.2).

a. Principle: Cyanate hydrolyzes to ammonia when heated at low pH.



The ammonia concentration must be determined on one sample portion before acidification. The ammonia content before and after hydrolysis of cyanate may be measured by phenate (Section 4500-NH₃.F), or ammonia-selective electrode (Section 4500-NH₃.D) method.¹ The test is applicable to cyanate compounds in natural waters and industrial waste.

b. Interferences:

1) Organic nitrogenous compounds may hydrolyze to ammonia (NH₃) upon acidification. To minimize this interference, control acidification and heating closely.

2) Reduce oxidants that oxidize cyanate to carbon dioxide and nitrogen with Na₂S₂O₃ (see Section 4500-CN⁻.G).

3) Industrial waste containing organic material may contain unknown interferences.

c. Detection limit: 1 to 2 mg CNO⁻/L.

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2. Apparatus

- a. *Expanded-scale pH meter or selective-ion meter.*
- b. *Ammonia-selective electrode.* *#(24)
- c. *Magnetic mixer*, with TFE-coated stirring bar.
- d. *Heat barrier:* Use a 3-mm-thick insulator under beaker to insulate against heat produced by stirrer motor.

3. Reagents

- a. *Stock ammonium chloride solution:* Dissolve 3.819 g anhydrous NH_4Cl , dried at 100°C , in water, and dilute to 1 L; 1.00 mL=1.00 mg N=1.22 mg NH_3 .
- b. *Standard ammonium chloride solution:* From the stock NH_4Cl solution prepare standard solutions containing 1.0, 10.0, and 100.0 mg $\text{NH}_3\text{-N/L}$ by diluting with ammonia-free water.
- c. *Sodium hydroxide, 10N:* Dissolve 400 g NaOH in water and dilute to 1 L.
- d. *Sulfuric acid solution, H_2SO_4 , 1 + 1.*
- e. *Ammonium chloride solution:* Dissolve 5.4 g NH_4Cl in distilled water and dilute to 1 L. (Use only for soaking electrodes.)

4. Procedure

- a. *Calibration:* Daily, calibrate the ammonia electrode as in Section 4500- $\text{NH}_3\text{.F.4b}$ and c using standard NH_4Cl solutions.
- b. *Treatment of sample:* Dilute sample, if necessary, so that the CNO^- concentration is 1 to 200 mg/L or $\text{NH}_3\text{-N}$ is 0.5 to 100 mg/L. Take or prepare at least 200 mL. From this 200 mL, take a 100-mL portion and, following the calibration procedure, establish the potential (millivolts) developed from the sample. Check electrode reading with prepared standards and adjust instrument calibration setting daily. Record $\text{NH}_3\text{-N}$ content of untreated sample (B).

Acidify 100 mL of prepared sample by adding 0.5 mL 1 + 1 H_2SO_4 to a pH of 2.0 to 2.5. Heat sample to 90 to 95°C and maintain temperature for 30 min. Cool to room temperature and restore to original volume by adding ammonia-free water. Pour into a 150-mL beaker, immerse electrode, start magnetic stirrer, then add 1 mL 10N NaOH solution. With pH paper check that pH is greater than 11. If necessary, add more NaOH until pH 11 is reached.

After equilibrium has been reached (30 s) record the potential reading. Estimate $\text{NH}_3\text{-N}$ content from calibration curve.

5. Calculations

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$$\text{mg NH}_3\text{-N derived from CNO}^-/\text{L} = A - B$$

where:

A = mg NH₃-N/L found in the acidified and heated sample portion and

B = mg NH₃-N/L found in untreated portion.

$$\text{mg CNO}^-/\text{L} = 3.0 \times (A - B)$$

6. Precision

No data on precision of this method are available. See Section 4500-NH₃.A.4 for precision of ammonia-selective electrode method.

7. Reference

1. THOMAS, R.F. & R.L. BOOTH. 1973. Selective electrode determination of ammonia in water and wastes. *Environ. Sci. Technol.* 7:523.

4500-CN⁻ M. Thiocyanate

1. General Discussion

When wastewater containing thiocyanate (SCN⁻) is chlorinated, highly toxic cyanogen chloride (CNCl) is formed. At an acidic pH, ferric ion (Fe³⁺) and SCN⁻ form an intense red color suitable for colorimetric determination.

a. Interference:

1) Hexavalent chromium (Cr⁶⁺) interferes and is removed by adding ferrous sulfate (FeSO₄) after adjusting to pH 1 to 2 with nitric acid (HNO₃). Raising the pH to 9 with 1N sodium hydroxide (NaOH) precipitates Fe³⁺ and Cr³⁺, which are then filtered out.

2) Reducing agents that reduce Fe³⁺ to Fe²⁺, thus preventing formation of ferric thiocyanate complex, are destroyed by adding a few drops of hydrogen peroxide (H₂O₂). Avoid excess H₂O₂ to prevent reaction with SCN⁻.

3) Industrial wastes may be highly colored or contain various interfering organic compounds. To eliminate these interferences,¹ use the pretreatment procedure given in ¶ 4c below. It is the analyst's responsibility to validate the method's applicability without pretreatment (¶ 4b). If in doubt, pretreat sample before proceeding with analysis (¶ 4c).

4) If sample contains cyanide amenable to chlorination and would be preserved for the cyanide determination at a high pH, sulfide could interfere by converting cyanide to SCN⁻. To

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preserve SCN^- and CN^- , precipitate the sulfide by adding lead salts according to Section 4500- CN^- .B.2 before adding alkali; filter to remove precipitate.

5) Thiocyanate is biodegradable. Preserve samples at $\text{pH} < 2$ by adding mineral acid and refrigerate.

6) If interferences from industrial wastes are not removed as directed in ¶ 4c below, consider adopting a solvent extraction technique with colorimetric or atomic absorption analysis of the extract.^{2,3}

b. Application: 0.1 to 2.0 mg SCN^-/L in natural or wastewaters. For higher concentrations, use a portion of diluted sample.

2. Apparatus

a. Spectrophotometer or filter photometer, for use at 460 nm, providing a light path of 5 cm.

b. Glass adsorption column: Use a 50-mL buret with a glass-wool plug, and pack with macroreticular resin (¶ 3) approximately 40 cm high. For convenience, apply a powder funnel of the same diameter as the buret to the top with a short piece of plastic tubing.

3. Reagents

a. Ferric nitrate solution: Dissolve 404 g $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ in about 800 mL distilled water. Add 80 mL conc HNO_3 and dilute to 1 L.

b. Nitric acid solution, 0.1N: Mix 6.4 mL conc HNO_3 in about 800 mL distilled water and dilute to 1 L.

c. Stock thiocyanate solution: Dissolve 1.673 g potassium thiocyanate (KSCN) in distilled water and dilute to 1000 mL; 1.00 mL 1.00 mg SCN^- .

d. Standard thiocyanate solution: Dilute 10 mL stock solution to 1 L with distilled water; 1.00 mL = 0.01 mg SCN^- .

e. Sodium hydroxide solution, 4 g/L: Dissolve 4 g NaOH in about 800 mL distilled water and dilute to 1 L.

f. Macroreticular resin, 18 to 50 mesh:(25)* The available resin may not be purified. Some samples have shown contamination with waxes and oil, giving poor permeability and adsorption. Purify as follows:

Place sufficient resin to fill the column or columns in a beaker and add 5 times the resin volume of acetone. Stir gently for 1 h. Pour off fines and acetone from settled resin and add 5 times the resin volume of hexane. Stir for 1 h. Pour off fines and hexane and add 5 times the resin volume of methanol. Stir for 15 min. Pour off methanol and add 3 times the resin volume of 0.1N NaOH . Stir for 15 min. Pour off NaOH solution and add 3 times the resin volume of 0.1N HNO_3 . Stir for 15 min. Pour off HNO_3 solution and add 3 times the resin volume of distilled water. Stir for 15 min. Drain excess water and use purified resin to fill the column. Store excess

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purified resin after covering it with distilled water. Keep in a closed jar.

g. Methyl alcohol.

4. Procedure

a. Preparation of calibration curve: Prepare a series of standards containing from 0.02 mg to 0.40 mg SCN^- by pipetting measured volumes of standard KSCN solution into 200-mL volumetric flasks and diluting with water. Mix well. Develop color according to ¶ b below. Plot absorbance against SCN^- concentration expressed as mg/50 mL sample. The absorbance plot should be linear.

b. Color development: Use a filtered sample or portion from a diluted solution so that the concentration of SCN^- is between 0.1 and 2 mg/L. Adjust pH to 2 with conc HNO_3 added dropwise. Pipet 50-mL portion to a beaker, add 2.5 mL ferric nitrate, and mix.

Fill a 5-cm absorption cell and measure absorbance against a reagent blank at 460 nm or close to the maximum absorbance found with the instrument being used. Measure absorbance of the developed color against a reagent blank within 5 min from adding the reagent. (The color develops within 30 s and fades on standing in light.)

c. Sample pretreatment:

1) Color and various organic compounds interfere with absorbance measurement. At pH 2, macroreticular resin removes these interfering materials by adsorption without affecting thiocyanate.

2) To prepare the adsorption column, fill it with resin, rinse with 100 mL methanol, and follow by rinses with 100 mL 0.1N NaOH, 100 mL 0.1N HNO_3 , and finally with 100 mL distilled water. If previously purified resin is used, omit these preparatory steps.

3) When washing, regenerating, or passing a sample through the column, as solution level approaches resin bed, add and drain five separate 5-mL volumes of solution or water (depending on which is used in next step) to approximate bed height. After last 5-mL volume, fill column with remaining liquid. This procedure prevents undue mixing of solutions and helps void the column of the previous solution.

4) Acidify 150 mL sample (or a dilution) to pH 2 by adding conc HNO_3 dropwise while stirring. Pass it through the column at a flow rate not to exceed 20 mL/min. If the resin becomes packed and the flow rate falls to 4 to 5 mL/min, use gentle pressure through a manually operated hand pump or squeeze bulb on the column. In this case, use a separator funnel for the liquid reservoir instead of the powder funnel. Alternatively use a vacuum bottle as a receiver and apply gentle vacuum. Do not let liquid level drop below the adsorbent in the column.

5) When passing a sample through the column, measure 90 mL of sample in a graduated cylinder, and from this use the five 5-mL additions as directed in ¶ 3), then pour the remainder of the 90 mL into the column. Add rest of sample and collect 60 mL eluate to be tested after the first 60 mL has passed through the column.

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6) Prepare a new calibration curve using standards prepared according to ¶ 4a, but acidify standards according to ¶ 4b, and pass them through the adsorption column. Develop color and measure absorbance according to ¶ 4b against a reagent blank prepared by passing acidified, distilled water through the adsorption column.

7) Pipet 50 mL from the collected eluate to a beaker, add 2.5 mL ferric nitrate solution, and mix. Measure absorbance according to ¶ 4b against a reagent blank [see ¶ 6) above].

8) From the measured absorbance value, determine thiocyanate content of the sample or dilution using the absorbance plot.

9) Each day the column is in use, test a mid-range standard to check absorption curve.

10) Regenerate column between samples by rinsing with 100 mL 0.1N NaOH; 50 mL 0.1N HNO₃; and 100 mL water. Insure that the water has rinsed empty glass section of the buret. Occasionally rinse with 100 mL methanol for complete regeneration. Adsorbed weak organic acids and thiocyanate residuals from earlier tests are eluted by the NaOH rinse. Leave the column covered with the last rinse water for storage.

5. Calculation

Compute slope (*m*) and intercept (*b*) of standard curve as directed in Section 4500-CN⁻.E.5. Calculate thiocyanate concentration as follows:

$$\text{mg SCN}^-/\text{L} = (ma_1 + b) \times \text{dilution factor}$$

where:

a_1 = absorbance of sample solution.

6. Precision and Bias⁴

a. Precision: Based on the results of twelve operators in nine laboratories, at four levels of concentration, the precision of the test method within its designated range is linear with concentration and may be expressed as follows:

$$\text{Reagent water: } S_T = 0.093x + 0.0426$$

$$S_o = 0.045x + 0.010$$

$$\text{Water matrix: } S_T = 0.055x + 0.0679$$

$$S_o = 0.024x + 0.182$$

where:

S_T = overall precision, mg/L,

S_o = pooled single-operator precision, mg/L, and

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x = thiocyanate concentration, mg/L.

b. Bias: Recoveries of known amounts of thiocyanate from reagent water and selected water matrices including natural waters, laboratory effluent, steel mill effluent, and dechlorinated and treated sanitary effluents were as follows:

Medium	Added mg/L	Recovered mg/L	n	S_T	Bias	% Bias
Reagent water	1.42	1.411	30	0.181	-0.009	-0.6
	0.71	0.683	27	0.091	-0.027	-4
	0.35	0.329	30	0.084	-0.021	-6
	0.07	0.068	30	0.052	-0.002	-3
Selected water matrices	1.42	1.408	26	0.151	-0.012	-0.8
	0.71	0.668	29	0.096	-0.042	-6
	0.35	0.320	29	0.085	-0.030	-9
	0.07	0.050	29	0.079	-0.020	-29

For other matrices these data may not apply.

7. References

1. SPENCER, R.R., J. LEENHEER & V.C. MARTI. 1980. Automated colorimetric determination of thiocyanate, thiosulfate and tetrathionate in water. 94th Annu. Meeting. Assoc. Official Agricultural Chemists, Washington, D.C. 1981.
2. DANCHICK, R.S. & D.F. BOLTZ. 1968. Indirect spectrophotometric and atomic absorption spectrometric methods in determination of thiocyanate. *Anal. Chem.* 43:2215.
3. LUTHY, R.G. 1978. Manual of Methods: Preservation and Analysis of Coal Gasification Wastewaters. FE-2496-16, U.S. Dep. Energy, National Technical Information Serv., Springfield, Va.
4. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1989. Research Rep. D4193:19-1099. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN⁻ N. Total Cyanide after Distillation, by Flow Injection Analysis (PROPOSED)

1. General Discussion

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a. Principle: Total cyanides are digested and steam-distilled from the sample as in Section 4500-CN⁻.C, cyanides amenable to chlorination are digested and steam-distilled from the sample as in Section 4500-CN⁻.G, or weak acid dissociable cyanides are digested and steam-distilled from the sample as in Section 4500-CN⁻.I, by using the apparatus described in Section 4500-CN⁻.C or an equivalent distillation apparatus. In any case, the distillate should consist of cyanide in 0.25M NaOH. The cyanide in this distillate is converted to cyanogen chloride, CNCl, by reaction with chloramine-T at pH less than 8. The CNCl then forms a red-blue dye by reacting with pyridine-barbituric acid reagent. The absorbance of this red dye is measured at 570 nm and is proportional to the total or weak acid dissociable cyanide in the sample.

Also see Section 4500-CN⁻.A and Section 4500-CN⁻.E, and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Nonvolatile interferences are eliminated or minimized by the distillation procedure. Some of the known interferences are aldehydes, nitrate-nitrite, and oxidizing agents such as chlorine, thiocyanate, thiosulfate, and sulfide. Multiple interferences may require the analysis of a series of laboratory fortified sample matrices (LFM) to verify the suitability of the chosen treatment. See Section 4500-CN⁻.B for a discussion of preliminary treatment of samples to be distilled.

2. Apparatus

Flow injection analysis equipment consisting of:

- a. FIA injection valve* with sample loop or equivalent.
- b. Multichannel proportioning pump.*
- c. FIA manifold* (Figure 4500-CN⁻:2) with tubing heater and flow cell. Relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*#(26)
- d. Absorbance detector*, 570 nm, 10-nm bandpass.
- e. Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and all reagents with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

- a. Carrier solution, 0.25M:* In a 1-L plastic container dissolve 10.0 g NaOH in 1.00 L water.
- b. Phosphate buffer, 0.71M:* To a 1-L tared container add 97.0 g potassium phosphate, monobasic, anhydrous, KH₂PO₄, and 975 g water. Stir or shake until dissolved. Prepare fresh

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monthly.

c. *Chloramine-T*: Dissolve 2.0 g chloramine-T hydrate (mol wt 227.65) in 500 mL water. Prepare fresh daily.

d. *Pyridine/barbituric acid*: In fume hood, place 15.0 g barbituric acid in a tared 1-L container and add 100 g water, rinsing down sides of beaker to wet the barbituric acid. Add 73 g pyridine (C_5H_5N) with stirring and mix until barbituric acid dissolves. Add 18 g conc HCl, then an additional 825 g water, and mix. Prepare fresh weekly.

e. *Stock cyanide standard*, 100 mg CN^-/L : In a 1-L container, dissolve 2.0 g potassium hydroxide (KOH) in approximately 800 mL water. Add 0.250 g potassium cyanide (KCN). CAUTION: KCN is highly toxic. Avoid inhalation of dust or contact with the solid or solutions. Make to final weight of 1000 g with water and mix. Prepare fresh weekly or standardize weekly using procedure in Section 4500- CN^- .D.4.

f. *Standard cyanide solution*: Prepare cyanide standards in the desired concentration range, using the stock cyanide standard (§ 3e) and diluting with the 0.25M NaOH carrier (§ 3a).

4. Procedure

Set up a manifold equivalent to that in Figure 4500- CN^- :2 and follow method supplied by manufacturer or laboratory standard operating procedure for this method. Follow quality control guidelines outlined in Section 4020.

5. Calculation

Prepare standard curves by plotting absorbance of standards processed through manifold versus cyanide concentration. The calibration curve is linear.

6. Precision and Bias

a. *Recovery and relative standard deviation*: The results of single-laboratory studies with various matrices are given in Table 4500- CN^- :I.

b. *MDL without distillation*: Using a published MDL method,¹ analysts ran 21 replicates of an undistilled 0.010-mg CN^-/L standard with a 780- μL sample loop. These gave a mean of 0.010 mg CN^-/L , a standard deviation of 0.00012 mg CN^-/L , and an MDL of 0.0003 mg CN^-/L . A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagent flow rate.

c. *MDL with distillation*: Using a published MDL method,¹ analysts ran 21 replicates of a 0.0050-mg CN^-/L standard distilled using the distillation device†#(27) equivalent to the apparatus specified in 4500- CN^- .C. When the 0.25M NaOH distillates were determined with a 780- μL sample loop, they gave a mean of 0.0045 mg CN^-/L , a standard deviation of 0.0002 mg CN^-/L , and an MDL of 0.0006 mg CN^-/L .

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d. Precision study: Ten injections of an undistilled 0.050-mg CN^-/L standard gave a relative standard deviation of 0.21%.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 12.11 amended June 30, 1986. 49 CFR 43430.

4500- CN^- O. Total Cyanide and Weak Acid Dissociable Cyanide by Flow Injection Analysis (PROPOSED)

1. General Discussion

a. Principle: Total cyanide consists of various metal-cyanide complexes. To break down or digest these complexes to yield HCN, the sample is mixed with heated phosphoric acid and then irradiated with ultraviolet radiation. The resulting “donor stream” contains the product HCN (aq). This donor stream is passed over a silicone rubber gas permeation membrane. The HCN from the donor stream is extracted by the membrane as HCN (g) and is trapped in a parallel “acceptor stream” that consists of dilute sodium hydroxide, the equivalent of the distillate resulting from the digesting distillations in the sample preparation methods Section 4500- CN^- .C, Section 4500- CN^- .G, and Section 4500- CN^- .I.

As in Section 4500- CN^- .N, the cyanide in this acceptor stream or distillate is converted to cyanogen chloride, CNCl , by reaction with chloramine-T at pH less than 8. The CNCl then forms a red-blue dye by reacting with pyridine-barbituric acid reagent. The absorbance of this red dye is measured at 570 nm and is proportional to the total or weak acid dissociable cyanide in the sample.

The weak acid dissociable (WAD) cyanide method is similar except that ultraviolet radiation and phosphoric acid are not used in the donor stream. Instead, a solution of dihydrogen phosphate is used as the donor stream.

Also see Section 4500- CN^- .A, Section 4500- CN^- .E, and Section 4500- CN^- .N and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering the sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Nonvolatile interferences are eliminated or minimized by the gas-permeable membrane.

Multiple interferences may require the analysis of a series of sample matrices with known additions to verify the suitability of the chosen treatment. See Section 4500- CN^- .B for a discussion of preliminary treatment of samples that will be distilled.

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1) Total cyanide interferences—Sulfide up to a concentration of 10 mg/L and thiocyanate up to a concentration of 20 mg/L do not interfere in the determination of 100 $\mu\text{g CN}^-/\text{L}$. When a sample containing nitrate at 100 mg NO_3^-/L and 20 mg/L thiocyanate was treated with sulfamic acid, the determined value was 138.2 $\mu\text{g CN}^-/\text{L}$ for a known concentration of 100 $\mu\text{g CN}^-/\text{L}$. When pretreated with ethylenediamine, a sample containing 50 mg formaldehyde/L did not interfere in the determination of cyanide.

2) WAD interferences—Sulfide up to 10 mg/L and thiocyanate up to 50 mg/L do not interfere in the determination of 0.1 mg/L cyanide.

2. Apparatus

Flow injection analysis equipment consisting of:

- a. *FIA injection valve* with sample loop or equivalent.
- b. *Multichannel proportioning pump*.
- c. *FIA manifold* (Figure 4500-CN⁻:3) with tubing heater, in-line ultraviolet digestion fluidics, a gas-permeable silicone rubber membrane and its holder, and flow cell. In Figure 4500-CN⁻:3, relative flow rates only are shown. The tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE. The ultraviolet unit should consist of TFE tubing irradiated by a mercury discharge ultraviolet lamp emitting radiation at 254 nm.
- d. *Absorbance detector*, 570 nm, 10-nm bandpass.
- e. *Injection valve control and data acquisition system*.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and all reagents with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L of solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

- a. *Phosphoric acid donor stream* (total cyanide): To a 1-L volumetric flask, add approximately 700 mL water, then add 30 mL conc phosphoric acid, H_3PO_4 . Mix and let solution cool. Dilute to mark. Prepare fresh monthly.
- b. *Dihydrogen phosphate donor stream* (WAD cyanide): To a tared 1-L container add 97 g anhydrous potassium dihydrogen phosphate, KH_2PO_4 , and 975 g water. Stir for 2 h or until the potassium phosphate has gone into solution. Degas with helium. Prepare fresh monthly.
- c. *NaOH acceptor stream, carrier, and diluent* (total and WAD cyanide), 0.025M NaOH: To a 1-L container add 1.0 g sodium hydroxide (NaOH) and 999 g water. Mix with a magnetic stirrer for about 5 min. Cover with a laboratory film. Degas with helium. Prepare fresh daily.
- d. *Buffer* (total and WAD cyanide), 0.71M phosphate: To a 1-L tared container add 97.0 g

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potassium phosphate, monobasic, anhydrous, KH_2PO_4 , and 975 g water. Stir or shake until dissolved. Prepare fresh monthly.

e. *Chloramine-T solution* (total and WAD cyanide): Dissolve 3 g chloramine-T hydrate in 500 mL water. Degas with helium. Prepare fresh daily. NOTE: Chloramine-T is an air-sensitive solid. Preferably discard this chemical 6 months after opening.

f. *Pyridine/barbituric acid solution* (total and WAD cyanide): In the fume hood, place 15.0 g barbituric acid in a tared 1-L container and add 100 g water, rinsing down the sides of the beaker to wet the barbituric acid. Add 73 g pyridine ($\text{C}_5\text{H}_5\text{N}$) with stirring and mix until the barbituric acid dissolves. Add 18 g conc HCl, then add an additional 825 mL water and mix. Prepare fresh weekly.

g. *Stock cyanide standard*, 100 mg CN^-/L : In a 1-L container dissolve 2.0 g potassium hydroxide, KOH, in approximately 800 mL water. Add 0.250 g potassium cyanide, KCN. CAUTION: KCN is highly toxic. Avoid inhalation of dust or contact with the solid or solutions. Make to final weight of 1000 g with water and invert three times to mix. Prepare fresh weekly or standardize weekly using the procedure in Section 4500-CN-D.4.

h. *Standard cyanide solutions*: Prepare cyanide standards in the desired concentration range, using the stock cyanide standard (¶ 3g) and diluting with the NaOH standards diluent (¶ 3c).

4. Procedure

Set up a manifold equivalent to that in Figure 4500-CN-3 and follow the method supplied by the manufacturer or laboratory standard operating procedure for this method. Follow quality control guidelines outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold vs. cyanide concentration. The calibration curve is linear.

6. Precision and Bias

a. *MDL, total cyanide*: A 420- μL sample loop was used in the total cyanide method. Using a published MDL method¹, analysts ran 21 replicates of a 10.0- μg CN^-/L standard. These gave a mean of 9.69 μg CN^-/L , a standard deviation of 0.86 μg CN^-/L , and an MDL of 2.7 μg CN^-/L .

b. *MDL, WAD cyanide*: A 420- μL sample loop was used in the WAD cyanide method. Using a published MDL method¹, analysts ran 21 replicates of a 10.0- μg CN^-/L standard. These gave a mean of 11.5 μg CN^-/L , a standard deviation of 0.73 μg CN^-/L , and an MDL of 2.3 μg CN^-/L .

c. *Precision study, total cyanide*: Seven injections of a 100.0- μg CN^-/L standard gave a relative standard deviation (RSD) of 1.0%.

d. *Precision study, WAD cyanide*: Ten injections of a 200.0- μg CN^-/L standard gave an

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RSD of 1.3%.

e. Recovery of total cyanide: Two injections each were made of solutions of potassium ferricyanide and potassium ferrocyanide, both at a concentration equivalent to 100 $\mu\text{g CN}^-/\text{L}$. Both gave an average recovery of 98%.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 12.11 amended June 30, 1986. 49 CFR 43430.

4500-Cl CHLORINE (RESIDUAL)*#(28)

4500-Cl A. Introduction

1. Effects of Chlorination

The chlorination of water supplies and polluted waters serves primarily to destroy or deactivate disease-producing microorganisms. A secondary benefit, particularly in treating drinking water, is the overall improvement in water quality resulting from the reaction of chlorine with ammonia, iron, manganese, sulfide, and some organic substances.

Chlorination may produce adverse effects. Taste and odor characteristics of phenols and other organic compounds present in a water supply may be intensified. Potentially carcinogenic chloroorganic compounds such as chloroform may be formed. Combined chlorine formed on chlorination of ammonia- or amine-bearing waters adversely affects some aquatic life. To fulfill the primary purpose of chlorination and to minimize any adverse effects, it is essential that proper testing procedures be used with a foreknowledge of the limitations of the analytical determination.

2. Chlorine Forms and Reactions

Chlorine applied to water in its molecular or hypochlorite form initially undergoes hydrolysis to form free chlorine consisting of aqueous molecular chlorine, hypochlorous acid, and hypochlorite ion. The relative proportion of these free chlorine forms is pH- and temperature-dependent. At the pH of most waters, hypochlorous acid and hypochlorite ion will predominate.

Free chlorine reacts readily with ammonia and certain nitrogenous compounds to form combined chlorine. With ammonia, chlorine reacts to form the chloramines: monochloramine, dichloramine, and nitrogen trichloride. The presence and concentrations of these combined forms depend chiefly on pH, temperature, initial chlorine-to-nitrogen ratio, absolute chlorine demand, and reaction time. Both free and combined chlorine may be present simultaneously. Combined

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chlorine in water supplies may be formed in the treatment of raw waters containing ammonia or by the addition of ammonia or ammonium salts. Chlorinated wastewater effluents, as well as certain chlorinated industrial effluents, normally contain only combined chlorine. Historically, the principal analytical problem has been to distinguish between free and combined forms of chlorine.

3. Selection of Method

In two separate but related studies, samples were prepared and distributed to participating laboratories to evaluate chlorine methods. Because of poor accuracy and precision and a high overall (average) total error in these studies, all orthotolidine procedures except one were dropped in the 14th edition of this work. The useful stabilized neutral orthotolidine method was deleted from the 15th edition because of the toxic nature of orthotolidine. The leuco crystal violet (LCV) procedure was dropped from the 17th edition because of its relative difficulty and the lack of comparative advantages.

a. Natural and treated waters: The iodometric methods (B and C) are suitable for measuring total chlorine concentrations greater than 1 mg/L, but the amperometric end point of Methods C and D gives greater sensitivity. All acidic iodometric methods suffer from interferences, generally in proportion to the quantity of potassium iodide (KI) and H^+ added.

The amperometric titration method (D) is a standard of comparison for the determination of free or combined chlorine. It is affected little by common oxidizing agents, temperature variations, turbidity, and color. The method is not as simple as the colorimetric methods and requires greater operator skill to obtain the best reliability. Loss of chlorine can occur because of rapid stirring in some commercial equipment. Clean and conditioned electrodes are necessary for sharp end points.

A low-level amperometric titration procedure (E) has been added to determine total chlorine at levels below 0.2 mg/L. This method is recommended only when quantification of such low residuals is necessary. The interferences are similar to those found with the standard amperometric procedure (D). The DPD methods (Methods F and G) are operationally simpler for determining free chlorine than the amperometric titration. Procedures are given for estimating the separate mono- and dichloramine and combined fractions. High concentrations of monochloramine interfere with the free chlorine determination unless the reaction is stopped with arsenite or thioacetamide. In addition, the DPD methods are subject to interference by oxidized forms of manganese unless compensated for by a blank.

The amperometric and DPD methods are unaffected by dichloramine concentrations in the range of 0 to 9 mg Cl as Cl_2/L in the determination of free chlorine. Nitrogen trichloride, if present, may react partially as free chlorine in the amperometric, DPD, and FACTS methods. The extent of this interference in the DPD methods does not appear to be significant.

The free chlorine test, syringaldazine (FACTS, Method H) was developed specifically for free chlorine. It is unaffected by significant concentrations of monochloramine, dichloramine, nitrate, nitrite, and oxidized forms of manganese.¹

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Sample color and turbidity may interfere in all colorimetric procedures.

Organic contaminants may produce a false free chlorine reading in most colorimetric methods (see ¶ 3b below). Many strong oxidizing agents interfere in the measurement of free chlorine in all methods. Such interferences include bromine, chlorine dioxide, iodine, permanganate, hydrogen peroxide, and ozone. However, the reduced forms of these compounds—bromide, chloride, iodide, manganous ion, and oxygen, in the absence of other oxidants, do not interfere. Reducing agents such as ferrous compounds, hydrogen sulfide, and oxidizable organic matter generally do not interfere.

b. Wastewaters: The determination of total chlorine in samples containing organic matter presents special problems. Because of the presence of ammonia, amines, and organic compounds, particularly organic nitrogen, residual chlorine exists in a combined state. A considerable residual may exist in this form, but at the same time there may be appreciable unsatisfied chlorine demand. Addition of reagents in the determination may change these relationships so that residual chlorine is lost during the analysis. Only the DPD method for total chlorine is performed under neutral pH conditions. In wastewater, the differentiation between free chlorine and combined chlorine ordinarily is not made because wastewater chlorination seldom is carried far enough to produce free chlorine.

The determination of residual chlorine in industrial wastes is similar to that in domestic wastewater when the waste contains organic matter, but may be similar to the determination in water when the waste is low in organic matter.

None of these methods is applicable to estuarine or marine waters because the bromide is converted to bromine and bromamines, which are detected as free or total chlorine. A procedure for estimating this interference is available for the DPD method.

Although the methods given below are useful for the determination of residual chlorine in wastewaters and treated effluents, select the method in accordance with sample composition. Some industrial wastes, or mixtures of wastes with domestic wastewater, may require special precautions and modifications to obtain satisfactory results.

Determine free chlorine in wastewater by any of the methods provided that known interfering substances are absent or appropriate correction techniques are used. The amperometric method is the method of choice because it is not subject to interference from color, turbidity, iron, manganese, or nitrite nitrogen. The DPD method is subject to interference from high concentrations of monochloramine, which is avoided by adding thioacetamide immediately after reagent addition. Oxidized forms of manganese at all levels encountered in water will interfere in all methods except in the free chlorine measurement of amperometric titrations and FACTS, but a blank correction for manganese can be made in Methods F and G.

The FACTS method is unaffected by concentrations of monochloramine, dichloramine, nitrite, iron, manganese, and other interfering compounds normally found in domestic wastewaters.

For total chlorine in samples containing significant amounts of organic matter, use either the DPD methods (F and G), amperometric, or iodometric back titration method (C) to prevent

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contact between the full concentration of liberated iodine and the sample. With Method C, do not use the starch-iodide end point if the concentration is less than 1 mg/L. In the absence of interference, the amperometric and starch-iodide end points give concordant results. The amperometric end point is inherently more sensitive and is free of interference from color and turbidity, which can cause difficulty with the starch-iodide end point. On the other hand, certain metals, surface-active agents, and complex anions in some industrial wastes interfere in the amperometric titration and indicate the need for another method for such wastewaters. Silver in the form of soluble silver cyanide complex, in concentrations of 1.0 mg Ag/L, poisons the cell at pH 4.0 but not at 7.0. The silver ion, in the absence of the cyanide complex, gives extensive response in the current at pH 4.0 and gradually poisons the cell at all pH levels. Cuprous copper in the soluble copper cyanide ion, in concentrations of 5 mg Cu/L or less, poisons the cell at pH 4.0 and 7.0. Although iron and nitrite may interfere with this method, minimize the interference by buffering to pH 4.0 before adding KI. Oxidized forms of manganese interfere in all methods for total chlorine including amperometric titration. An unusually high content of organic matter may cause uncertainty in the end point.

Regardless of end-point detection, either phenylarsine oxide or thiosulfate may be used as the standard reducing reagent at pH 4. The former is more stable and is preferred.

The DPD titrimetric and colorimetric methods (F and G, respectively) are applicable to determining total chlorine in polluted waters. In addition, both DPD procedures and the amperometric titration method allow for estimating monochloramine and dichloramine fractions. Because all methods for total chlorine depend on the stoichiometric production of iodine, waters containing iodine-reducing substances may not be analyzed accurately by these methods, especially where iodine remains in the solution for a significant time. This problem occurs in Methods B and D. The back titration procedure (C) and Methods F and G cause immediate reaction of the iodine generated so that it has little chance to react with other iodine-reducing substances.

In all colorimetric procedures, compensate for color and turbidity by using color and turbidity blanks.

A method (I) for total residual chlorine using a potentiometric iodide electrode is proposed. This method is suitable for analysis of chlorine residuals in natural and treated waters and wastewater effluents. No differentiation of free and combined chlorine is possible. This procedure is an adaptation of other iodometric techniques and is subject to the same inferences.

4. Sampling and Storage

Chlorine in aqueous solution is not stable, and the chlorine content of samples or solutions, particularly weak solutions, will decrease rapidly. Exposure to sunlight or other strong light or agitation will accelerate the reduction of chlorine. Therefore, start chlorine determinations immediately after sampling, avoiding excessive light and agitation. Do not store samples to be analyzed for chlorine.

5. Reference

Standard Methods for the Examination of Water and Wastewater

1. COOPER, W.J., N.M. ROSCHER & R.A. SLIFER. 1982. Determining free available chlorine by DPD-colorimetric, DPD-steadifac (colorimetric) and FACTS procedures. *J. Amer. Water Works Assoc.* 74:362.

6. Bibliography

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4500-Cl B. Iodometric Method I

1. General Discussion

a. Principle: Chlorine will liberate free iodine from potassium iodide (KI) solutions at pH 8 or less. The liberated iodine is titrated with a standard solution of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) with starch as the indicator. Titrate at pH 3 to 4 because the reaction is not stoichiometric at neutral pH due to partial oxidation of thiosulfate to sulfate.

b. Interference: Oxidized forms of manganese and other oxidizing agents interfere. Reducing agents such as organic sulfides also interfere. Although the neutral titration minimizes the interfering effect of ferric and nitrite ions, the acid titration is preferred because some forms of combined chlorine do not react at pH 7. Use only acetic acid for the acid titration; sulfuric acid (H_2SO_4) will increase interferences; *never use hydrochloric acid (HCl)*. See ¶ A.3 for discussion of other interferences.

c. Minimum detectable concentration: The minimum detectable concentration approximates 40 $\mu\text{g Cl as Cl}_2/\text{L}$ if 0.01N $\text{Na}_2\text{S}_2\text{O}_3$ is used with a 1000-mL sample. Concentrations below 1 mg/L cannot be determined accurately by the starch-iodide end point used in this method. Lower concentrations can be measured with the amperometric end point in Methods C and D.

2. Reagents

- a. Acetic acid*, conc (glacial).
- b. Potassium iodide*, KI, crystals.
- c. Standard sodium thiosulfate*, 0.1N: Dissolve 25 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in 1 L freshly boiled

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distilled water and standardize against potassium bi-iodate or potassium dichromate after at least 2 weeks storage. This initial storage is necessary to allow oxidation of any bisulfite ion present. Use boiled distilled water and add a few milliliters chloroform (CHCl_3) to minimize bacterial decomposition.

Standardize 0.1N $\text{Na}_2\text{S}_2\text{O}_3$ by one of the following:

1) Iodate method—Dissolve 3.249 g anhydrous potassium bi-iodate, $\text{KH}(\text{IO}_3)_2$, primary standard quality, or 3.567 g KIO_3 dried at $103 \pm 2^\circ\text{C}$ for 1 h, in distilled water and dilute to 1000 mL to yield a 0.1000N solution. Store in a glass-stoppered bottle.

To 80 mL distilled water, add, with constant stirring, 1 mL conc H_2SO_4 , 10.00 mL 0.1000N $\text{KH}(\text{IO}_3)_2$, and 1 g KI. Titrate immediately with 0.1N $\text{Na}_2\text{S}_2\text{O}_3$ titrant until the yellow color of the liberated iodine almost is discharged. Add 1 mL starch indicator solution and continue titrating until the blue color disappears.

2) Dichromate method—Dissolve 4.904 g anhydrous potassium dichromate, $\text{K}_2\text{Cr}_2\text{O}_7$, of primary standard quality, in distilled water and dilute to 1000 mL to yield a 0.1000N solution. Store in a glass-stoppered bottle.

Proceed as in the iodate method, with the following exceptions: Substitute 10.00 mL 0.1000N $\text{K}_2\text{Cr}_2\text{O}_7$ for iodate and let reaction mixture stand 6 min in the dark before titrating with 0.1N $\text{Na}_2\text{S}_2\text{O}_3$ titrant.

$$\text{Normality } \text{Na}_2\text{S}_2\text{O}_3 = \frac{1}{\text{mL } \text{Na}_2\text{S}_2\text{O}_3 \text{ consumed}}$$

d. Standard sodium thiosulfate titrant, 0.01N or 0.025N: Improve the stability of 0.01N or 0.025N $\text{Na}_2\text{S}_2\text{O}_3$ by diluting an aged 0.1N solution, made as directed above, with freshly boiled distilled water. Add 4 g sodium borate and 10 mg mercuric iodide/L solution. For accurate work, standardize this solution daily in accordance with the directions given above, using 0.01N or 0.025N iodate or $\text{K}_2\text{Cr}_2\text{O}_7$. Use sufficient volumes of these standard solutions so that their final dilution is not greater than 1 + 4. To speed up operations where many samples must be titrated use an automatic buret of a type in which rubber does not come in contact with the solution. Standard titrants, 0.0100N and 0.0250N, are equivalent, respectively, to 354.5 μg and 886.3 μg Cl_2 as Cl_2 /1.00 mL.

e. Starch indicator solution: To 5 g starch (potato, arrowroot, or soluble), add a little cold water and grind in a mortar to a thin paste. Pour into 1 L of boiling distilled water, stir, and let settle overnight. Use clear supernate. Preserve with 1.25 g salicylic acid, 4 g zinc chloride, or a combination of 4 g sodium propionate and 2 g sodium azide/L starch solution. Some commercial starch substitutes are satisfactory.

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f. *Standard iodine, 0.1N*: See ¶ C.3g.

g. *Dilute standard iodine, 0.0282N*: See ¶ C.3h.

3. Procedure

a. *Volume of sample*: Select a sample volume that will require no more than 20 mL 0.01N $\text{Na}_2\text{S}_2\text{O}_3$ and no less than 0.2 mL for the starch-iodide end point. For a chlorine range of 1 to 10 mg/L, use a 500-mL sample; above 10 mg/L, use proportionately less sample. Use smaller samples and volumes of titrant with the amperometric end point.

b. *Preparation for titration*: Place 5 mL acetic acid, or enough to reduce the pH to between 3.0 and 4.0, in a flask or white porcelain casserole. Add about 1 g KI estimated on a spatula. Pour sample in and mix with a stirring rod.

c. *Titration*: Titrate away from direct sunlight. Add 0.025N or 0.01N $\text{Na}_2\text{S}_2\text{O}_3$ from a buret until the yellow color of the liberated iodine almost is discharged. Add 1 mL starch solution and titrate until blue color is discharged.

If the titration is made with 0.025N $\text{Na}_2\text{S}_2\text{O}_3$ instead of 0.01N, then, with a 1-L sample, 1 drop is equivalent to about 50 $\mu\text{g/L}$. It is not possible to discern the end point with greater accuracy.

d. *Blank titration*: Correct result of sample titration by determining blank contributed by oxidizing or reducing reagent impurities. The blank also compensates for the concentration of iodine bound to starch at the end point.

Take a volume of distilled water corresponding to the sample used for titration in ¶s 3a–c, add 5 mL acetic acid, 1 g KI, and 1 mL starch solution. Perform blank titration as in 1) or 2) below, whichever applies.

1) If a blue color develops, titrate with 0.01N or 0.025N $\text{Na}_2\text{S}_2\text{O}_3$ to disappearance of blue color and record result. *B* (see ¶ 4, below) is negative.

2) If no blue color occurs, titrate with 0.0282N iodine solution until a blue color appears. Back-titrate with 0.01N or 0.025N $\text{Na}_2\text{S}_2\text{O}_3$ and record the difference. *B* is positive.

Before calculating the chlorine concentration, subtract the blank titration of ¶ 1) from the sample titration; or, if necessary, add the net equivalent value of the blank titration of ¶ 2).

4. Calculation

For standardizing chlorine solution for temporary standards:

$$\text{mg Cl as Cl}_2/\text{mL} = \frac{(A \pm B) \times N \times 35.45}{\text{mL sample}}$$

For determining total available residual chlorine in a water sample:

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$$\text{mg Cl as Cl}_2/\text{L} = \frac{(A \pm B) \times N \times 35\,450}{\text{mL sample}}$$

where:

A = mL titration for sample,

B = mL titration for blank (positive or negative), and

N = normality of $\text{Na}_2\text{S}_2\text{O}_3$.

5. Precision and Bias

Published studies^{1,2} give the results of nine methods used to analyze synthetic water samples without interferences; variations of some of the methods appear in this edition. More current data are not now available.

6. References

1. Water Chlorine (Residual) No. 1. 1969. Analytical Reference Service Rep. No. 35, U.S. Environmental Protection Agency, Cincinnati, Ohio.
2. Water Chlorine (Residual) No. 2. 1971. Analytical Reference Service Rep. No. 40, U.S. Environmental Protection Agency, Cincinnati, Ohio.

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4500-Cl C. Iodometric Method II

Standard Methods for the Examination of Water and Wastewater

1. General Discussion

a. Principle: In this method, used for wastewater analysis, the end-point signal is reversed because the unreacted standard reducing agent (phenylarsine oxide or thiosulfate) remaining in the sample is titrated with standard iodine or standard iodate, rather than the iodine released being titrated directly. This indirect procedure is necessary regardless of the method of end-point detection, to avoid contact between the full concentration of liberated iodine and the wastewater.

When iodate is used as a back titrant, use only phosphoric acid. Do not use acetate buffer.

b. Interference: Oxidized forms of manganese and other oxidizing agents give positive interferences. Reducing agents such as organic sulfides do not interfere as much as in Method B. Minimize iron and nitrite interference by buffering to pH 4.0 before adding potassium iodide (KI). An unusually high content of organic matter may cause some uncertainty in the end point. Whenever manganese, iron, and other interferences definitely are absent, reduce this uncertainty and improve precision by acidifying to pH 1.0. Control interference from more than 0.2 mg nitrite/L with phosphoric acid-sulfamic acid reagent. A larger fraction of organic chloramines will react at lower pH along with interfering substances. See ¶ A.3 for a discussion of other interferences.

2. Apparatus

For a description of the amperometric end-point detection apparatus and a discussion of its use, see ¶ D.2a.

3. Reagents

a. Standard phenylarsine oxide solution, 0.005 64N: Dissolve approximately 0.8 g phenylarsine oxide powder in 150 mL 0.3N NaOH solution. After settling, decant 110 mL into 800 mL distilled water and mix thoroughly. Bring to pH 6 to 7 with 6N HCl and dilute to 950 mL with distilled water. CAUTION: *Severe poison, cancer suspect agent.*

Standardization—Accurately measure 5 to 10 mL freshly standardized 0.0282N iodine solution into a flask and add 1 mL KI solution. Titrate with phenylarsine oxide solution, using the amperometric end point (Method D) or starch solution (see ¶ B.2e) as an indicator. Adjust to 0.005 64N and recheck against the standard iodine solution; 1.00 mL = 200 µg available chlorine. (CAUTION: *Toxic—take care to avoid ingestion.*)

b. Standard sodium thiosulfate solution, 0.1N: See ¶ B.2c.

c. Standard sodium thiosulfate solution, 0.005 64N: Prepare by diluting 0.1N Na₂S₂O₃. For maximum stability of the dilute solution, prepare by diluting an aged 0.1N solution with freshly boiled distilled water (to minimize bacterial action) and add 4 g Na₄B₄O₇/L. To inhibit mold formation optionally add either 10 mg HgI₂ or 2 drops toluene per liter of solution. Standardize daily as directed in B.2c using 0.005 64N K₂Cr₂O₇ or iodate solution. Use sufficient volume of sample so that the final dilution does not exceed 1 + 2. Use an automatic buret of a type in which rubber does not come in contact with the solution. 1.00 mL = 200 µg available chlorine.

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d. *Potassium iodide*, KI, crystals.

e. *Acetate buffer solution*, pH 4.0: Dissolve 146 g anhydrous $\text{NaC}_2\text{H}_3\text{O}_2$, or 243 g $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$, in 400 mL distilled water, add 480 g conc acetic acid, and dilute to 1 L with chlorine-demand-free water.

f. *Standard arsenite solution*, 0.1N: Accurately weigh a stoppered weighing bottle containing approximately 4.95 g arsenic trioxide, As_2O_3 . Transfer without loss to a 1-L volumetric flask and again weigh bottle. Do not attempt to brush out adhering oxide. Moisten As_2O_3 with water and add 15 g NaOH and 100 mL distilled water. Swirl flask contents gently to dissolve. Dilute to 250 mL with distilled water and saturate with CO_2 , thus converting all NaOH to NaHCO_3 . Dilute to mark, stopper, and mix thoroughly. This solution will preserve its titer almost indefinitely. (CAUTION: *Severe poison. Cancer suspect agent.*)

$$\text{Normality} = \frac{\text{g As}_2\text{O}_3}{49.455}$$

g. *Standard iodine solution*, 0.1N: Dissolve 40 g KI in 25 mL chlorine-demand-free water, add 13 g resublimed iodine, and stir until dissolved. Transfer to a 1-L volumetric flask and dilute to mark.

Standardization—Accurately measure 40 to 50 mL 0.1N arsenite solution into a flask and titrate with 0.1N iodine solution, using starch solution as indicator. To obtain accurate results, insure that the solution is saturated with CO_2 at end of titration by passing current of CO_2 through solution for a few minutes just before end point is reached, or add a few drops of HCl to liberate sufficient CO_2 to saturate solution. Alternatively standardize against $\text{Na}_2\text{S}_2\text{O}_3$; see ¶ B.2c1).

Optionally, prepare 0.1000N iodine solution directly as a standard solution by weighing 12.69 g primary standard resublimed iodine. Because I_2 may be volatilized and lose from both solid and solution, transfer the solid immediately to KI as specified above. Never let solution stand in open containers for extended periods.

h. *Standard iodine titrant*, 0.0282N: Dissolve 25 g KI in a little distilled water in a 1-L volumetric flask, add correct amount of 0.1N iodine solution exactly standardized to yield a 0.0282N solution, and dilute to 1 L with chlorine-demand-free water. For accurate work, standardize daily according to directions in ¶ 3g above, using 5 to 10 mL of arsenite or $\text{Na}_2\text{S}_2\text{O}_3$ solution. Store in amber bottles or in the dark; protect solution from direct sunlight at all times and keep from all contact with rubber.

i. *Starch indicator*: See ¶ B.2e.

j. *Standard iodate titrant*, 0.005 64N: Dissolve 201.2 mg primary standard grade KIO_3 , dried

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for 1 h at 103°C, or 183.3 mg primary standard anhydrous potassium bi-iodate in distilled water and dilute to 1 L.

k. Phosphoric acid solution, H_3PO_4 , 1 + 9.

l. Phosphoric acid-sulfamic acid solution: Dissolve 20 g $\text{NH}_2\text{SO}_3\text{H}$ in 1 L 1 + 9 phosphoric acid.

m. Chlorine-demand-free water: Prepare chlorine-demand-free water from good-quality distilled or deionized water by adding sufficient chlorine to give 5 mg/L free chlorine. After standing 2 d this solution should contain at least 2 mg/L free chlorine; if not, discard and obtain better-quality water. Remove remaining free chlorine by placing container in sunlight or irradiating with an ultraviolet lamp. After several hours take sample, add KI, and measure total chlorine with a colorimetric method using a nessler tube to increase sensitivity. Do not use before last trace of free and combined chlorine has been removed.

Distilled water commonly contains ammonia and also may contain reducing agents. Collect good-quality distilled or deionized water in a sealed container from which water can be drawn by gravity. To the air inlet of the container add an H_2SO_4 trap consisting of a large test tube half filled with 1 + 1 H_2SO_4 connected in series with a similar but empty test tube. Fit both test tubes with stoppers and inlet tubes terminating near the bottom of the tubes and outlet tubes terminating near the top of the tubes. Connect outlet tube of trap containing H_2SO_4 to the distilled water container, connect inlet tube to outlet of empty test tube. The empty test tube will prevent discharge to the atmosphere of H_2SO_4 due to temperature-induced pressure changes. Stored in such a container, chlorine-demand-free water is stable for several weeks unless bacterial growth occurs.

4. Procedure

a. Preparation for titration:

1) Volume of sample—For chlorine concentration of 10 mg/L or less, titrate 200 mL. For greater chlorine concentrations, use proportionately less sample and dilute to 200 mL with chlorine-demand-free water. Use a sample of such size that not more than 10 mL phenylarsine oxide solution is required.

2) Preparation for titration—Measure 5 mL 0.005 64N phenylarsine oxide or thiosulfate for chlorine concentrations from 2 to 5 mg/L, and 10 mL for concentrations of 5 to 10 mg/L, into a flask or casserole for titration with standard iodine or iodate. Start stirring. For titration by amperometry or standard iodine, also add excess KI (approximately 1 g) and 4 mL acetate buffer solution or enough to reduce the pH to between 3.5 and 4.2.

b. Titration: Use one of the following:

1) Amperometric titration—Add 0.0282N iodine titrant in small increments from a 1-mL buret or pipet. Observe meter needle response as iodine is added: the pointer remains practically stationary until the end point is approached, whereupon each iodine increment causes a

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temporary deflection of the microammeter, with the pointer dropping back to its original position. Stop titration at end point when a small increment of iodine titrant gives a definite pointer deflection upscale and the pointer does not return promptly to its original position. Record volume of iodine titrant used to reach end point.

2) Colorimetric (iodine) titration—Add 1 mL starch solution and titrate with 0.0282*N* iodine to the first appearance of blue color that persists after complete mixing.

3) Colorimetric (iodate) titration—To suitable flask or casserole add 200 mL chlorine-demand-free water and add, with agitation, the required volume of reductant, an excess of KI (approximately 0.5 g), 2 mL 10% H₃PO₄ solution, and 1 mL starch solution in the order given, and titrate immediately with 0.005 64*N* iodate solution to the first appearance of a blue color that persists after complete mixing. Designate volume of iodate solution used as *A*. Repeat procedure, substituting 200 mL sample for the 200 mL chlorine-demand-free water. If sample is colored or turbid, titrate to the first change in color, using for comparison another portion of sample with H₃PO₄ added. Designate this volume of iodate solution as *B*.

5. Calculation

a. Titration with standard iodine:

$$\text{mg Cl as Cl}_2/\text{L} = \frac{(A - 5B) \times 200}{C}$$

where:

A = mL 0.005 64*N* reductant,

B = mL 0.0282 *N* I₂, and

C = mL sample.

b. Titration with standard iodate:

$$\text{mg Cl as Cl}_2/\text{L} = \frac{(A - B) \times 200}{C}$$

where:

A = mL Na₂S₂O₃,

B = mL iodate required to titrate Na₂S₂O₃, and

C = mL sample.

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6. Bibliography

See Section 4500-Cl.B.7.

4500-Cl D. Amperometric Titration Method

1. General Discussion

Amperometric titration requires a higher degree of skill and care than the colorimetric methods. Chlorine residuals over 2 mg/L are measured best by means of smaller samples or by dilution with water that has neither residual chlorine nor a chlorine demand. The method can be used to determine total chlorine and can differentiate between free and combined chlorine. A further differentiation into monochloramine and dichloramine fractions is possible by control of KI concentration and pH.

a. Principle: The amperometric method is a special adaptation of the polarographic principle. Free chlorine is titrated at a pH between 6.5 and 7.5, a range in which the combined chlorine reacts slowly. The combined chlorine, in turn, is titrated in the presence of the proper amount of KI in the pH range 3.5 to 4.5. When free chlorine is determined, the pH must not be greater than 7.5 because the reaction becomes sluggish at higher pH values, nor less than 6.5 because at lower pH values some combined chlorine may react even in the absence of iodide. When combined chlorine is determined, the pH must not be less than 3.5 because of increased interferences at lower pH values, nor greater than 4.5 because the iodide reaction is not quantitative at higher pH values. The tendency of monochloramine to react more readily with iodide than does dichloramine provides a means for further differentiation. The addition of a small amount of KI in the neutral pH range enables estimation of monochloramine content. Lowering the pH into the acid range and increasing the KI concentration allows the separation determination of dichloramine.

Organic chloramines can be measured as free chlorine, monochloramine, or dichloramine, depending on the activity of chlorine in the organic compound.

Phenylarsine oxide is stable even in dilute solution and each mole reacts with two equivalents of halogen. A special amperometric cell is used to detect the end point of the residual chlorine-phenylarsine oxide titration. The cell consists of a nonpolarizable reference electrode that is immersed in a salt solution and a readily polarizable noble-metal electrode that is in contact with both the salt solution and the sample being titrated. In some applications, end-point selectivity is improved by adding +200 mV to the platinum electrode versus silver, silver chloride. Another approach to end-point detection uses dual platinum electrodes, a mercury cell with voltage divider to impress a potential across the electrodes, and a microammeter. If there is no chlorine residual in the sample, the microammeter reading will be comparatively low because of cell polarization. The greater the residual, the greater the microammeter reading. The meter acts merely as a null-point indicator—that is, the actual meter reading is not important, but rather the relative readings as the titration proceeds. The gradual addition of phenylarsine oxide causes

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the cell to become more and more polarized because of the decrease in chlorine. The end point is recognized when no further decrease in meter reading can be obtained by adding more phenylarsine oxide.

b. Interference: Accurate determinations of free chlorine cannot be made in the presence of nitrogen trichloride, NCl_3 , or chlorine dioxide, which titrate partly as free chlorine. When present, NCl_3 can titrate partly as free chlorine and partly as dichloramine, contributing a positive error in both fractions at a rate of approximately 0.1%/min. Some organic chloramines also can be titrated in each step. Monochloramine can intrude into the free chlorine fraction and dichloramine can interfere in the monochloramine fraction, especially at high temperatures and prolonged titration times. Free halogens other than chlorine also will titrate as free chlorine. Combined chlorine reacts with iodide ions to produce iodine. When titration for free chlorine follows a combined chlorine titration, which requires addition of KI, erroneous results may occur unless the measuring cell is rinsed thoroughly with distilled water between titrations.

Interference from copper has been noted in samples taken from copper pipe or after heavy copper sulfate treatment of reservoirs, with metallic copper plating out on the electrode. Silver ions also poison the electrode. Interference occurs in some highly colored waters and in waters containing surface-active agents. Very low temperatures slow response of measuring cell and longer time is required for the titration, but precision is not affected. A reduction in reaction rate is caused by pH values above 7.5; overcome this by buffering all samples to pH 7.0 or less. On the other hand, some substances, such as manganese, nitrite, and iron, do not interfere. The violent stirring of some commercial titrators can lower chlorine values by volatilization. When dilution is used for samples containing high chlorine content, take care that the dilution water is free of chlorine and ammonia and possesses no chlorine demand.

See ¶ A.3 for a discussion of other interferences.

2. Apparatus

a. End-point detection apparatus, consisting of a cell unit connected to a microammeter, with necessary electrical accessories. The cell unit includes a noble-metal electrode of sufficient surface area, a salt bridge to provide an electrical connection without diffusion of electrolyte, and a reference electrode of silver-silver chloride in a saturated sodium chloride solution connected into the circuit by means of the salt bridge. Numerous commercial systems are available.

Keep platinum electrode free of deposits and foreign matter. Vigorous chemical cleaning generally is unnecessary. Occasional mechanical cleaning with a suitable abrasive usually is sufficient. Keep salt bridge in good operating condition; do not allow it to become plugged nor permit appreciable flow of electrolyte through it. Keep solution surrounding reference electrode free of contamination and maintain it at constant composition by insuring an adequate supply of undissolved salt at all times. A cell with two metal electrodes polarized by a small DC potential also may be used. (See Bibliography.)

b. Agitator, designed to give adequate agitation at the noble-metal electrode surface to insure proper sensitivity. Thoroughly clean agitator and exposed electrode system to remove all

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chlorine-consuming contaminants by immersing them in water containing 1 to 2 mg/L free chlorine for a few minutes. Add KI to the same water and let agitator and electrodes remain immersed for 5 min. After thorough rinsing with chlorine-demand-free water or the sample to be tested, sensitized electrodes and agitator are ready for use. Remove iodide reagent completely from cell.

c. *Buret*: Commercial titrators usually are equipped with suitable burets (1 mL). Manual burets are available.*#(30)

d. *Glassware*, exposed to water containing at least 10 mg/L chlorine for 3 h or more before use and rinsed with chlorine-demand-free water.

3. Reagents

a. *Standard phenylarsine oxide titrant*: See ¶ C.3a.

b. *Phosphate buffer solution*, pH 7: Dissolve 25.4 g anhydrous KH_2PO_4 and 34.1 g anhydrous Na_2HPO_4 in 800 mL distilled water. Add 2 mL sodium hypochlorite solution containing 1% chlorine and mix thoroughly. Protect from sunlight for 2 d. Determine that free chlorine still remains in the solution. Then expose to sunlight until no chlorine remains. If necessary, carry out the final dechlorination with an ultraviolet lamp. Determine that no total chlorine remains by adding KI and measuring with one of the colorimetric tests. Dilute to 1 L with distilled water and filter if any precipitate is present.

c. *Potassium iodide solution*: Dissolve 50 g KI and dilute to 1 L with freshly boiled and cooled distilled water. Store in the dark in a brown glass-stoppered bottle, preferably in the refrigerator. Discard when solution becomes yellow.

d. *Acetate buffer solution*, pH 4: See ¶ C.3e.

4. Procedure

a. *Sample volume*: Select a sample volume requiring no more than 2 mL phenylarsine oxide titrant. Thus, for chlorine concentrations of 2 mg/L or less, take a 200-mL sample; for chlorine levels in excess of 2 mg/L, use 100 mL or proportionately less.

b. *Free chlorine*: Unless sample pH is known to be between 6.5 and 7.5, add 1 mL pH 7 phosphate buffer solution to produce a pH of 6.5 to 7.5. Titrate with standard phenylarsine oxide titrant, observing current changes on microammeter. Add titrant in progressively smaller increments until all needle movement ceases. Make successive buret readings when needle action becomes sluggish, signaling approach of end point. Subtract last very small increment that causes no needle response because of overtitration. Alternatively, use a system involving continuous current measurements and determine end point mathematically.

Continue titrating for combined chlorine as described in ¶ 4c below or for the separate monochloramine and dichloramine fractions as detailed in ¶s 4e and 4f.

c. *Combined chlorine*: To sample remaining from free-chlorine titration add 1.00 mL KI solution and 1 mL acetate buffer solution, in that order. Titrate with phenylarsine oxide titrant to

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the end point, as above. Do not refill buret but simply continue titration after recording figure for free chlorine. Again subtract last increment to give amount of titrant actually used in reaction with chlorine. (If titration was continued without refilling buret, this figure represents total chlorine. Subtracting free chlorine from total gives combined chlorine.) Wash apparatus and sample cell thoroughly to remove iodide ion to avoid inaccuracies when the titrator is used subsequently for a free chlorine determination.

d. Separate samples: If desired, determine total chlorine and free chlorine on separate samples. If sample pH is between 3.5 and 9.5 and total chlorine alone is required, treat sample immediately with 1 mL KI solution followed by 1 mL acetate buffer solution, and titrate with phenylarsine oxide titrant as described in ¶ 4c preceding.

e. Monochloramine: After titrating for free chlorine, add 0.2 mL KI solution to same sample and, without refilling buret, continue titration with phenylarsine oxide titrant to end point. Subtract last increment to obtain net volume of titrant consumed by monochloramine.

f. Dichloramine: Add 1 mL acetate buffer solution and 1 mL KI solution to same sample and titrate final dichloramine fraction as described above.

5. Calculation

Convert individual titrations for free chlorine, combined chlorine, total chlorine, monochloramine, and dichloramine by the following equation:

$$\text{mg Cl as Cl}_2/\text{L} = \frac{A \times 200}{\text{mL sample}}$$

where:

A = mL phenylarsine oxide titration.

6. Precision and Bias

See ¶ B.5.

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4500-Cl E. Low-Level Amperometric Titration Method

1. General Discussion

Detection and quantification of chlorine residuals below 0.2 mg/L require special modifications to the amperometric titration procedure. With these modifications chlorine concentrations at the 10-μg/L level can be measured. It is not possible to differentiate between free and combined chlorine forms. Oxidizing agents that interfere with the amperometric titration method (D) will interfere.

a. Principle: This method modifies D by using a more dilute titrant and a graphical procedure to determine the end point.

b. Interference: See ¶ D.1b.

2. Apparatus

See ¶ D.2.

3. Reagents

a. Potassium bi-iodate, 0.002 256N: Dissolve 0.7332 g anhydrous potassium bi-iodate, $\text{KH}(\text{IO}_3)_2$, in 500 mL chlorine-free distilled water and dilute to 1000 mL. Dilute 10.00 mL to 100.0 mL with chlorine-free distilled water. Use only freshly prepared solution for the standardization of phenylarsine oxide.

b. Potassium iodide, KI crystals.

c. Low-strength phenylarsine oxide titrant, 0.000 564N: Dilute 10.00 mL of 0.005 64N phenylarsine oxide (see C.3a) to 100.0 mL with chlorine-demand-free water (see C.3m).

Standardization—Dilute 5.00 mL 0.002 256N potassium bi-iodate to 200 mL with chlorine-free water. Add approximately 1.5 g KI and stir to dissolve. Add 1 mL acetate buffer and let stand in the dark for 6 min. Titrate using the amperometric titrator and determine the equivalence point as indicated below.

$$\text{Normality} = 0.002256 \times 5/A$$

where:

A = mL phenylarsine oxide titrant required to reach the equivalence point of standard bi-iodate.

d. Acetate buffer solution, pH 4: See ¶ C.3e.

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4. Procedure

Select a sample volume requiring no more than 2 mL phenylarsine oxide titrant. A 200-mL sample will be adequate for samples containing less than 0.2 mg total chlorine/L.

Before beginning titration, rinse buret with titrant several times. Rinse sample container with distilled water and then with sample. Add 200 mL sample to sample container and approximately 1.5 g KI. Dissolve, using a stirrer or mixer. Add 1 mL acetate buffer and place container in end-point detection apparatus. When the current signal stabilizes, record the reading. Initially adjust meter to a near full-scale deflection. Titrate by adding small, known, volumes of titrant. After each addition, record cumulative volume added and current reading when the signal stabilizes. If meter reading falls to near or below 10% of full-scale deflection, record low reading, readjust meter to near full-scale deflection, and record difference between low amount and readjusted high deflection. Add this value to all deflection readings for subsequent titrant additions. Continue adding titrant until no further meter deflection occurs. If fewer than three titrant additions were made before meter deflection ceased, discard sample and repeat analysis using smaller titrant increments.

Determine equivalence point by plotting total meter deflection against titrant volume added. Draw straight line through the first several points in the plot and a second, horizontal straight line corresponding to the final total deflection in the meter. Read equivalence point as the volume of titrant added at the intersection of these two lines.

5. Calculation

$$\text{mg Cl as Cl}_2/\text{L} = \frac{A \times 200 \times N}{B \times 0.00564}$$

where:

A = mL titrant at equivalence point,

B = sample volume, mL, and

N = phenylarsine oxide normality.

6. Bibliography

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4500-Cl F. DPD Ferrous Titrimetric Method

1. General Discussion

a. Principle: *N,N*-diethyl-*p*-phenylenediamine (DPD) is used as an indicator in the titrimetric procedure with ferrous ammonium sulfate (FAS). Where complete differentiation of chlorine

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species is not required, the procedure may be simplified to give only free and combined chlorine or total chlorine.

In the absence of iodide ion, free chlorine reacts instantly with DPD indicator to produce a red color. Subsequent addition of a small amount of iodide ion acts catalytically to cause monochloramine to produce color. Addition of iodide ion to excess evokes a rapid response from dichloramine. In the presence of iodide ion, part of the nitrogen trichloride (NCl_3) is included with dichloramine and part with free chlorine. A supplementary procedure based on adding iodide ion before DPD permits estimating proportion of NCl_3 appearing with free chlorine.

Chlorine dioxide (ClO_2) appears, to the extent of one-fifth of its total chlorine content, with free chlorine. A full response from ClO_2 , corresponding to its total chlorine content, may be obtained if the sample first is acidified in the presence of iodide ion and subsequently is brought back to an approximately neutral pH by adding bicarbonate ion. Bromine, bromamine, and iodine react with DPD indicator and appear with free chlorine.

Addition of glycine before determination of free chlorine converts free chlorine to unreactive forms, with only bromine and iodine residuals remaining. Subtractions of these residuals from the residual measured without glycine permits differentiation of free chlorine from bromine and iodine.

b. pH control: For accurate results careful pH control is essential. At the proper pH of 6.2 to 6.5, the red colors produced may be titrated to sharp colorless end points. *Titrate as soon as the red color is formed in each step.* Too low a pH in the first step tends to make the monochloramine show in the free-chlorine step and the dichloramine in the monochloramine step. Too high a pH causes dissolved oxygen to give a color.

c. Temperature control: In all methods for differentiating free chlorine from chloramines, higher temperatures increase the tendency for chloramines to react and lead to increased apparent free-chlorine results. Higher temperatures also increase color fading. Complete measurements rapidly, especially at higher temperature.

d. Interference: The most significant interfering substance likely to be encountered in water is oxidized manganese. To correct for this, place 5 mL buffer solution and 0.5 mL sodium arsenite solution in the titration flask. Add 100 mL sample and mix. Add 5 mL DPD indicator solution, mix, and titrate with standard FAS titrant until red color is discharged. Subtract reading from Reading A obtained by the normal procedure as described in ¶ 3a1) of this method or from the total chlorine reading obtained in the simplified procedure given in ¶ 3a4). If the combined reagent in powder form (see below) is used, first add KI and arsenite to the sample and mix, then add combined buffer-indicator reagent.

As an alternative to sodium arsenite use a 0.25% solution of thioacetamide, adding 0.5 mL to 100 mL sample.

Interference by copper up to approximately 10 mg Cu/L is overcome by the EDTA incorporated in the reagents. EDTA enhances stability of DPD indicator solution by retarding deterioration due to oxidation, and in the test itself, provides suppression of dissolved oxygen

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errors by preventing trace metal catalysis.

Chromate in excess of 2 mg/L interferes with end-point determination. Add barium chloride to mask this interference by precipitation.

High concentrations of combined chlorine can break through into the free chlorine fraction. *If free chlorine is to be measured in the presence of more than 0.5 mg/L combined chlorine, use the thioacetamide modification.* If this modification is not used, a color-development time in excess of 1 min leads to progressively greater interference from monochloramine. Adding thioacetamide (0.5 mL 0.25% solution to 100 mL) immediately after mixing DPD reagent with sample completely stops further reaction with combined chlorine in the free chlorine measurement. Continue immediately with FAS titration to obtain free chlorine. Obtain total chlorine from the normal procedure, i.e., without thioacetamide.

Because high concentrations of iodide are used to measure combined chlorine and only traces of iodide greatly increase chloramine interference in free chlorine measurements, take care to avoid iodide contamination by rinsing between samples or using separate glassware.

See ¶ A.3 for a discussion of other interferences.

e. Minimum detectable concentration: Approximately 18 µg Cl as Cl₂/L. This detection limit is achievable under ideal conditions; normal working detection limits typically are higher.

2. Reagents

a. Phosphate buffer solution: Dissolve 24 g anhydrous Na₂HPO₄ and 46 g anhydrous KH₂PO₄ in distilled water. Combine with 100 mL distilled water in which 800 mg disodium ethylenediamine tetraacetate dihydrate (EDTA) have been dissolved. Dilute to 1 L with distilled water and optionally add either 20 mg HgCl₂ or 2 drops toluene to prevent mold growth. Interference from trace amounts of iodide in the reagents can be negated by optional addition of 20 mg HgCl₂ to the solution. (CAUTION: HgCl₂ is toxic—take care to avoid ingestion.)

b. N,N-Diethyl-p-phenylenediamine (DPD) indicator solution: Dissolve 1 g DPD oxalate, *#(31) or 1.5 g DPD sulfate pentahydrate, †#(32) or 1.1 g anhydrous DPD sulfate in chlorine-free distilled water containing 8 mL 1 + 3 H₂SO₄ and 200 mg disodium EDTA. Make up to 1 L, store in a brown glass-stoppered bottle in the dark, and discard when discolored. Periodically check solution blank for absorbance and discard when absorbance at 515 nm exceeds 0.002/cm. (The buffer and indicator sulfate are available commercially as a combined reagent in stable powder form.) CAUTION: *The oxalate is toxic—take care to avoid ingestion.*

c. Standard ferrous ammonium sulfate (FAS) titrant: Dissolve 1.106 g Fe(NH₄)₂(SO₄)₂·6H₂O in distilled water containing 1 mL 1 + 3 H₂SO₄ and make up to 1 L with freshly boiled and cooled distilled water. This standard may be used for 1 month, and the titer checked by potassium dichromate. For this purpose add 10 mL 1 + 5 H₂SO₄, 5 mL conc H₃PO₄, and 2 mL 0.1% barium diphenylamine sulfonate indicator to a 100-mL sample of FAS and titrate with potassium dichromate to a violet end point that persists for 30 s. FAS titrant equivalent to

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100 µg Cl as Cl_2 /1.00 mL requires 20.00 mL dichromate for titration.

d. *Potassium iodide*, KI, crystals.

e. *Potassium iodide solution*: Dissolve 500 mg KI and dilute to 100 mL, using freshly boiled and cooled distilled water. Store in a brown glass-stoppered bottle, preferably in a refrigerator. Discard when solution becomes yellow.

f. *Potassium dichromate solution*, 0.691 g to 1000 mL.

g. *Barium diphenylaminesulfonate*, 0.1%: Dissolve 0.1 g $(\text{C}_6\text{H}_5\text{NHC}_6\text{H}_4\text{-4-SO}_3)_2\text{Ba}$ in 100 mL distilled water.

h. *Sodium arsenite solution*: Dissolve 5.0 g NaAsO_2 in distilled water and dilute to 1 L.
(CAUTION: *Toxic—take care to avoid ingestion.*)

i. *Thioacetamide solution*: Dissolve 250 mg CH_3CSNH_2 in 100 mL distilled water.
(CAUTION: *Cancer suspect agent. Take care to avoid skin contact or ingestion.*)

j. *Chlorine-demand-free water*: See ¶ C.3m.

k. *Glycine solution*: Dissolve 20 g glycine (aminoacetic acid) in sufficient chlorine-demand-free water to bring to 100 mL total volume. Store under refrigerated conditions and discard if cloudiness develops.

l. *Barium chloride crystals*, $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$.

3. Procedure

The quantities given below are suitable for concentrations of total chlorine up to 5 mg/L. If total chlorine exceeds 5 mg/L, use a smaller sample and dilute to a total volume of 100 mL. Mix usual volumes of buffer reagent and DPD indicator solution, or usual amount of DPD powder, with distilled water *before* adding sufficient sample to bring total volume to 100 mL. (If sample is added before buffer, test does not work.)

If chromate is present (>2 mg/L) add and mix 0.2 g $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ /100 mL sample before adding other reagents. If, in addition, sulfate is >500 mg/L, use 0.4 g $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ /100 mL sample.

a. *Free chlorine or chloramine*: Place 5 mL each of buffer reagent and DPD indicator solution in titration flask and mix (or use about 500 mg DPD powder). Add 100 mL sample, or diluted sample, and mix.

1) Free chlorine—Titrate rapidly with standard FAS titrant until red color is discharged (Reading A).

2) Monochloramine—Add one very small crystal of KI (about 0.5 mg) or 0.1 mL (2 drops) KI solution and mix. Continue titrating until red color is discharged again (Reading B).

3) Dichloramine—Add several crystals KI (about 1 g) and mix to dissolve. Let stand for 2 min and continue titrating until red color is discharged (Reading C). For dichloramine

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concentrations greater than 1 mg/L, let stand 2 min more if color driftback indicates slightly incomplete reaction. When dichloramine concentrations are not expected to be high, use half the specified amount of KI.

4) Simplified procedure for free and combined chlorine or total chlorine—Omit 2) above to obtain monochloramine and dichloramine together as combined chlorine. To obtain total chlorine in one reading, add full amount of KI at the start, with the specified amounts of buffer reagent and DPD indicator, and titrate after 2 min standing.

b. Nitrogen trichloride: Place one very small crystal of KI (about 0.5 mg) or 0.1 mL KI solution in a titration flask. Add 100 mL sample and mix. Add contents to a second flask containing 5 mL each of buffer reagent and DPD indicator solution (or add about 500 mg DPD powder direct to the first flask). Titrate rapidly with standard FAS titrant until red color is discharged (Reading *N*).

c. Free chlorine in presence of bromine or iodine: Determine free chlorine as in ¶ 3a1). To a second 100-mL sample, add 1 mL glycine solution before adding DPD and buffer. Titrate according to ¶ 3a1). Subtract the second reading from the first to obtain Reading *A*.

4. Calculation

For a 100-mL sample, 1.00 mL standard FAS titrant = 1.00 mg Cl as Cl_2/L .

Reading	NCl_3 Absent	NCl_3 Present
<i>A</i>	Free Cl	Free Cl
<i>B – A</i>	NH_2Cl	NH_2Cl
<i>C – B</i>	NHCl_2	$\text{NHCl}_2 + \frac{1}{2}\text{NCl}_3$
<i>N</i>	—	Free Cl + $\frac{1}{2}\text{NCl}_3$
$2(N – A)$	—	NCl_3
<i>C – N</i>	—	NHCl_2

In the event that monochloramine is present with NCl_3 , it will be included in *N*, in which case obtain NCl_3 from $2(N – B)$.

Chlorine dioxide, if present, is included in *A* to the extent of one-fifth of its total chlorine content.

In the simplified procedure for free and combined chlorine, only *A* (free Cl) and *C* (total Cl) are required. Obtain combined chlorine from $C – A$.

The result obtained in the simplified total chlorine procedure corresponds to *C*.

5. Precision and Bias

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See ¶ B.5.

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4500-Cl G. DPD Colorimetric Method

1. General Discussion

a. Principle: This is a colorimetric version of the DPD method and is based on the same principles. Instead of titration with standard ferrous ammonium sulfate (FAS) solution as in the titrimetric method, a colorimetric procedure is used.

b. Interference: See ¶ A.3 and ¶ F.1d. Compensate for color and turbidity by using sample to zero photometer. Minimize chromate interference by using the thioacetamide blank correction.

c. Minimum detectable concentration: Approximately 10 µg Cl as Cl₂/L. This detection limit is achievable under ideal conditions; normal working detection limits typically are higher.

2. Apparatus

a. Photometric equipment: One of the following is required:

1) *Spectrophotometer*, for use at a wavelength of 515 nm and providing a light path of 1 cm or longer.

2) *Filter photometer*, equipped with a filter having maximum transmission in the wavelength

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range of 490 to 530 nm and providing a light path of 1 cm or longer.

b. Glassware: Use separate glassware, including separate spectrophotometer cells, for free and combined (dichloramine) measurements, to avoid iodide contamination in free chlorine measurement.

3. Reagents

See ¶s F.2a, b, c, d, e, h, i, and j.

4. Procedure

a. Calibration of photometric equipment: Calibrate instrument with chlorine or potassium permanganate solutions.

1) Chlorine solutions—Prepare chlorine standards in the range of 0.05 to 4 mg/L from about 100 mg/L chlorine water standardized as follows: Place 2 mL acetic acid and 10 to 25 mL chlorine-demand-free water in a flask. Add about 1 g KI. Measure into the flask a suitable volume of chlorine solution. In choosing a convenient volume, note that 1 mL 0.025N $\text{Na}_2\text{S}_2\text{O}_3$ titrant (see ¶ B.2d) is equivalent to about 0.9 mg chlorine. Titrate with standardized 0.025N $\text{Na}_2\text{S}_2\text{O}_3$ titrant until the yellow iodine color almost disappears. Add 1 to 2 mL starch indicator solution and continue titrating to disappearance of blue color.

Determine the blank by adding identical quantities of acid, KI, and starch indicator to a volume of chlorine-demand-free water corresponding to the sample used for titration. Perform blank titration A or B, whichever applies, according to ¶ B.3d.

$$\text{mg Cl as Cl}_2/\text{mL} = \frac{(A + B) \times N \times 35.45}{\text{mL sample}}$$

where:

N = normality of $\text{Na}_2\text{S}_2\text{O}_3$,

A = mL titrant for sample,

B = mL titrant for blank (to be added or subtracted according to required blank titration. See B.3d).

Use chlorine-demand-free water and glassware to prepare these standards. Develop color by first placing 5 mL phosphate buffer solution and 5 mL DPD indicator reagent in flask and then adding 100 mL chlorine standard with thorough mixing as described in *b* and *c* below. Fill photometer or colorimeter cell from flask and read color at 515 nm. Return cell contents to flask and titrate with standard FAS titrant as a check on chlorine concentration.

2) Potassium permanganate solutions—Prepare a stock solution containing 891 mg KMnO_4 /1000 mL. Dilute 10.00 mL stock solution to 100 mL with distilled water in a volumetric flask. When 1 mL of this solution is diluted to 100 mL with distilled water, a chlorine equivalent

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of 1.00 mg/L will be produced in the DPD reaction. Prepare a series of KMnO_4 standards covering the chlorine equivalent range of 0.05 to 4 mg/L. Develop color by first placing 5 mL phosphate buffer and 5 mL DPD indicator reagent in flask and adding 100 mL standard with thorough mixing as described in *b* and *c* below. Fill photometer or colorimeter cell from flask and read color at 515 nm. Return cell contents to flask and titrate with FAS titrant as a check on any absorption of permanganate by distilled water.

Obtain all readings by comparison to color standards or the standard curve before use in calculation.

b. Volume of sample: Use a sample volume appropriate to the photometer or colorimeter. The following procedure is based on using 10-mL volumes; adjust reagent quantities proportionately for other sample volumes. Dilute sample with chlorine-demand-free water when total chlorine exceeds 4 mg/L.

c. Free chlorine: Place 0.5 mL each of buffer reagent and DPD indicator reagent in a test tube or photometer cell. Add 10 mL sample and mix. Read color immediately (Reading A).

d. Monochloramine: Continue by adding one very small crystal of KI (about 0.1 mg) and mix. If dichloramine concentration is expected to be high, instead of small crystal add 0.1 mL (2 drops) freshly prepared KI solution (0.1 g/100 mL). Read color immediately (Reading B).

e. Dichloramine: Continue by adding several crystals of KI (about 0.1 g) and mix to dissolve. Let stand about 2 min and read color (Reading C).

f. Nitrogen trichloride: Place a very small crystal of KI (about 0.1 mg) in a clean test tube or photometer cell. Add 10 mL sample and mix. To a second tube or cell add 0.5 mL each of buffer and indicator reagents; mix. Add contents to first tube or cell and mix. Read color immediately (Reading N).

g. Chromate correction using thioacetamide: Add 0.5 mL thioacetamide solution (F.2i) to 100 mL sample. After mixing, add buffer and DPD reagent. Read color immediately. Add several crystals of KI (about 0.1 g) and mix to dissolve. Let stand about 2 min and read color. Subtract the first reading from Reading A and the second reading from Reading C and use in calculations.

h. Simplified procedure for total chlorine: Omit Step *d* above to obtain monochloramine and dichloramine together as combined chlorine. To obtain total chlorine in one reading, add the full amount of KI at the start, with the specified amounts of buffer reagent and DPD indicator. Read color after 2 min.

5. Calculation

Reading	NCl_3 Absent	NCl_3 Present
A	Free Cl	Free Cl
B – A	NH_2Cl	NH_2Cl

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Reading	NCl_3 Absent	NCl_3 Present
$C - B$	NHCl_2	$\text{NHCl}_2 + \frac{1}{2}\text{NCl}_3$
N	—	Free Cl + $\frac{1}{2}\text{NCl}_3$
$2(N - A)$	—	NCl_3
$C - N$	—	NHCl_2

In the event that monochloramine is present with NCl_3 , it will be included in Reading N , in which case obtain NCl_3 from $2(N-B)$.

6. Bibliography

See ¶ F.6.

4500-Cl H. Syringaldazine (FACTS) Method

1. General Discussion

a. Principle: The free (available) chlorine test, syringaldazine (FACTS) measures free chlorine over the range of 0.1 to 10 mg/L. A saturated solution of syringaldazine (3,5-dimethoxy-4-hydroxybenzaldazine) in 2-propanol is used. Syringaldazine is oxidized by free chlorine on a 1:1 molar basis to produce a colored product with an absorption maximum of 530 nm. The color product is only slightly soluble in water; therefore, at chlorine concentrations greater than 1 mg/L, the final reaction mixture must contain 2-propanol to prevent product precipitation and color fading.

The optimum color and solubility (minimum fading) are obtained in a solution having a pH between 6.5 and 6.8. At a pH less than 6, color development is slow and reproducibility is poor. At a pH greater than 7, the color develops rapidly but fades quickly. A buffer is required to maintain the reaction mixture pH at approximately 6.7. Take care with waters of high acidity or alkalinity to assure that the added buffer maintains the proper pH.

Temperature has a minimal effect on the color reaction. The maximum error observed at temperature extremes of 5 and 35°C is $\pm 10\%$.

b. Interferences: Interferences common to other methods for determining free chlorine do not affect the FACTS procedure. Monochloramine concentrations up to 18 mg/L, dichloramine concentrations up to 10 mg/L, and manganese concentrations (oxidized forms) up to 1 mg/L do not interfere. Trichloramine at levels above 0.6 mg/L produces an apparent free chlorine reaction. Very high concentrations of monochloramine (≥ 35 mg/L) and oxidized manganese (≥ 2.6 mg/L) produce a color with syringaldazine slowly. Ferric iron can react with syringaldazine; however, concentrations up to 10 mg/L do not interfere. Nitrite (≤ 250 mg/L), nitrate (≤ 100 mg/L), sulfate (≤ 1000 mg/L), and chloride (≤ 1000 mg/L) do not interfere. Waters

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with high hardness (≥ 500 mg/L) will produce a cloudy solution that can be compensated for by using a blank. Oxygen does not interfere.

Other strong oxidizing agents, such as iodine, bromine, and ozone, will produce a color.

c. Minimum detectable concentration: The FACTS procedure is sensitive to free chlorine concentrations of 0.1 mg/L or less.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Filter photometer, providing a light path of 1 cm for chlorine concentrations ≤ 1 mg/L or a light path from 1 to 10 mm for chlorine concentrations above 1 mg/L; also equipped with a filter having a band pass of 500 to 560 nm.

b. Spectrophotometer, for use at 530 nm, providing the light paths noted above.

3. Reagents

a. Chlorine-demand-free water: See C.3m. Use to prepare reagent solutions and sample dilutions.

b. Syringaldazine indicator: Dissolve 115 mg 3,5-dimethoxy-4-hydroxybenzaldazine*#(33) in 1 L 2-propanol.

c. 2-Propanol: To aid in dissolution use ultrasonic agitation or gentle heating and stirring. Redistill reagent-grade 2-propanol to remove chlorine demand. Use a 30.5-cm Vigreux column and take the middle 75% fraction. Alternatively, chlorinate good-quality 2-propanol to maintain a free residual overnight; then expose to UV light or sunlight to dechlorinate. CAUTION: 2-Propanol is extremely flammable.

d. Buffer: Dissolve 17.01 g KH_2PO_4 in 250 mL water; pH should be 4.4. Dissolve 17.75 g Na_2HPO_4 in 250 mL water; the pH should be 9.9. Mix equal volumes of these solutions to obtain FACTS buffer, pH 6.6. Verify pH with pH meter. For waters containing considerable hardness or high alkalinity other pH 6.6 buffers can be used, for example, 23.21 g maleic acid and 16.5 mL 50% NaOH per liter of water.

e. Hypochlorite solution: Dilute household hypochlorite solution, which contains about 30 000 to 50 000 mg Cl equivalent/L, to a strength between 100 and 1000 mg/L. Standardize as directed in ¶ G.4a1).

4. Procedure

a. Calibration of photometer: Prepare a calibration curve by making dilutions of a standardized hypochlorite solution (¶ 3e). Develop and measure colors as described in ¶ 4b, below. Check calibration regularly, especially as reagent ages.

b. Free chlorine analysis: Add 3 mL sample and 0.1 mL buffer to a 5-mL-capacity test tube. Add 1 mL syringaldazine indicator, cap tube, and invert twice to mix. Transfer to a photometer tube or spectrophotometer cell and measure absorbance. Compare absorbance value obtained

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with calibration curve and report corresponding value as milligrams free chlorine per liter.

5. Bibliography

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4500-Cl I. Iodometric Electrode Technique

1. General Discussion

a. Principle: This method involves the direct potentiometric measurement of iodine released on the addition of potassium iodide to an acidified sample. A platinum-iodide electrode pair is used in combination with an expanded-scale pH meter.

b. Interference: All oxidizing agents that interfere with other iodometric procedures interfere. These include oxidized manganese and iodate, bromine, and cupric ions. Silver and mercuric ions above 10 and 20 mg/L interfere.

2. Apparatus

a. Electrodes: Use either a combination electrode consisting of a platinum electrode and an iodide ion-selective electrode or two individual electrodes. Both systems are available commercially.

b. pH/millivolt meter: Use an expanded-scale pH/millivolt meter with 0.1 mV readability or a direct-reading selective ion meter.

3. Reagents

a. pH 4 buffer solution: See ¶ C.3e.

b. Chlorine-demand-free water: See ¶ C.3m.

c. Potassium iodide solution: Dissolve 42 g KI and 0.2 g Na₂CO₃ in 500 mL chlorine-demand-free, distilled water. Store in a dark bottle.

d. Standard potassium iodate 0.002 81N: Dissolve 0.1002 g KIO₃ in chlorine-demand-free, distilled water and dilute to 1000 mL. Each 1.0 mL, when diluted to 100 mL, produces a solution equivalent to 1 mg/L as Cl₂.

4. Procedure

a. Standardization: Pipet into three 100-mL stoppered volumetric flasks 0.20, 1.00, and 5.00

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mL standard iodate solution. Add to each flask, and a fourth flask to be used as a reagent blank, 1 mL each of acetate buffer solution and KI solution. Stopper, swirl to mix, and let stand 2 min before dilution. Dilute each standard to 100 mL with chlorine-demand-free distilled water. Stopper, invert flask several times to mix, and pour into separate 150-mL beakers. Stir gently without turbulence, using a magnetic stirrer, and immerse electrode(s) in the 0.2-mg/L (0.2-mL) standard. Wait for the potential to stabilize and record potential in mV. Rinse electrodes with chlorine-demand-free water and repeat for each standard and for the reagent blank. Prepare a calibration curve by plotting, on semilogarithmic paper, potential (linear axis) against concentration. Determine apparent chlorine concentration in the reagent blank from this graph (Reading B).

b. Analysis: Select a volume of sample containing no more than 0.5 mg chlorine. Pipet 1 mL acetate buffer solution and 1 mL KI into a 100-mL glass-stoppered volumetric flask. Stopper, swirl and let stand for at least 2 min. Adjust sample pH to 4 to 5, if necessary (mid-range pH paper is adequate for pH measurement), by adding acetic acid. Add pH-adjusted sample to volumetric flask and dilute to mark. Stopper and mix by inversion several times. Let stand for 2 min. Pour into a 150-mL beaker, immerse the electrode(s), wait for the potential to stabilize, and record. If the mV reading is greater than that recorded for the 5-mg/L standard, repeat analysis with a smaller volume of sample.

5. Calculation

Determine chlorine concentration (mg/L) corresponding to the recorded mV reading from the standard curve. This is Reading A. Determine total residual chlorine from the following:

$$\text{Total residual chlorine} = A \times 100/V$$

where V = sample volume, mL. If total residual chlorine is below 0.2 mg/L, subtract apparent chlorine in reagent blank (Reading B) to obtain the true total residual chlorine value.

6. Bibliography

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4500-Cl⁻ CHLORIDE*#(34)

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4500-Cl⁻ A. Introduction

1. Occurrence

Chloride, in the form of chloride (Cl⁻) ion, is one of the major inorganic anions in water and wastewater. The salty taste produced by chloride concentrations is variable and dependent on the chemical composition of water. Some waters containing 250 mg Cl⁻/L may have a detectable salty taste if the cation is sodium. On the other hand, the typical salty taste may be absent in waters containing as much as 1000 mg/L when the predominant cations are calcium and magnesium.

The chloride concentration is higher in wastewater than in raw water because sodium chloride (NaCl) is a common article of diet and passes unchanged through the digestive system. Along the sea coast, chloride may be present in high concentrations because of leakage of salt water into the sewerage system. It also may be increased by industrial processes.

A high chloride content may harm metallic pipes and structures, as well as growing plants.

2. Selection of Method

Six methods are presented for the determination of chloride. Because the first two are similar in most respects, selection is largely a matter of personal preference. The argentometric method (B) is suitable for use in relatively clear waters when 0.15 to 10 mg Cl⁻ are present in the portion titrated. The end point of the mercuric nitrate method (C) is easier to detect. The potentiometric method (D) is suitable for colored or turbid samples in which color-indicated end points might be difficult to observe. The potentiometric method can be used without a pretreatment step for samples containing ferric ions (if not present in an amount greater than the chloride concentration), chromic, phosphate, and ferrous and other heavy-metal ions. The ferricyanide method (E) is an automated technique. Flow injection analysis (G), an automated colorimetric technique, is useful for analyzing large numbers of samples. Preferably determine chloride by ion chromatography (Section 4110). Chloride also can be determined by the capillary ion electrophoresis method (Section 4140). Methods (C and G) in which mercury, a highly toxic reagent, is used require special disposal practices to avoid improper sewage discharges. Follow appropriate regulatory procedures (see Section 1090).

3. Sampling and Storage

Collect representative samples in clean, chemically resistant glass or plastic bottles. The maximum sample portion required is 100 mL. No special preservative is necessary if the sample is to be stored.

4500-Cl⁻ B. Argentometric Method

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1. General Discussion

a. Principle: In a neutral or slightly alkaline solution, potassium chromate can indicate the end point of the silver nitrate titration of chloride. Silver chloride is precipitated quantitatively before red silver chromate is formed.

b. Interference: Substances in amounts normally found in potable waters will not interfere. Bromide, iodide, and cyanide register as equivalent chloride concentrations. Sulfide, thiosulfate, and sulfite ions interfere but can be removed by treatment with hydrogen peroxide. Orthophosphate in excess of 25 mg/L interferes by precipitating as silver phosphate. Iron in excess of 10 mg/L interferes by masking the end point.

2. Apparatus

a. Erlenmeyer flask, 250-mL.

b. Buret, 50-mL.

3. Reagents

a. Potassium chromate indicator solution: Dissolve 50 g K_2CrO_4 in a little distilled water. Add $AgNO_3$ solution until a definite red precipitate is formed. Let stand 12 h, filter, and dilute to 1 L with distilled water.

b. Standard silver nitrate titrant, 0.0141M (0.0141N): Dissolve 2.395 g $AgNO_3$ in distilled water and dilute to 1000 mL. Standardize against NaCl by the procedure described in ¶ 4b below; 1.00 mL = 500 μg Cl^- . Store in a brown bottle.

c. Standard sodium chloride, 0.0141M (0.0141N): Dissolve 824.0 mg NaCl (dried at 140°C) in distilled water and dilute to 1000 mL; 1.00 mL = 500 μg Cl^- .

d. Special reagents for removal of interference:

1) *Aluminum hydroxide suspension:* Dissolve 125 g aluminum potassium sulfate or aluminum ammonium sulfate, $AlK(SO_4)_2 \cdot 12H_2O$ or $AlNH_4(SO_4)_2 \cdot 12H_2O$, in 1 L distilled water. Warm to 60°C and add 55 mL conc ammonium hydroxide (NH_4OH) slowly with stirring. Let stand about 1 h, transfer to a large bottle, and wash precipitate by successive additions, with thorough mixing and decanting with distilled water, until free from chloride. When freshly prepared, the suspension occupies a volume of approximately 1 L.

2) *Phenolphthalein indicator solution.*

3) *Sodium hydroxide, NaOH, 1N.*

4) *Sulfuric acid, H_2SO_4 , 1N.*

5) *Hydrogen peroxide, H_2O_2 , 30%.*

4. Procedure

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a. Sample preparation: Use a 100-mL sample or a suitable portion diluted to 100 mL. If the sample is highly colored, add 3 mL $\text{Al}(\text{OH})_3$ suspension, mix, let settle, and filter.

If sulfide, sulfite, or thiosulfate is present, add 1 mL H_2O_2 and stir for 1 min.

b. Titration: Directly titrate samples in the pH range 7 to 10. Adjust sample pH to 7 to 10 with H_2SO_4 or NaOH if it is not in this range. For adjustment, preferably use a pH meter with a non-chloride-type reference electrode. (If only a chloride-type electrode is available, determine amount of acid or alkali needed for adjustment and discard this sample portion. Treat a separate portion with required acid or alkali and continue analysis.) Add 1.0 mL K_2CrO_4 indicator solution. Titrate with standard AgNO_3 titrant to a pinkish yellow end point. Be consistent in end-point recognition.

Standardize AgNO_3 titrant and establish reagent blank value by the titration method outlined above. A blank of 0.2 to 0.3 mL is usual.

5. Calculation

$$\text{mg Cl}^-/\text{L} = \frac{(A - B) \times N \times 35\,450}{\text{mL sample}}$$

where:

A = mL titration for sample,

B = mL titration for blank, and

N = normality of AgNO_3 .

$$\text{mg NaCl/L} = (\text{mg Cl}^-/\text{L}) \times 1.65$$

6. Precision and Bias

A synthetic sample containing 241 mg Cl^-/L , 108 mg Ca/L , 82 mg Mg/L ; 3.1 mg K/L , 19.9 mg Na/L , 1.1 mg $\text{NO}_3^-/\text{N}/\text{L}$, 0.25 mg $\text{NO}_2^-/\text{N}/\text{L}$, 259 mg $\text{SO}_4^{2-}/\text{L}$, and 42.5 mg total alkalinity/L (contributed by NaHCO_3) in distilled water was analyzed in 41 laboratories by the argentometric method, with a relative standard deviation of 4.2% and a relative error of 1.7%.

7. Bibliography

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November 16-20, 1986, Portland, Ore., p. 673. American Water Works Assoc., Denver, Colo.

4500-Cl⁻ C. Mercuric Nitrate Method

1. General Discussion

a. Principle: Chloride can be titrated with mercuric nitrate, $\text{Hg}(\text{NO}_3)_2$, because of the formation of soluble, slightly dissociated mercuric chloride. In the pH range 2.3 to 2.8, diphenylcarbazone indicates the titration end point by formation of a purple complex with the excess mercuric ions. Xylene cyanol FF serves as a pH indicator and end-point enhancer. Increasing the strength of the titrant and modifying the indicator mixtures extend the range of measurable chloride concentrations.

b. Interference: Bromide and iodide are titrated with $\text{Hg}(\text{NO}_3)_2$ in the same manner as chloride. Chromate, ferric, and sulfite ions interfere when present in excess of 10 mg/L.

2. Apparatus

a. Erlenmeyer flask, 250-mL.

b. Microburet, 5-mL with 0.01-mL graduation intervals.

3. Reagents

a. Standard sodium chloride, 0.0141M (0.0141N): See Method B, ¶ B.3c above.

b. Nitric acid, HNO_3 , 0.1N.

c. Sodium hydroxide, NaOH, 0.1N.

d. Reagents for chloride concentrations below 100 mg/L:

1) *Indicator-acidifier reagent:* The HNO_3 concentration of this reagent is an important factor in the success of the determination and can be varied as indicated in a) or b) to suit the alkalinity range of the sample. Reagent a) contains sufficient HNO_3 to neutralize a total alkalinity of 150 mg as CaCO_3 /L to the proper pH in a 100-mL sample. Adjust amount of HNO_3 to accommodate samples of alkalinity different from 150 mg/L.

a) Dissolve, in the order named, 250 mg s-diphenylcarbazone, 4.0 mL conc HNO_3 , and 30 mg xylene cyanol FF in 100 mL 95% ethyl alcohol or isopropyl alcohol. Store in a dark bottle in a refrigerator. This reagent is not stable indefinitely. Deterioration causes a slow end point and high results.

b) Because pH control is critical, adjust pH of highly alkaline or acid samples to 2.5 ± 0.1 with 0.1N HNO_3 or NaOH, not with sodium carbonate (Na_2CO_3). Use a pH meter with a nonchloride type of reference electrode for pH adjustment. If only the usual chloride-type reference electrode is available for pH adjustment, determine amount of acid or alkali required to

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obtain a pH of 2.5 ± 0.1 and discard this sample portion. Treat a separate sample portion with the determined amount of acid or alkali and continue analysis. Under these circumstances, omit HNO_3 from indicator reagent.

2) *Standard mercuric nitrate titrant*, 0.007 05M (0.0141N): Dissolve 2.3 g $\text{Hg}(\text{NO}_3)_2$ or 2.5 g $\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$ in 100 mL distilled water containing 0.25 mL conc HNO_3 . Dilute to just under 1 L. Make a preliminary standardization by following the procedure described in ¶ 4a. Use replicates containing 5.00 mL standard NaCl solution and 10 mg sodium bicarbonate (NaHCO_3) diluted to 100 mL with distilled water. Adjust titrant to 0.0141N and make a final standardization; 1.00 mL = 500 $\mu\text{g Cl}^-$. Store away from light in a dark bottle.

e. Reagent for chloride concentrations greater than 100 mg/L:

1) *Mixed indicator reagent*: Dissolve 0.50 g diphenylcarbazone powder and 0.05 g bromphenol blue powder in 75 mL 95% ethyl or isopropyl alcohol and dilute to 100 mL with the same alcohol.

2) *Strong standard mercuric nitrate titrant*, 0.0705M (0.141N) Dissolve 25 g $\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$ in 900 mL distilled water containing 5.0 mL conc HNO_3 . Dilute to just under 1 L and standardize by following the procedure described in ¶ 4b. Use replicates containing 25.00 mL standard NaCl solution and 25 mL distilled water. Adjust titrant to 0.141N and make a final standardization; 1.00 mL = 5.00 mg Cl^- .

4. Procedure

a. Titration of chloride concentrations less than 100 mg/L: Use a 100-mL sample or smaller portion so that the chloride content is less than 10 mg.

Add 1.0 mL indicator-acidifier reagent. (The color of the solution should be green-blue at this point. A light green indicates pH less than 2.0; a pure blue indicates pH more than 3.8.) For most potable waters, the pH after this addition will be 2.5 ± 0.1 . For highly alkaline or acid waters, adjust pH to about 8 before adding indicator-acidifier reagent.

Titrate with 0.0141N $\text{Hg}(\text{NO}_3)_2$ titrant to a definite purple end point. The solution turns from green-blue to blue a few drops before the end point.

Determine blank by titrating 100 mL distilled water containing 10 mg NaHCO_3 .

b. Titration of chloride concentrations greater than 100 mg/L: Use a sample portion (5 to 50 mL) requiring less than 5 mL titrant to reach the end point. Measure into a 150-mL beaker. Add approximately 0.5 mL mixed indicator reagent and mix well. The color should be purple. Add 0.1N HNO_3 dropwise until the color just turns yellow. Titrate with strong $\text{Hg}(\text{NO}_3)_2$ titrant to first permanent dark purple. Titrate a distilled water blank using the same procedure.

5. Calculation

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$$\text{mg Cl}^{-}/\text{L} = \frac{(A - B) \times N \times 35\,450}{\text{mL sample}}$$

where:

A = mL titration for sample,
 B = mL titration for blank, and
 N = normality of $\text{Hg}(\text{NO}_3)_2$.

$$\text{mg NaCl/L} = (\text{mg Cl}^{-}/\text{L}) \times 1.65$$

6. Precision and Bias

A synthetic sample containing 241 mg Cl^{-}/L , 108 mg Ca/L , 82 mg Mg/L , 3.1 mg K/L , 19.9 mg Na/L , 1.1 mg $\text{NO}_3^{-}\text{-N}/\text{L}$, 0.25 mg $\text{NO}_2^{-}\text{-N}/\text{L}$, 259 mg $\text{SO}_4^{2-}/\text{L}$, and 42.5 mg total alkalinity/L (contributed by NaHCO_3) in distilled water was analyzed in 10 laboratories by the mercurimetric method, with a relative standard deviation of 3.3% and a relative error of 2.9%.

7. Bibliography

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4500- Cl^{-} D. Potentiometric Method

1. General Discussion

a. Principle: Chloride is determined by potentiometric titration with silver nitrate solution with a glass and silver-silver chloride electrode system. During titration an electronic voltmeter is used to detect the change in potential between the two electrodes. The end point of the titration is that instrument reading at which the greatest change in voltage has occurred for a small and constant increment of silver nitrate added.

b. Interference: Iodide and bromide also are titrated as chloride. Ferricyanide causes high results and must be removed. Chromate and dichromate interfere and should be reduced to the chromic state or removed. Ferric iron interferes if present in an amount substantially higher than the amount of chloride. Chromic ion, ferrous ion, and phosphate do not interfere.

Grossly contaminated samples usually require pretreatment. Where contamination is minor,

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some contaminants can be destroyed simply by adding nitric acid.

2. Apparatus

a. Glass and silver-silver chloride electrodes: Prepare in the laboratory or purchase a silver electrode coated with AgCl for use with specified instruments. Instructions on use and care of electrodes are supplied by the manufacturer.

b. Electronic voltmeter, to measure potential difference between electrodes: A pH meter may be converted to this use by substituting the appropriate electrode.

c. Mechanical stirrer, with plastic-coated or glass impeller.

3. Reagents

a. Standard sodium chloride solution, 0.0141M (0.0141N): See Section 4500-Cl⁻.B.3c.

b. Nitric acid, HNO₃, conc.

c. Standard silver nitrate titrant, 0.0141M (0.0141N): See Section 4500-Cl⁻.B.3b.

d. Pretreatment reagents:

1) *Sulfuric acid,* H₂SO₄, 1 + 1.

2) *Hydrogen peroxide,* H₂O₂, 30%.

3) *Sodium hydroxide,* NaOH, 1N.

4. Procedure

a. Standardization: The various instruments that can be used in this determination differ in operating details; follow the manufacturer's instructions. Make necessary mechanical adjustments. Then, after allowing sufficient time for warmup (10 min), balance internal electrical components to give an instrument setting of 0 mV or, if a pH meter is used, a pH reading of 7.0.

1) Place 10.0 mL standard NaCl solution in a 250-mL beaker, dilute to about 100 mL, and add 2.0 mL conc HNO₃. Immerse stirrer and electrodes.

2) Set instrument to desired range of millivolts or pH units. Start stirrer.

3) Add standard AgNO₃ titrant, recording scale reading after each addition. At the start, large increments of AgNO₃ may be added; then, as the end point is approached, add smaller and equal increments (0.1 or 0.2 mL) at longer intervals, so that the exact end point can be determined. Determine volume of AgNO₃ used at the point at which there is the greatest change in instrument reading per unit addition of AgNO₃.

4) Plot a differential titration curve if the exact end point cannot be determined by inspecting the data. Plot change in instrument reading for equal increments of AgNO₃ against volume of AgNO₃ added, using average of buret readings before and after each addition. The procedure is illustrated in Figure 4500-Cl⁻:1.

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b. Sample analysis:

1) Pipet 100.0 mL sample, or a portion containing not more than 10 mg Cl^- , into a 250-mL beaker. In the absence of interfering substances, proceed with ¶ 3) below.

2) In the presence of organic compounds, sulfite, or other interferences (such as large amounts of ferric iron, cyanide, or sulfide) acidify sample with H_2SO_4 , using litmus paper. Boil for 5 min to remove volatile compounds. Add more H_2SO_4 , if necessary, to keep solution acidic. Add 3 mL H_2O_2 and boil for 15 min, adding chloride-free distilled water to keep the volume above 50 mL. Dilute to 100 mL, add NaOH solution dropwise until alkaline to litmus, then 10 drops in excess. Boil for 5 min, filter into a 250-mL beaker, and wash precipitate and paper several times with hot distilled water.

3) Add conc HNO_3 dropwise until acidic to litmus paper, then 2.0 mL in excess. Cool and dilute to 100 mL if necessary. Immerse stirrer and electrodes and start stirrer. Make any necessary adjustments according to the manufacturer's instructions and set selector switch to appropriate setting for measuring the difference of potential between electrodes.

4) Complete determination by titrating according to ¶ 4a4). If an end-point reading has been established from previous determinations for similar samples and conditions, use this predetermined end point. For the most accurate work, make a blank titration by carrying chloride-free distilled water through the procedure.

5. Calculation

$$\text{mg Cl}^-/\text{L} = \frac{(A - B) \times N \times 35\,450}{\text{mL sample}}$$

where:

A = mL AgNO_3 ,

B = mL blank, and

N = normality of titrant.

6. Precision and Bias

In the absence of interfering substances, the precision and bias are estimated to be about 0.12 mg for 5 mg Cl^- , or 2.5% of the amount present. When pretreatment is required to remove interfering substances, the precision and bias are reduced to about 0.25 mg for 5 mg Cl^- , or 5% of amount present.

7. Bibliography

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4500-Cl⁻ E. Automated Ferricyanide Method

1. General Discussion

a. Principle: Thiocyanate ion is liberated from mercuric thiocyanate by the formation of soluble mercuric chloride. In the presence of ferric ion, free thiocyanate ion forms a highly colored ferric thiocyanate, of which the intensity is proportional to the chloride concentration.

b. Interferences: Remove particulate matter by filtration or centrifugation before analysis. Guard against contamination from reagents, water, glassware, and sample preservation process. No chemical interferences are significant.

c. Application: The method is applicable to potable, surface, and saline waters, and domestic and industrial wastewaters. The concentration range is 1 to 200 mg Cl⁻/L; it can be extended by dilution.

2. Apparatus

a. Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-Cl⁻:2.

b. Filters, 480-nm.

3. Reagents

a. Stock mercuric thiocyanate solution: Dissolve 4.17 g Hg(SCN)₂ in about 500 mL methanol, dilute to 1000 mL with methanol, mix, and filter through filter paper.

b. Stock ferric nitrate solution: Dissolve 202 g Fe(NO₃)₃·9H₂O in about 500 mL distilled water, then carefully add 21 mL conc HNO₃. Dilute to 1000 mL with distilled water and mix. Filter through paper and store in an amber bottle.

c. Color reagent: Add 150 mL stock Hg(SCN)₂ solution to 150 mL stock Fe(NO₃)₃ solution.

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Mix and dilute to 1000 mL with distilled water. Add 0.5 mL polyoxyethylene 23 lauryl ether.*#(35)

d. Stock chloride solution: Dissolve 1.6482 g NaCl, dried at 140°C, in distilled water and dilute to 1000 mL; 1.00 mL = 1.00 mg Cl⁻.

e. Standard chloride solutions: Prepare chloride standards in the desired concentration range, such as 1 to 200 mg/L, using stock chloride solution.

4. Procedure

Set up manifold as shown in Figure 4500-Cl⁻:2 and follow general procedure described by the manufacturer.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against chloride concentrations in standards. Compute sample chloride concentration by comparing sample response with standard curve.

6. Precision and Bias

With an automated system in a single laboratory six samples were analyzed in septuplicate. At a concentration ranging from about 1 to 50 mg Cl⁻/L the average standard deviation was 0.39 mg/L. The coefficient of variation was 2.2%. In two samples with added chloride, recoveries were 104% and 97%.

7. Bibliography

ZALL, D.M., D. FISHER & M.D. GARNER. 1956. Photometric determination of chlorides in water.

Anal. Chem. 28:1665.

O'BRIEN, J.E. 1962. Automatic analysis of chlorides in sewage. *Wastes Eng.* 33:670.

4500-Cl⁻ F. (RESERVED)

4500-Cl⁻ G. Mercuric Thiocyanate Flow Injection Analysis (PROPOSED)

1. General Discussion

a. Principle: A water sample containing chloride is injected into a carrier stream to which mercuric thiocyanate and ferric nitrate are added. The chloride complexes with the Hg(II), displacing the thiocyanate anion, which forms the highly colored ferric thiocyanate complex anion. The resulting peak's absorbance is measured at 480 nm. The peak area is proportional to the concentration of chloride in the original sample.

Also see Section 4500-Cl⁻.A and Section 4130, Flow Injection Analysis (FIA).

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b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Substances such as sulfite and thiosulfate, which reduce iron(III) to iron(II) and mercury(II) to mercury(I), can interfere. Halides, which also form strong complexes with mercuric ion (e.g., Br^- , I^-), give a positive interference.

2. Apparatus

Flow injection analysis equipment consisting of:

- a. FIA injection valve* with sample loop.
- b. Multichannel proportioning pump.*
- c. FIA manifold with flow cell* (Figure 4500-Cl⁻:3). Relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*#(36)
- d. Absorbance detector*, 480 nm, 10-nm bandpass.
- e. Valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions.

a. Stock mercuric thiocyanate solution: In a 1-L volumetric flask, dissolve 4.17 g mercuric thiocyanate, $\text{Hg}(\text{SCN})_2$, in about 500 mL methanol. Dilute to mark with methanol and mix.

CAUTION: *Mercuric thiocyanate is toxic. Wear gloves!*

b. Stock ferric nitrate reagent, 0.5M: In a 1-L volumetric flask, dissolve 202 g ferric nitrate, $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, in approximately 800 mL water. Add 25 mL conc HNO_3 and dilute to mark. Invert to mix.

c. Color reagent: In a 500-mL volumetric flask, mix 75 mL stock mercuric thiocyanate solution with 75 mL stock ferric nitrate reagent and dilute to mark with water. Invert to mix. Vacuum filter through a 0.45- μm membrane filter. The color reagent also is available as a commercially prepared solution that is stable for several months.

d. Stock chloride standard, 1000 mg Cl^-/L : In a 105°C oven, dry 3 g primary standard grade sodium chloride, NaCl , overnight. In a 1-L volumetric flask, dissolve 1.648 g primary standard grade sodium chloride in about 500 mL water. Dilute to mark and invert to mix.

e. Standard chloride solutions: Prepare chloride standards for the calibration curve in the desired concentration range, using the stock standard (§ 3d), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-Cl⁻:3 and follow method supplied by

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manufacturer, or laboratory standard operating procedure for this method. Follow quality control procedures described in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus chloride concentration. The calibration curve gives a good fit to a second-order polynomial.

6. Precision and Bias

a. Recovery and relative standard deviation: The results of single-laboratory studies with various matrices are given in Table 4500-Cl⁻:I .

b. MDL: A 100- μ L sample loop was used in the method described above. Using a published MDL method¹ analysts ran 21 replicates of a 1.0-mg Cl⁻/L standard. These gave a mean of 1.19 mg Cl⁻/L, a standard deviation of 0.027 mg Cl⁻/L, and an MDL of 0.07 mg Cl⁻/L. This is only an estimate because the ratio of standard to the MDL is above guidelines (see Section 1030). A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagents flow rate. A higher MDL may be obtained by decreasing the sample loop volume and decreasing this ratio.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and Procedure for the Determination of Method Detection Limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-ClO₂ CHLORINE DIOXIDE*#(37)

4500-ClO₂ A. Introduction

Because the physical and chemical properties of chlorine dioxide resemble those of chlorine in many respects, read the entire discussion of Residual Chlorine (Section 4500-Cl) before attempting a chlorine dioxide determination.

1. Occurrence and Significance

Chlorine dioxide, ClO₂, has been used widely as a bleaching agent in the paper and pulp industry. It has been applied to water supplies to combat tastes and odors due to phenolic-type wastes, actinomycetes, and algae, as well as to oxidize soluble iron and manganese to a more easily removable form. It is a disinfectant, and some results suggest that it may be stronger than

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free chlorine or hypochlorite.

Chlorine dioxide is a deep yellow, volatile, unpleasant-smelling gas that is toxic and under certain conditions may react explosively. It should be handled with care in a vented area. The use of odor to warn of exposure to concentrations of health significance may not be adequate.

There are several methods of generating ClO_2 ; for laboratory purposes the acidification of a solution of sodium chlorite followed by suitable scrubbing and capture of the released gaseous ClO_2 is the most practical. CAUTION: *Sodium chlorite is a powerful oxidizer; keep out of direct contact with oxidizable material to avoid possibility of explosion.*

2. Selection of Method

The iodometric method (B) gives a very precise measure of total available strength of a solution in terms of its ability to liberate iodine from iodide. However, ClO_2 , chlorine, chlorite, and hypochlorite are not distinguished easily by this technique. It is designed primarily, and best used, for standardizing ClO_2 solutions needed for preparation of temporary standards. It often is inapplicable to industrial wastes.

The amperometric methods (C and E) are useful when a knowledge of the various chlorine fractions in a water sample is desired. They distinguish various chlorine compounds of interest with good accuracy and precision, but require specialized equipment and considerable analytical skill.

The *N,N*-diethyl-*p*-phenylenediamine (DPD) method (D) has the advantages of a relatively easy-to-perform colorimetric test with the ability to distinguish between ClO_2 and some forms of chlorine. This technique is not as accurate as the amperometric method, but should yield results adequate for many common applications. NOTE: Reports in the literature indicate that the DPD method is subject to interference from monochloramine and chloraminoacetic acid, and the chlorite anion.¹

3. Sampling and Storage

Determine ClO_2 promptly after collecting the sample. Do not expose sample to sunlight or strong artificial light and do not aerate to mix. Most of these methods can be performed on site, with prior calibration in the laboratory. Minimum ClO_2 losses occur when the determination is completed immediately at the site of sample collection.

4. Reference

1. CHISWELL, B. & K.R. O'HALLORAN. 1991. Use of Lissamine Green B as a spectrophotometric reagent for the determination of low residuals of chlorine dioxide. *Analyst* 116:657.

5. Bibliography

INGOLS, R.S. & G.M. RIDENOUR. 1948. Chemical properties of chlorine dioxide in water

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- FEUSS, J.V. 1964. Problems in determination of chlorine dioxide residuals. *J. Amer. Water Works Assoc.* 56:607.
- MASSCHELEIN, W. 1966. Spectrophotometric determination of chlorine dioxide with acid chrome violet K. *Anal. Chem.* 38:1839.
- MASSCHELEIN, W. 1969. Les Oxydes de Chlore et le Chlorite de Sodium. Dunod, Paris, Chapter XI.

4500-ClO₂ B. Iodometric Method

1. General Discussion

a. Principle: A pure solution of ClO₂ is prepared from gaseous ClO₂ by slowly adding dilute H₂SO₄ to a sodium chlorite (NaClO₂) solution. Contaminants such as chlorine are removed from the gas stream by a NaClO₂ scrubber; the gas is passed into distilled water in a steady stream of air. See CAUTION, ¶ A.1.

ClO₂ releases free iodine from a KI solution acidified with acetic acid or H₂SO₄. The liberated iodine is titrated with a standard solution of sodium thiosulfate (Na₂S₂O₃), with starch as the indicator.

b. Interference: There is little interference in this method, but temperature and strong light affect solution stability. Minimize ClO₂ losses by storing stock ClO₂ solution in a dark refrigerator and by preparing and titrating dilute ClO₂ solutions for standardization purposes at the lowest practicable temperature and in subdued light.

c. Minimum detectable concentration: One drop (0.05 mL) of 0.01N (0.01M) Na₂S₂O₃ is equivalent to 20 µg ClO₂/L (or 40 µg/L in terms of available chlorine) when a 500-mL sample is titrated.

2. Reagents

All reagents listed for the determination of residual chlorine in Section 4500-Cl.B.2a - Section 4500-Cl.B.2g are required. Also needed are the following:

a. Stock chlorine dioxide solution: Prepare a gas generating and absorbing system as illustrated in Figure 4500-ClO₂:1. Connect aspirator flask, 500-mL capacity, with rubber tubing to a source of purified compressed air. Let air bubble through a layer of 300 mL distilled water in flask and then pass through a glass tube ending within 5 mm of the bottom of the 1-L

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gas-generating bottle. Conduct evolved gas via glass tubing through a scrubber bottle containing saturated NaClO_2 solution or a tower packed with flaked NaClO_2 , and finally, via glass tubing, into a 2-L borosilicate glass collecting bottle where the gas is absorbed in 1500 mL distilled water. Provide an air outlet tube on collecting bottle for escape of air. Select for gas generation a bottle constructed of strong borosilicate glass and having a mouth wide enough to permit insertion of three separate glass tubes: the first leading almost to the bottom for admitting air, the second reaching below the liquid surface for gradual introduction of H_2SO_4 , and the third near the top for exit of evolved gas and air. Fit to second tube a graduated cylindrical separatory funnel to contain H_2SO_4 . Locate this system in a fume hood with an adequate shield.

Dissolve 10 g NaClO_2 in 750 mL distilled water and place in generating bottle. Carefully add 2 mL conc H_2SO_4 to 18 mL distilled water and mix. Transfer to funnel. Connect flask to generating bottle, generating bottle to scrubber, and the latter to collecting bottle. Pass a smooth current of air through the system, as evidenced by the bubbling rate in all bottles.

Introduce 5-mL increments of H_2SO_4 from funnel into generating bottle at 5-min intervals. Continue air flow for 30 min after last portion of acid has been added.

Store yellow stock solution in glass-stoppered dark-colored bottle in a dark refrigerator. The concentration of ClO_2 thus prepared varies between 250 and 600 mg/L, corresponding to approximately 500 to 1200 mg free chlorine/L.

b. Standard chlorine dioxide solution: Use this solution for preparing temporary ClO_2 standards. Dilute required volume of stock ClO_2 solution to desired strength with chlorine-demand-free water (see Section 4500-Cl.C.3*m*). Standardize solution by titrating with standard 0.01*N* (0.01*M*) or 0.025*N* (0.025*M*) $\text{Na}_2\text{S}_2\text{O}_3$ titrant in the presence of KI, acid, and starch indicator by following the procedure given in ¶ 3 below. A full or nearly full bottle of chlorine or ClO_2 solution retains its titer longer than a partially full one. When repeated withdrawals reduce volume to a critical level, standardize diluted solution at the beginning, midway in the series of withdrawals, and at the end of the series. Shake contents thoroughly before drawing off needed solution from middle of the glass-stoppered dark-colored bottle. Prepare this solution frequently.

3. Procedure

Select volume of sample, prepare for titration, and titrate sample and blank as described in Section 4500-Cl.B.3. The only exception is the following: *Let ClO_2 react in the dark with acid and KI for 5 min before starting titration.*

4. Calculations

Express ClO_2 concentrations in terms of ClO_2 or as free chlorine content. Free chlorine is defined as the total oxidizing power of ClO_2 measured by titrating iodine released by ClO_2 from an acidic solution of KI. Calculate result in terms of chlorine itself.

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For standardizing ClO_2 solution:

$$\text{mg ClO}_2/\text{mL} = \frac{(A \pm B) \times N \times 13.49}{\text{mL sample titrated}}$$

For determining ClO_2 temporary standards:

$$\text{mg ClO}_2 \text{ as Cl}_2/\text{mL} = \frac{(A \pm B) \times N \times 35.45}{\text{mL sample titrated}}$$

where:

A = mL titration for sample,

B = mL titration for blank (positive or negative, see Section 4500-Cl.B.3d), and

N = normality of $\text{Na}_2\text{S}_2\text{O}_3$ = molarity of $\text{Na}_2\text{S}_2\text{O}_3$.

5. Bibliography

POST, M.A. & W.A. MOORE. 1959. Determination of chlorine dioxide in treated surface waters. *Anal. Chem.* 31:1872.

4500- ClO_2 C. Amperometric Method I

1. General Discussion

a. Principle: The amperometric titration of ClO_2 is an extension of the amperometric method for chlorine. By performing four titrations with phenylarsine oxide, free chlorine (including hypochlorite and hypochlorous acid), chloramines, chlorite, and ClO_2 may be determined separately. The first titration step consists of conversion of ClO_2 to chlorite and chlorate through addition of sufficient NaOH to produce a pH of 12, followed by neutralization to a pH of 7 and titration of free chlorine. In the second titration KI is added to a sample that has been treated similarly with alkali and had the pH readjusted to 7; titration yields free chlorine and monochloramine. The third titration involves addition of KI and pH adjustment to 7, followed by titration of free chlorine, monochloramine, and one-fifth of the available ClO_2 . In the fourth titration, addition of sufficient H_2SO_4 to lower the pH to 2 enables all available ClO_2 and chlorite, as well as the total free chlorine, to liberate an equivalent amount of iodine from the added KI and thus be titrated.

b. Interference: The interferences described in Section 4500-Cl.D.1b apply also to determination of ClO_2 .

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2. Apparatus

The apparatus required is given in Section 4500-Cl.D.2a through d.

3. Reagents

All reagents listed for the determination of chlorine in Section 4500-Cl.D.3 are required. Also needed are the following:

- a. *Sodium hydroxide*, NaOH, 6N (6M).
- b. *Sulfuric acid*, H₂SO₄, 6N (3M), 1 + 5.

4. Procedure

Minimize effects of pH, time, and temperature of reaction by standardizing all conditions.

a. *Titration of free available chlorine (hypochlorite and hypochlorous acid)*: Add sufficient 6N (6M) NaOH to raise sample pH to 12. After 10 min, add 6N (3M) H₂SO₄ to lower pH to 7. Titrate with standard phenylarsine oxide titrant to the amperometric end point as given in Section 4500-Cl.D. Record result as A.

b. *Titration of free available chlorine and chloramine*: Add 6N (6M) NaOH to raise sample pH to 12. After 10 min, add 6N (3M) H₂SO₄ to reduce pH to 7. Add 1 mL KI solution. Titrate with standard phenylarsine oxide titrant to the amperometric end point. Record result as B.

c. *Titration of free available chlorine, chloramine, and one-fifth of available ClO₂*: Adjust sample pH to 7 with pH 7 phosphate buffer solution. Add 1 mL KI solution. Titrate with standard phenylarsine oxide titrant to the amperometric end point. Record result as C.

d. *Titration of free available chlorine, chloramines, ClO₂, and chlorite*: Add 1 mL KI solution to sample. Add sufficient 6N (3M) H₂SO₄ to lower pH to 2. After 10 min, add sufficient 6N (6M) NaOH to raise pH to 7. Titrate with standard phenylarsine oxide titrant to the amperometric end point. Record result as D.

5. Calculation

Convert individual titrations (A, B, C, and D) into chlorine concentration by the following equation:

$$\text{mg Cl as Cl}_2/\text{L} = \frac{E \times 200}{\text{mL sample}}$$

where:

E = mL phenylarsine oxide titration for each individual sample A, B, C, or D.

Calculate ClO₂ and individual chlorine fractions as follows:

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$$\begin{aligned}\text{mg ClO}_2 \text{ as ClO}_2/\text{L} &= 1.9 (C - B) \\ \text{mg ClO}_2 \text{ as Cl}_2/\text{L} &= 5 (C - B) \\ \text{mg free available chlorine/L} &= A \\ \text{mg chloramine/L as chlorine} &= B - A \\ \text{mg chlorite/L as chlorine} &= 4B - 5C + D\end{aligned}$$

6. Bibliography

HALLER, J.F. & S.S. LISTEK. 1948. Determination of chlorine dioxide and other active chlorine compounds in water. *Anal. Chem.* 20:639.

4500-ClO₂ D. DPD Method

1. General Discussion

a. Principle: This method is an extension of the *N,N*-diethyl-*p*-phenylenediamine (DPD) method for determining free chlorine and chloramines in water. ClO₂ appears in the first step of this procedure but only to the extent of one-fifth of its total available chlorine content corresponding to reduction of ClO₂ to chlorite ion. If the sample is then acidified in the presence of iodide the chlorite also reacts. When neutralized by subsequent addition of bicarbonate, the color thus produced corresponds to the total available chlorine content of the ClO₂. If chlorite is present in the sample, this will be included in the step involving acidification and neutralization. Chlorite that did not result from ClO₂ reduction by the procedure will cause a positive error equal to twice this chlorite concentration. In evaluating mixtures of these various chloro-compounds, it is necessary to suppress free chlorine by adding glycine before reacting the sample with DPD reagent. Differentiation is based on the fact that glycine converts free chlorine instantaneously into chloroaminoacetic acid but has no effect on ClO₂.

b. Interference: The interference by oxidized manganese described in Section 4500-Cl.F.1d applies also to ClO₂ determination. Manganese interference appears as an increase in the first titrations after addition of DPD, with or without KI, and irrespective of whether there has been prior addition of glycine. Titration readings must be corrected suitably. Interference by chromate in wastewaters may be corrected similarly.

Iron contributed to the sample by adding ferrous ammonium sulfate (FAS) titrant may activate chlorite so as to interfere with the first end point of the titration. Suppress this effect with additional EDTA, disodium salt.

Exercise caution in the selection of this method, because of interferences from monochloramine and chloraminoacetic acid and the chlorite anion.

2. Reagents

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Reagents required in addition to those for the DPD free-combined chlorine method as listed in Section 4500-Cl.F.2 are as follows:

- a. *Glycine solution*: Dissolve 10 g $\text{NH}_2\text{CH}_2\text{COOH}$ in 100 mL distilled water.
- b. *Sulfuric acid solution*: Dilute 5 mL conc H_2SO_4 to 100 mL with distilled water.
- c. *Sodium bicarbonate solution*: Dissolve 27.5 g NaHCO_3 in 500 mL distilled water.
- d. *EDTA*: Disodium salt of ethylenediamine tetraacetic acid, solid.

3. Procedure

For samples containing more than 5 mg/L total available chlorine follow the dilution procedure given in Section 4500-Cl.F.3.

a. *Chlorine dioxide*: Add 2 mL glycine solution to 100 mL sample and mix. Place 5 mL each of buffer reagent and DPD indicator solution in a separate titration flask and mix (or use about 500 mg DPD powder). Add about 200 mg EDTA, disodium salt. Then add glycine-treated sample and mix. Titrate rapidly with standard FAS titrant until red color is discharged (Reading G).

b. *Free available chlorine and chloramine*: Using a second 100-mL sample follow the procedures of Section 4500-Cl.F.3a adding about 200 mg EDTA, disodium salt, initially with the DPD reagents (Readings A, B, and C).

c. *Total available chlorine including chlorite*: After obtaining Reading C add 1 mL H_2SO_4 solution to the same sample in titration flask, mix, and let stand about 2 min. Add 5 mL NaHCO_3 solution, mix, and titrate (Reading D).

d. *Colorimetric procedure*: Instead of titration with standard FAS solution, colorimetric procedures may be used to obtain the readings at each stage. Calibrate colorimeters with standard permanganate solution as directed in Section 4500-Cl.G.4a. Use of additional EDTA, disodium salt, with the DPD reagents is not required in colorimetric procedures.

4. Calculations

For 100 mL sample, 1 mL FAS solution = 1 mg available chlorine/L.

In the absence of chlorite:

$$\text{Chlorine dioxide} = 5G \text{ (or } 1.9G \text{ expressed as } \text{ClO}_2\text{)}$$

$$\text{Free available chlorine} = A - G$$

$$\text{Monochloramine} = B - A$$

$$\text{Dichloramine} = C - B$$

$$\text{Total available chlorine} = C + 4G$$

If the step leading to Reading B is omitted, monochloramine and dichloramine are obtained together when:

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$$\text{Combined available chlorine} = C - A$$

If it is desired to check for presence of chlorite in sample, obtain Reading *D*. Chlorite is indicated if *D* is greater than $C + 4G$.

In the presence of chlorite:

$$\text{Chlorine dioxide} = 5G \text{ (or } 1.9G \text{ expressed as } \text{ClO}_2\text{)}$$

$$\text{Chlorite} = D - (C + 4G)$$

$$\text{Free available chlorine} = A - G$$

$$\text{Monochloramine} = B - A$$

$$\text{Dichloramine} = C - B$$

$$\text{Total available chlorine} = D$$

If *B* is omitted,

$$\text{Combined available chlorine} = C - A$$

5. Bibliography

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4500-ClO₂ E. Amperometric Method II

1. General Discussion

a. Principle: Like Amperometric Method I (Section 4500-ClO₂.C), this procedure entails successive titrations of combinations of chlorine species. Subsequent calculations determine the concentration of each species. The equilibrium for reduction of the chlorine species of interest by iodide is pH-dependent.

The analysis of a sample for chlorine, chlorine dioxide, chlorite, and chlorate requires the following steps: determination of all of the chlorine (free plus combined) and one-fifth of the chlorine dioxide at pH 7; lowering sample pH to 2 and determination of the remaining four-fifths

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of the ClO_2 and all of the chlorite (the chlorite measured in this step comes from the chlorite originally present in the sample and that formed in the first titration); preparation of a second sample by purging with nitrogen to remove ClO_2 and by reacting with iodide at pH 7 to remove any chlorine remaining; lowering latter sample pH to 2 and determination of all chlorite present (this chlorite only comes from the chlorite originally present in the sample); and, in a third sample, determination of all of the relevant, oxidized chlorine species—chlorine, chlorine dioxide, chlorite, and chlorate—after reduction in hydrochloric acid.¹

This procedure can be applied to concentrated solutions (10 to 100 mg/L) or dilute solutions (0.1 to 10 mg/L) by appropriate selection of titrant concentration and sample size.

b. Interferences: At pH values above 4, significant iodate formation is possible if iodine is formed in the absence of iodide;² this results in a negative bias in titrating the first and second samples. Acidification of these samples causes reduction of iodate to iodine and a positive bias. To prevent formation of iodate add 1 g KI granules to stirred sample.

A positive bias results from oxidation of iodide to iodine by dissolved oxygen in strongly acidic solutions.¹ To minimize this bias, use bromide as the reducing agent in titrating the third sample (bromide is not oxidized by oxygen under these conditions). After reaction is completed, add iodide, which will be oxidized to iodine by the bromine formed from the reduction of the original chlorine species. Add iodide carefully so that bromine gas is not lost. Rapid dilution of the sample with sodium phosphate decreases sample acidity and minimizes oxidation of iodide by oxygen. The pH of the solution to be titrated should be between 1.0 and 2.0. Carry a blank through the procedure as a check on iodide oxidation.

The potential for interferences from manganese, copper, and nitrate is minimized by buffering the sample to pH ≥ 4 .^{3,4} For the method presented here, the low pH required for the chlorite and chlorate analyses provides conditions favorable to manganese, copper, and nitrite interferences.

2. Apparatus

a. Titrators: See Section 4500-Cl.D.2a through *d*. Amperometric titrators with a platinum-platinum electrode system are more stable and require less maintenance. (NOTE: Chlorine dioxide may attack adhesives used to connect the platinum plate to the electrode, resulting in poor readings.)

If a potentiometric titrator is used, provide a platinum sensing electrode and a silver chloride reference electrode for end-point detection.

b. Glassware: Store glassware used in this method separately from other laboratory glassware and do not use for other purposes because ClO_2 reacts with glass to form a hydrophobic surface coating. To satisfy any ClO_2 demand, before first use immerse all glassware in a strong ClO_2 solution (200 to 500 mg/L) for 24 h and rinse only with water between uses.

c. Sampling: ClO_2 is volatile and will vaporize easily from aqueous solution. When

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sampling a liquid stream, minimize contact with air by placing a flexible sample line to reach the bottom of the sample container, letting several container volumes overflow, slowly removing sample line, and capping container with minimum headspace. Protect from sunlight. Remove sample portions with a volumetric pipet with pipet tip placed at bottom of container. Drain pipet by placing its tip below the surface of reagent or dilution water.

3. Reagents

- a. *Standard sodium thiosulfate*, 0.100N (0.100M): See Section 4500-Cl.B.2c.
- b. *Standard phenylarsine oxide*, 0.005 64N (0.005 64M): See Section 4500-Cl.C.3a. (Weigh out 1.25 g phenylarsine oxide and standardize to 0.005 64M.)
- c. *Phosphate buffer solution*, pH 7: See Section 4500-Cl.D.3b.
- d. *Potassium iodide*, KI, granules.
- e. *Saturated sodium phosphate solution*: Prepare a saturated solution of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ with cold deionized-distilled water.
- f. *Potassium bromide solution*, 5%: Dissolve 5 g KBr and dilute to 100 mL. Store in a brown glass-stoppered bottle. Make fresh weekly.
- g. *Hydrochloric acid*, HCl, conc.
- h. *Hydrochloric acid*, HCl, 2.5N (2.5M): Cautiously add 200 mL conc HCl, with mixing, to distilled water, diluting to 1000 mL.
- i. *Purge gas*: Use nitrogen gas for purging ClO_2 from samples. Assure that gas is free of contaminants and pass it through a 5% KI scrub solution. Discard solution at first sign of color.

4. Procedure

Use either sodium thiosulfate or phenylarsine oxide as titrant. Select concentration on basis of concentration range expected. The total mass of oxidant species should be no greater than about 15 mg. Make appropriate sample dilutions if necessary. A convenient volume for titration is 200 to 300 mL. Preferably analyze all samples and blanks in triplicate.

Minimize effects of pH, time, and temperature of reaction by standardizing all conditions.

a. *Titration of residual chlorine and one-fifth of available ClO_2* : Place 1 mL pH 7 phosphate buffer in beaker and add distilled-deionized dilution water if needed. Introduce sample with minimum aeration and add 1 g KI granules while stirring. Titrate to end point (see Section 4500-Cl.D). Record reading $A = \text{mL titrant/mL sample}$.

b. *Titration of four-fifths of available ClO_2 and chlorite*: Continuing with same sample, add 2 mL 2.5N (2.5M) HCl. Let stand in the dark for 5 min. Titrate to end point. Record reading $B = \text{mL titrant/mL sample}$.

c. *Titration of nonvolatilized chlorine*: Place 1 mL pH 7 phosphate buffer in purge vessel and add distilled-deionized dilution water if needed. Add sample and purge with nitrogen gas for 15 min. Use a gas-dispersion tube to give good gas-liquid contact. Add 1 g KI granules while

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stirring and titrate to end point. Record reading $C = \text{mL titrant/mL sample}$.

d. *Titration of chlorite*: Continuing with same sample, add 2 mL 2.5N (2.5M) HCl. Let stand in the dark for 5 min. Titrate to end point, and record reading $D = \text{mL titrant/mL sample}$.

e. *Titration of chlorine, ClO_2 , chlorate, and chlorite*: Add 1 mL KBr and 10 mL conc HCl to 50-mL reaction flask and mix. Carefully add 15 mL sample, with minimum aeration. Mix and stopper immediately. Let stand in the dark for 20 min. Rapidly add 1 g KI granules and shake vigorously for 5 s. Rapidly transfer to titration flask containing 25 mL saturated Na_2HPO_4 solution. Rinse reaction flask thoroughly and add rinse water to titration flask. Final titration volume should be about 200 to 300 mL. Titrate to end point.

Repeat procedure of preceding paragraph using distilled-deionized water in place of sample to determine blank value.

Record reading $E = (\text{mL titrant sample} - \text{mL titrant blank}) / \text{mL sample}$.

NOTE: The 15-mL sample volume can be adjusted to provide an appropriate dilution, but maintain the ratio of sample to HCl.

5. Calculations

Because the combining power of the titrants is pH-dependent, all calculations are based on the equivalents of reducing titrant required to react with equivalents of oxidant present. Use Table 4500- ClO_2 :I to obtain the equivalent weights to be used in the calculations.

In the following equations, N is the normality of the titrant used in equivalents per liter and A through E are as defined previously.

$$\text{Chlorite, mg } \text{ClO}_2^-/\text{L} = D \times N \times 16\,863$$

$$\text{Chlorate, mg } \text{ClO}_2^{2-}/\text{L} = [E - (A + B)] \times N \times 13\,909$$

$$\text{Chlorine dioxide, mg } \text{ClO}_2/\text{L} = (5/4) \times (B - D) \times N \times 13\,490$$

$$\text{Chlorine, mg } \text{Cl}_2/\text{L} = A - [(B - D)/4] \times N \times 35\,453$$

6. References

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4500-F⁻ FLUORIDE*#(38)

4500-F⁻ A. Introduction

A fluoride concentration of approximately 1.0 mg/L in drinking water effectively reduces dental caries without harmful effects on health. Fluoride may occur naturally in water or it may be added in controlled amounts. Some fluorosis may occur when the fluoride level exceeds the recommended limits. In rare instances the naturally occurring fluoride concentration may approach 10 mg/ L; such waters should be defluoridated.

Accurate determination of fluoride has increased in importance with the growth of the practice of fluoridation of water supplies as a public health measure. Maintenance of an optimal fluoride concentration is essential in maintaining effectiveness and safety of the fluoridation procedure.

1. Preliminary Treatment

Among the methods suggested for determining fluoride ion (F⁻) in water, the electrode and colorimetric methods are the most satisfactory. Because both methods are subject to errors due to interfering ions (Table 4500-F⁻:I), it may be necessary to distill the sample as directed in Section 4500-F-B before making the determination. When interfering ions are not present in excess of the tolerance of the method, the fluoride determination may be made directly without distillation.

2. Selection of Method

The electrode methods (C and G) are suitable for fluoride concentrations from 0.1 to more than 10 mg/L. Adding the prescribed buffer frees the electrode method from most interferences that adversely effect the SPADNS colorimetric method and necessitate preliminary distillation. Some substances in industrial wastes, such as fluoborates, may be sufficiently concentrated to present problems in electrode measurements and will not be measured without a preliminary distillation. Fluoride measurements can be made with an ion-selective electrode and either an

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expanded-scale pH meter or a specific ion meter, usually without distillation, in the time necessary for electrode equilibration.

The SPADNs method (D) has a linear analytical range of 0 to 1.40 mg F⁻/L. Use of a nonlinear calibration can extend the range to 3.5 mg F⁻/L. Color development is virtually instantaneous. Color determinations are made photometrically, using either a filter photometer or a spectrophotometer. A curve developed from standards is used for determining the fluoride concentration of a sample.

Fluoride also may be determined by the automated complexone method, Method E.

Ion chromatography (Section 4110) is an acceptable method if weaker eluents are used to separate fluoride from interfering peaks or fluoride can be determined by capillary ion electrophoresis (Section 4140).

The flow injection method (G) is a convenient automated technique for analyzing large numbers of samples.

3. Sampling and Storage

Preferably use polyethylene bottles for collecting and storing samples for fluoride analysis. Glass bottles are satisfactory if previously they have not contained high-fluoride solutions. Always rinse bottle with a portion of sample.

For the SPADNs method, never use an excess of dechlorinating agent. Dechlorinate with sodium arsenite rather than sodium thiosulfate when using the SPADNS method because the latter may produce turbidity that causes erroneous readings.

4500-F⁻ B. Preliminary Distillation Step

1. Discussion

Fluoride can be separated from other nonvolatile constituents in water by conversion to hydrofluoric or fluosilicic acid and subsequent distillation. The conversion is accomplished by using a strong, high-boiling acid. To protect against glassware etching, hydrofluoric acid is converted to fluosilicic acid by using soft glass beads. Quantitative fluoride recovery is approached by using a relatively large sample. Acid and sulfate carryover are minimized by distilling over a controlled temperature range.

Distillation will separate fluoride from most water samples. Some tightly bound fluoride, such as that in biological materials, may require digestion before distillation, but water samples seldom require such drastic treatment. Distillation produces a distillate volume equal to that of the original water sample so that usually it is not necessary to incorporate a dilution factor when expressing analytical results. The distillate will be essentially free of substances that might interfere with the fluoride determination if the apparatus used is adequate and distillation has been carried out properly. The only common volatile constituent likely to cause interference with colorimetric analysis of the distillate is chloride. When the concentration of chloride is high

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enough to interfere, add silver sulfate to the sulfuric acid distilling mixture to minimize the volatilization of hydrogen chloride.

Heating an acid-water mixture can be hazardous if precautions are not taken: *Mix acid and water thoroughly before heating.* Use of a quartz heating mantle and a magnetic stirrer in the distillation apparatus simplifies the mixing step.

2. Apparatus

a. *Distillation apparatus* consisting of a 1-L round-bottom long-neck borosilicate glass boiling flask, a connecting tube, an efficient condenser, a thermometer adapter, and a thermometer that can be read to 200°C. Use standard taper joints for all connections in the direct vapor path. Position the thermometer so that the bulb always is immersed in boiling mixture. The apparatus should be disassembled easily to permit adding sample. Substituting a thermoregulator and necessary circuitry for the thermometer is acceptable and provides some automation.

Alternative types of distillation apparatus may be used. Carefully evaluate any apparatus for fluoride recovery and sulfate carryover. The critical points are obstructions in the vapor path and trapping of liquid in the adapter and condenser. (The condenser should have a vapor path with minimum obstruction. A double-jacketed condenser, with cooling water in the outer jacket and the inner spiral tube, is ideal, but other condensers are acceptable if they have minimum obstructions. Avoid using Graham-type condensers.) Avoid using an open flame as a heat source if possible, because heat applied to the boiling flask above the liquid level causes superheating of vapor and subsequent sulfate carryover.

CAUTION: *Regardless of apparatus used, provide for thorough mixing of sample and acid; heating a non-homogenous acid-water mixture will result in bumping or possibly a violent explosion.*

The preferred apparatus is illustrated in Figure 4500-F⁻:1.

- b. *Quartz hemispherical heating mantle*, for full-voltage operation.
- c. *Magnetic stirrer*, with TFE-coated stirring bar.
- d. *Soft glass beads*.

3. Reagents

- a. *Sulfuric acid*, H₂SO₄, conc, reagent grade.
- b. *Silver sulfate*, Ag₂SO₄, crystals, reagent grade.

4. Procedure

a. Place 400 mL distilled water in the distilling flask and, with the magnetic stirrer operating, carefully add 200 mL conc H₂SO₄. Keep stirrer in operation throughout distillation. Add a few glass beads and connect the apparatus as shown in Figure 4500-F⁻:1, making sure all joints are tight. Begin heating and continue until flask contents reach 180°C (because of heat retention by the mantle, it is necessary to discontinue heating when the temperature reaches 178°C to prevent

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overheating). Discard distillate. This process removes fluoride contamination and adjusts the acid-water ratio for subsequent distillations.

b. After the acid mixture remaining in the steps outlined in ¶ 4a, or previous distillations, has cooled to 80°C or below, add 300 mL sample, with stirrer operating, and distill until the temperature reaches 180°C. To prevent sulfate carryover, turn off heat before 178°C. Retain the distillate for analysis.

c. Add Ag_2SO_4 to the distilling flask at the rate of 5 mg/mg Cl^- when the chloride concentration is high enough to interfere (see Table 4500-F⁻:I).

d. Use H_2SO_4 solution in the flask repeatedly until contaminants from samples accumulate to such an extent that recovery is affected or interferences appear in the distillate. Check acid suitability periodically by distilling standard fluoride samples and analyzing for both fluoride and sulfate. After distilling samples containing more than 3 mg F^-/L , flush still by adding 300 mL distilled water, redistill, and combine the two fluoride distillates. If necessary, repeat flushing until the fluoride content of the last distillate is at a minimum. Include additional fluoride recovered with that of the first distillation. After periods of inactivity, similarly flush still and discard distillate.

5. Interpretation of Results

The recovery of fluoride is quantitative within the accuracy of the methods used for its measurement.

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4500-F⁻ C. Ion-Selective Electrode Method

1. General Discussion

a. *Principle:* The fluoride electrode is an ion-selective sensor. The key element in the fluoride electrode is the laser-type doped lanthanum fluoride crystal across which a potential is established by fluoride solutions of different concentrations. The crystal contacts the sample solution at one face and an internal reference solution at the other. The cell may be represented by:



The fluoride electrode can be used with a standard calomel reference electrode and almost any

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modern pH meter having an expanded millivolt scale. Calomel electrodes contain both metallic and dissolved mercury; therefore, dispose of them only in approved sites or recycle. For this reason, the Ag/AgCl reference electrode is preferred.

The fluoride electrode measures the ion activity of fluoride in solution rather than concentration. Fluoride ion activity depends on the solution total ionic strength and pH, and on fluoride complexing species. Adding an appropriate buffer provides a nearly uniform ionic strength background, adjusts pH, and breaks up complexes so that, in effect, the electrode measures concentration.

b. Interference: Table 4500-F⁻:I lists common interferences. Fluoride forms complexes with several polyvalent cations, notably aluminum and iron. The extent to which complexation takes place depends on solution pH, relative levels of fluoride, and complexing species. However, CDTA (cyclohexylenediaminetetraacetic acid), a component of the buffer, preferentially will complex interfering cations and release free fluoride ions. Concentrations of aluminum, the most common interference, up to 3.0 mg/L can be complexed preferentially. In acid solution, F⁻ forms a poorly ionized HF·HF complex but the buffer maintains a pH above 5 to minimize hydrogen fluoride complex formation. In alkaline solution hydroxide ion also can interfere with electrode response to fluoride ion whenever the hydroxide ion concentration is greater than one-tenth the concentration of fluoride ion. At the pH maintained by the buffer, no hydroxide interference occurs.

Fluoborates are widely used in industrial processes. Dilute solutions of fluoborate or fluoboric acid hydrolyze to liberate fluoride ion but in concentrated solutions, as in electroplating wastes, hydrolysis does not occur completely. Distill such samples or measure fluoborate with a fluoborate-selective electrode. Also distill the sample if the dissolved solids concentration exceeds 10 000 mg/L.

2. Apparatus

- a. Expanded-scale or digital pH meter or ion-selective meter.*
- b. Sleeve-type reference electrode:* Do not use fiber-tip reference electrodes because they exhibit erratic behavior in very dilute solutions.
- c. Fluoride electrode.*
- d. Magnetic stirrer, with TFE-coated stirring bar.*
- e. Timer.*

3. Reagents

- a. Stock fluoride solution:* Dissolve 221.0 mg anhydrous sodium fluoride, NaF, in distilled water and dilute to 1000 mL; 1.00 mL = 100 µg F⁻.
- b. Standard fluoride solution:* Dilute 100 mL stock fluoride solution to 1000 mL with distilled water; 1.00 mL = 10.0 µg F⁻.
- c. Fluoride buffer:* Place approximately 500 mL distilled water in a 1-L beaker and add 57

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mL glacial acetic acid, 58 g NaCl, and 4.0 g 1,2 cyclohexylenediaminetetraacetic acid (CDTA).*(39) Stir to dissolve. Place beaker in a cool water bath and add slowly 6N NaOH (about 125 mL) with stirring, until pH is between 5.3 and 5.5. Transfer to a 1-L volumetric flask and add distilled water to the mark. This buffer, as well as a more concentrated version, is available commercially. In using the concentrated buffer follow the manufacturer's directions.

4. Procedure

a. Instrument calibration: No major adjustment of any instrument normally is required to use electrodes in the range of 0.2 to 2.0 mg F⁻/L. For those instruments with zero at center scale adjust calibration control so that the 1.0 mg F⁻/L standard reads at the center zero (100 mV) when the meter is in the expanded-scale position. This cannot be done on some meters that do not have a millivolt calibration control. To use a selective-ion meter follow the manufacturer's instructions.

b. Preparation of fluoride standards: Prepare a series of standards by diluting with distilled water 5.0, 10.0, and 20.0 mL of standard fluoride solution to 100 mL with distilled water. These standards are equivalent to 0.5, 1.0, and 2.0 mg F⁻/L.

c. Treatment of standards and sample: In 100-mL beakers or other convenient containers add by volumetric pipet from 10 to 25 mL standard or sample. Bring standards and sample to same temperature, preferably room temperature. Add an equal volume of buffer. The total volume should be sufficient to immerse the electrodes and permit operation of the stirring bar.

d. Measurement with electrode: Immerse electrodes in each of the fluoride standard solutions and measure developed potential while stirring on a magnetic stirrer. Avoid stirring before immersing electrodes because entrapped air around the crystal can produce erroneous readings or needle fluctuations. Let electrodes remain in the solution 3 min (or until reading is constant) before taking a final millivolt reading. A layer of insulating material between stirrer and beaker minimizes solution heating. Withdraw electrodes, rinse with distilled water, and *blot dry* between readings. (CAUTION: Blotting may poison electrode if not done gently.) Repeat measurements with samples.

When using an expanded-scale pH meter or selective-ion meter, frequently recalibrate the electrode by checking potential reading of the 1.00-mg F⁻/L standard and adjusting the calibration control, if necessary, until meter reads as before.

If a direct-reading instrument is not used, plot potential measurement of fluoride standards against concentration on two-cycle semilogarithmic graph paper. Plot milligrams F⁻ per liter on the logarithmic axis (ordinate), with the lowest concentration at the bottom of the graph. Plot millivolts on the abscissa. From the potential measurement for each sample, read the corresponding fluoride concentration from the standard curve.

The known-additions method may be substituted for the calibration method described. Follow the directions of the instrument manufacturer.

Selective-ion meters may necessitate using a slightly altered procedure, such as preparing

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1.00 and 10.0 mg F⁻/L standards or some other concentration. Follow the manufacturer's directions. Commercial standards, often already diluted with buffer, frequently are supplied with the meter. Verify the stated fluoride concentration of these standards by comparing them with standards prepared by the analyst.

5. Calculation

$$\text{mg F}^{-}/\text{L} = \frac{\mu\text{g F}^{-}}{\text{mL sample}}$$

6. Precision and Bias

A synthetic sample containing 0.850 mg F⁻/L in distilled water was analyzed in 111 laboratories by the electrode method, with a relative standard deviation of 3.6% and a relative error of 0.7%.

A second synthetic sample containing 0.750 mg F⁻/L, 2.5 mg (NaPO₃)₆/L, and 300 mg alkalinity/L added as NaHCO₃, was analyzed in 111 laboratories by the electrode method, with a relative standard deviation of 4.8% and a relative error of 0.2%.

A third synthetic sample containing 0.900 mg F⁻/L, 0.500 mg Al/L, and 200 mg SO₄²⁻/L was analyzed in 13 laboratories by the electrode method, with a relative standard deviation of 2.9% and a relative error of 4.9%.

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4500-F⁻ D. SPADNS Method

1. General Discussion

a. *Principle:* The SPADNS colorimetric method is based on the reaction between fluoride and a zirconium-dye lake. Fluoride reacts with the dye lake, dissociating a portion of it into a colorless complex anion (ZrF₆²⁻); and the dye. As the amount of fluoride increases, the color produced becomes progressively lighter.

The reaction rate between fluoride and zirconium ions is influenced greatly by the acidity of the reaction mixture. If the proportion of acid in the reagent is increased, the reaction can be made almost instantaneous. Under such conditions, however, the effect of various ions differs

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from that in the conventional alizarin methods. The selection of dye for this rapid fluoride method is governed largely by the resulting tolerance to these ions.

b. Interference: Table 4500-F⁻:I lists common interferences. Because these are neither linear in effect nor algebraically additive, mathematical compensation is impossible. Whenever any one substance is present in sufficient quantity to produce an error of 0.1 mg/ L or whenever the total interfering effect is in doubt, distill the sample. Also distill colored or turbid samples. In some instances, sample dilution or adding appropriate amounts of interfering substances to the standards may be used to compensate for the interference effect. If alkalinity is the only significant interference, neutralize it with either hydrochloric or nitric acid. Chlorine interferes and provision for its removal is made.

Volumetric measurement of sample and reagent is extremely important to analytical accuracy. Use samples and standards at the same temperature or at least within 2°C. Maintain constant temperature throughout the color development period. Prepare different calibration curves for different temperature ranges.

2. Apparatus

Colorimetric equipment: One of the following is required:

- a. Spectrophotometer,* for use at 570 nm, providing a light path of at least 1 cm.
- b. Filter photometer,* providing a light path of at least 1 cm and equipped with a greenish yellow filter having maximum transmittance at 550 to 580 nm.

3. Reagents

a. Standard fluoride solution: Prepare as directed in the electrode method, Section 4500-F⁻.C.3b.

b. SPADNS solution: Dissolve 958 mg SPADNS, sodium 2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalene disulfonate, also called 4,5-dihydroxy-3-(parasulfophenylazo)-2,7-naphthalenedisulfonic acid trisodium salt, in distilled water and dilute to 500 mL. This solution is stable for at least 1 year if protected from direct sunlight.

c. Zirconyl-acid reagent: Dissolve 133 mg zirconyl chloride octahydrate, $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$, in about 25 mL distilled water. Add 350 mL conc HCl and dilute to 500 mL with distilled water.

d. Acid zirconyl-SPADNS reagent: Mix equal volumes of SPADNS solution and zirconyl-acid reagent. The combined reagent is stable for at least 2 years.

e. Reference solution: Add 10 mL SPADNS solution to 100 mL distilled water. Dilute 7 mL conc HCl to 10 mL and add to the diluted SPADNS solution. The resulting solution, used for setting the instrument reference point (zero), is stable for at least 1 year. Alternatively, use a prepared standard of 0 mg F⁻/L as a reference.

f. Sodium arsenite solution: Dissolve 5.0 g NaAsO_2 and dilute to 1 L with distilled water.

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(CAUTION: *Toxic—avoid ingestion.*)

4. Procedure

a. Preparation of standard curve: Prepare fluoride standards in the range of 0 to 1.40 mg F⁻/L by diluting appropriate quantities of standard fluoride solution to 50 mL with distilled water. Pipet 5.00 mL each of SPADNS solution and zirconyl-acid reagent, or 10.00 mL mixed acid-zirconyl-SPADNS reagent, to each standard and mix well. Avoid contamination. Set photometer to zero absorbance with the reference solution and obtain absorbance readings of standards. Plot a curve of the milligrams fluoride-absorbance relationship. Prepare a new standard curve whenever a fresh reagent is made or a different standard temperature is desired. As an alternative to using a reference, set photometer at some convenient point (0.300 or 0.500 absorbance) with the prepared 0 mg F⁻/L standard.

b. Sample pretreatment: If the sample contains residual chlorine, remove it by adding 1 drop (0.05 mL) NaAsO₂ solution/ 0.1 mg residual chlorine and mix. (Sodium arsenite concentrations of 1300 mg/L produce an error of 0.1 mg/L at 1.0 mg F⁻/L.)

c. Color development: Use a 50.0-mL sample or a portion diluted to 50 mL with distilled water. Adjust sample temperature to that used for the standard curve. Add 5.00 mL each of SPADNS solution and zirconyl-acid reagent, or 10.00 mL acid-zirconyl-SPADNS reagent; mix well and read absorbance, first setting the reference point of the photometer as above. If the absorbance falls beyond the range of the standard curve, repeat using a diluted sample.

5. Calculation

$$\text{mg F}^{-}/\text{L} = \frac{A}{\text{mL sample}} \times \frac{B}{C}$$

where:

A = $\mu\text{g F}^{-}$ determined from plotted curve,

B = final volume of diluted sample, mL, and

C = volume of diluted sample used for color development, mL.

When the prepared 0 mg F⁻/L standard is used to set the photometer, alternatively calculate fluoride concentration as follows:

$$\text{mg F}^{-}/\text{L} = \frac{A_0 - A_x}{A_0 - A_1}$$

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where:

A_0 = absorbance of the prepared 0 mg F⁻/L standard,

A_1 = absorbance of a prepared 1.0 mg F⁻/L standard, and

A_x = absorbance of the prepared sample.

6. Precision and Bias

A synthetic sample containing 0.830 mg F⁻/L and no interference in distilled water was analyzed in 53 laboratories by the SPADNS method, with a relative standard deviation of 8.0% and a relative error of 1.2%. After direct distillation of the sample, the relative standard deviation was 11.0% and the relative error 2.4%.

A synthetic sample containing 0.570 mg F⁻/L, 10 mg Al/L, 200 mg SO₄²⁻/L, and 300 mg total alkalinity/L was analyzed in 53 laboratories by the SPADNS method without distillation, with a relative standard deviation of 16.2% and a relative error of 7.0%. After direct distillation of the sample, the relative standard deviation was 17.2% and the relative error 5.3%.

A synthetic sample containing 0.680 mg F⁻/L, 2 mg Al/L, 2.5 mg (NaPO₃)₆/L, 200 mg SO₄²⁻/L, and 300 mg total alkalinity/L was analyzed in 53 laboratories by direct distillation and SPADNS methods with a relative standard deviation of 2.8% and a relative error of 5.9%.

7. Bibliography

BELLACK, E. & P.J. SCHOUBOE. 1968. Rapid photometric determination of fluoride with SPADNS-zirconium lake. *Anal. Chem.* 30:2032.

4500-F⁻ E. Complexone Method

1. General Discussion

a. Principle: The sample is distilled in the automated system, and the distillate is reacted with alizarin fluorine blue-lanthanum reagent to form a blue complex that is measured colorimetrically at 620 nm.

b. Interferences: Interferences normally associated with the determination of fluoride are removed by distillation.

c. Application: This method is applicable to potable, surface, and saline waters as well as domestic and industrial wastewaters. The range of the method, which can be modified by using the adjustable colorimeter, is 0.1 to 2.0 mg F⁻/L.

2. Apparatus

An example of the required continuous-flow analytical instrument consists of the interchangeable components in the number and manner indicated in Figure 4500-F⁻:2.

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3. Reagents

a. *Standard fluoride solution:* Prepare in appropriate concentrations from 0.10 to 2.0 mg F⁻/L using the stock fluoride solution (see Section 4500-F⁻.C.3a).

b. *Distillation reagent:* Add 50 mL conc H₂SO₄ to about 600 mL distilled water. Add 10.00 mL stock fluoride solution (see Section 4500-F⁻.C.3a; 1.00 mL = 100 µg F⁻) and dilute to 1000 mL.

c. *Acetate buffer solution:* Dissolve 60 g anhydrous sodium acetate, NaC₂H₃O₂, in about 600 mL distilled water. Add 100 mL conc (glacial) acetic acid and dilute to 1 L.

d. *Alizarin fluorine blue stock solution:* Add 960 mg alizarin fluorine,*†(40) C₁₄H₇O₄·CH₂N(CH₂·COOH)₂, to 100 mL distilled water. Add 2 mL conc NH₄OH and mix until dye is dissolved. Add 2 mL conc (glacial) acetic acid, dilute to 250 mL and store in an amber bottle in the refrigerator.

e. *Lanthanum nitrate stock solution:* Dissolve 1.08 g La(NO₃)₃ in about 100 mL distilled water, dilute to 250 mL, and store in refrigerator.

f. *Working color reagent:* Mix in the following order: 300 mL acetate buffer solution, 150 mL acetone, 50 mL tertiary butanol, 36 mL alizarin fluorine blue stock solution, 40 mL lanthanum nitrate stock solution, and 2 mL polyoxyethylene 23 lauryl ether.†‡(41) Dilute to 1 L with distilled water. This reagent is stable for 2 to 4 d.

4. Procedure

No special handling or preparation of sample is required.

Set up manifold as shown in Figure 4500-F⁻:2 and follow the manufacturer's instructions.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against constituent concentrations in standards. Compute sample concentrations by comparing sample response with standard curve.

6. Precision and Bias

In a single laboratory four samples of natural water containing from 0.40 to 0.82 mg F⁻/L were analyzed in septuplicate. Average precision was ± 0.03 mg F⁻/L. To two of the samples, additions of 0.20 and 0.80 mg F⁻/L were made. Average recovery of the additions was 98%.

7. Bibliography

WEINSTEIN, L.H., R.H. MANDL, D.C. MCCUNE, J.S. JACOBSON & A.E. HITCHCOCK. 1963. A semi-automated method for the determination of fluorine in air and plant tissues. *Boyce Thompson Inst.* 22:207.

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4500-F⁻ F. (Reserved)

4500-F⁻ G. Ion-Selective Electrode Flow Injection Analysis (PROPOSED)

1. General Discussion

a. *Principle:* Fluoride is determined potentiometrically by using a combination fluoride-selective electrode in a flow cell. The fluoride electrode consists of a lanthanum fluoride crystal across which a potential is developed by fluoride ions. The reference cell is a Ag/AgCl/Cl⁻ cell. The reference junction is of the annular liquid-junction type and encloses the fluoride-sensitive crystal.

Also see Section 4500-F⁻.C and Section 4130, Flow Injection Analysis (FIA).

b. *Interferences:* Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

The polyvalent cations Si⁴⁺, Al³⁺, and Fe³⁺ interfere by forming complexes with fluoride. As part of the buffer reagent, 1,2-cyclohexyldiaminetetraacetic acid (CDTA) is added to preferentially complex these cations and eliminate this interference when these concentrations do not exceed 3.0 mg Al³⁺/L and 20 mg Fe³⁺/L.

Some interferents are removed by distillation; see Section 4500-F⁻.B. Drinking water samples generally do not require sample distillation.

2. Apparatus

Flow injection analysis equipment consisting of:

a. *FIA injection valve* with sample loop or equivalent.

b. *Multichannel proportioning pump.*

c. *FIA manifold* (Figure 4500-F⁻:3) with tubing heater and ion-selective electrode flow cell.

In Figure 4500-F⁻:3, relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

d. *Combination ion-selective electrode.*

e. *Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min.

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a. *Carrier*, 1.0 mg F⁻/L: Add 10 mL or 10 g stock fluoride standard (§ 3d) to 990 mL water and mix well.

b. *Buffer*: To a tared 1-L polyethylene container add 929.5 g water, 59.8 g glacial acetic acid, 30.0 g sodium hydroxide, NaOH, 58.0 g sodium chloride, NaCl, 0.5 g stock fluoride standard (§ 3d), and 4.0 g 1,2-cyclohexyldiaminetetraacetic acid (CDTA) (also called trans-1,2-diaminocyclohexane). Stir on a magnetic stir plate until all material has dissolved.

c. *Electrode conditioning solution*: To a tared 1-L container, add 534 g buffer (§ 3b) and 500 g carrier (§ 3a). Shake or stir to mix thoroughly. Store fluoride electrode in this solution when it is not in use.

d. *Stock fluoride standard*, 100.0 mg F⁻/L: In a 1-L volumetric flask, dissolve 0.2210 g sodium fluoride, NaF, in approximately 950 mL water. Dilute to mark with water and mix well. Store in a polyethylene bottle.

e. *Standard fluoride solutions*: Prepare fluoride standards in the desired concentration range, using the stock standard (§ 3d), and diluting with water. A blank or zero concentration standard cannot be prepared for this method because it will give an undefined response from the fluoride electrode.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-F⁻:3 and follow method supplied by manufacturer or laboratory standard operating procedure for this method. Follow quality control procedures outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting the electrode response to standards processed through the manifold vs. fluoride concentration. Standards greater than 1.0 mg F⁻/L will give positive peaks, standards less than 1.0 mg F⁻/L will give negative peaks, and the 1.0 mg F⁻/L standard having the same concentration as the carrier will give no peak. The calibration curve gives a good fit to a second-order polynomial.

It is not necessary to plot the response versus log[F⁻]; if this is done the calibration curve will still be a second-order polynomial because there is a concentration-dependent kinetic effect in the flowing stream electrode system.

6. Precision and Bias

The samples used in the studies described below were not distilled.

a. *Recovery and relative standard deviation*: The results of single-laboratory studies with various matrices are given in Table 4500-F⁻:II.

b. *MDL*: A 390-μL sample loop was used in the method described above. Ten replicates of a 1.0-mg F⁻/L standard were run to obtain an MDL of 0.02 mg F⁻/L.

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c. *Precision:* Ten replicate standards of 2.0 mg F⁻/L gave a % RSD of 0.5%.

4500-H⁺ PH VALUE*(42)

4500-H⁺ A. Introduction

1. Principles

Measurement of pH is one of the most important and frequently used tests in water chemistry. Practically every phase of water supply and wastewater treatment, e.g., acid-base neutralization, water softening, precipitation, coagulation, disinfection, and corrosion control, is pH-dependent. pH is used in alkalinity and carbon dioxide measurements and many other acid-base equilibria. At a given temperature the *intensity* of the acidic or basic character of a solution is indicated by pH or hydrogen ion activity. Alkalinity and acidity are the acid- and base-neutralizing capacities of a water and usually are expressed as milligrams CaCO₃ per liter. Buffer capacity is the amount of strong acid or base, usually expressed in moles per liter, needed to change the pH value of a 1-L sample by 1 unit. pH as defined by Sorenson¹ is $-\log [H^+]$; it is the “intensity” factor of acidity. Pure water is very slightly ionized and at equilibrium the ion product is

$$\begin{aligned} [H^+][OH^-] &= K_w \\ &= 1.01 \times 10^{-14} \text{ at } 25^\circ\text{C} \end{aligned} \quad (1)$$

and

$$\begin{aligned} [H^+] &= [OH^-] \\ &= 1.005 \times 10^{-7} \end{aligned}$$

where:

$[H^+]$ = activity of hydrogen ions, moles/L,
 $[OH^-]$ = activity of hydroxyl ions, moles/L, and
 K_w = ion product of water.

Because of ionic interactions in all but very dilute solutions, it is necessary to use the “activity” of an ion and not its molar concentration. Use of the term pH assumes that the activity of the hydrogen ion, a_{H^+} , is being considered. The *approximate* equivalence to molarity, $[H^+]$ can be presumed only in very dilute solutions (ionic strength <0.1).

A logarithmic scale is convenient for expressing a wide range of ionic activities. Equation 1

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in logarithmic form and corrected to reflect activity is:

$$(-\log_{10} a_{\text{H}^+}) + (-\log_{10} a_{\text{OH}^-}) = 14 \quad (2)$$

or

$$\text{pH} + \text{pOH} = \text{p}K_{\text{w}}$$

where:

$$\text{pH} = -\log_{10} a_{\text{H}^+} \text{ and}$$

$$\text{pOH} = -\log_{10} a_{\text{OH}^-}.$$

Equation 2 states that as pH increases pOH decreases correspondingly and vice versa because $\text{p}K_{\text{w}}$ is constant for a given temperature. At 25°C, pH 7.0 is neutral, the activities of the hydrogen and hydroxyl ions are equal, and each corresponds to an approximate activity of 10^{-7} moles/L. The neutral point is temperature-dependent and is pH 7.5 at 0°C and pH 6.5 at 60°C.

The pH value of a highly dilute solution is approximately the same as the negative common logarithm of the hydrogen ion concentration. Natural waters usually have pH values in the range of 4 to 9, and most are slightly basic because of the presence of bicarbonates and carbonates of the alkali and alkaline earth metals.

2. Reference

1. SORENSON, S. 1909. Über die Messung und die Bedeutung der Wasserstoff ionen Konzentration bei Enzymatischen Prozessen. *Biochem. Z.* 21:131.

4500-H⁺ B. Electrometric Method

1. General Discussion

a. Principle: The basic principle of electrometric pH measurement is determination of the activity of the hydrogen ions by potentiometric measurement using a standard hydrogen electrode and a reference electrode. The hydrogen electrode consists of a platinum electrode across which hydrogen gas is bubbled at a pressure of 101 kPa. Because of difficulty in its use and the potential for poisoning the hydrogen electrode, the glass electrode commonly is used. The electromotive force (emf) produced in the glass electrode system varies linearly with pH. This linear relationship is described by plotting the measured emf against the pH of different buffers. Sample pH is determined by extrapolation.

Because single ion activities such as a_{H^+} cannot be measured, pH is defined operationally on a potentiometric scale. The pH measuring instrument is calibrated potentiometrically with an

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indicating (glass) electrode and a reference electrode using National Institute of Standards and Technology (NIST) buffers having assigned values so that:

$$\text{pH}_B = -\log_{10} a_{\text{H}^+}$$

where:

pH_B = assigned pH of NIST buffer.

The operational pH scale is used to measure sample pH and is defined as:

$$\text{pH}_x = \text{pH}_B \pm \frac{F(E_x - E_s)}{2.303 RT}$$

where:

pH_x = potentiometrically measured sample pH,

F = Faraday: 9.649×10^4 coulomb/mole,

E_x = sample emf, V,

E_s = buffer emf, V,

R = gas constant; 8.314 joule/(mole °K), and

T = absolute temperature, °K.

NOTE: Although the equation for pH_x appears in the literature with a plus sign, the sign of emf readings in millivolts for most pH meters manufactured in the U.S. is negative. The choice of negative sign is consistent with the IUPAC Stockholm convention concerning the sign of electrode potential.^{1,2}

The activity scale gives values that are higher than those on Sorenson's scale by 0.04 units:

$$\text{pH (activity)} = \text{pH (Sorenson)} + 0.04$$

The equation for pH_x assumes that the emf of the cells containing the sample and buffer is due solely to hydrogen ion activity unaffected by sample composition. In practice, samples will have varying ionic species and ionic strengths, both affecting H^+ activity. This imposes an experimental limitation on pH measurement; thus, to obtain meaningful results, the differences between E_x and E_s should be minimal. Samples must be dilute aqueous solutions of simple solutes (<0.2M). (Choose buffers to bracket the sample.) Determination of pH cannot be made accurately in nonaqueous media, suspensions, colloids, or high-ionic-strength solutions.

b. Interferences: The glass electrode is relatively free from interference from color, turbidity, colloidal matter, oxidants, reductants, or high salinity, except for a sodium error at $\text{pH} > 10$.

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Reduce this error by using special “low sodium error” electrodes.

pH measurements are affected by temperature in two ways: mechanical effects that are caused by changes in the properties of the electrodes and chemical effects caused by equilibrium changes. In the first instance, the Nernstian slope increases with increasing temperature and electrodes take time to achieve thermal equilibrium. This can cause long-term drift in pH. Because chemical equilibrium affects pH, standard pH buffers have a specified pH at indicated temperatures.

Always report temperature at which pH is measured.

2. Apparatus

a. pH meter consisting of potentiometer, a glass electrode, a reference electrode, and a temperature-compensating device. A circuit is completed through the potentiometer when the electrodes are immersed in the test solution. Many pH meters are capable of reading pH or millivolts and some have scale expansion that permits reading to 0.001 pH unit, but most instruments are not that precise.

For routine work use a pH meter accurate and reproducible to 0.1 pH unit with a range of 0 to 14 and equipped with a temperature-compensation adjustment.

Although manufacturers provide operating instructions, the use of different descriptive terms may be confusing. For most instruments, there are two controls: intercept (set buffer, asymmetry, standardize) and slope (temperature, offset); their functions are shown diagrammatically in Figure 4500-H⁺:1 and Figure 4500-H⁺:2. The intercept control shifts the response curve laterally to pass through the isopotential point with no change in slope. This permits bringing the instrument on scale (0 mV) with a pH 7 buffer that has no change in potential with temperature.

The slope control rotates the emf/pH slope about the isopotential point (0 mV/pH 7). To adjust slope for temperature without disturbing the intercept, select a buffer that brackets the sample with pH 7 buffer and adjust slope control to pH of this buffer. The instrument will indicate correct millivolt change per unit pH at the test temperature.

b. Reference electrode consisting of a half cell that provides a constant electrode potential. Commonly used are calomel and silver: silver-chloride electrodes. Either is available with several types of liquid junctions.

The liquid junction of the reference electrode is critical because at this point the electrode forms a salt bridge with the sample or buffer and a liquid junction potential is generated that in turn affects the potential produced by the reference electrode. Reference electrode junctions may be annular ceramic, quartz, or asbestos fiber, or the sleeve type. The quartz type is most widely used. The asbestos fiber type is not recommended for strongly basic solutions. Follow the manufacturer's recommendation on use and care of the reference electrode.

Refill nonsealed electrodes with the correct electrolyte to proper level and make sure junction is properly wetted.

c. Glass electrode: The sensor electrode is a bulb of special glass containing a fixed concentration of HCl or a buffered chloride solution in contact with an internal reference

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electrode. Upon immersion of a new electrode in a solution the outer bulb surface becomes hydrated and exchanges sodium ions for hydrogen ions to build up a surface layer of hydrogen ions. This, together with the repulsion of anions by fixed, negatively charged silicate sites, produces at the glass-solution interface a potential that is a function of hydrogen ion activity in solution.

Several types of glass electrodes are available. Combination electrodes incorporate the glass and reference electrodes into a single probe. Use a “low sodium error” electrode that can operate at high temperatures for measuring pH over 10 because standard glass electrodes yield erroneously low values. For measuring pH below 1 standard glass electrodes yield erroneously high values; use liquid membrane electrodes instead.

d. Beakers: Preferably use polyethylene or TFE*#(44) beakers.

e. Stirrer: Use either a magnetic, TFE-coated stirring bar or a mechanical stirrer with inert plastic-coated impeller.

f. Flow chamber: Use for continuous flow measurements or for poorly buffered solutions.

3. Reagents

a. General preparation: Calibrate the electrode system against standard buffer solutions of known pH. Because buffer solutions may deteriorate as a result of mold growth or contamination, prepare fresh as needed for accurate work by weighing the amounts of chemicals specified in Table 4500-H+:I, dissolving in distilled water at 25°C, and diluting to 1000 mL. This is particularly important for borate and carbonate buffers.

Boil and cool distilled water having a conductivity of less than 2 $\mu\text{mhos/cm}$. To 50 mL add 1 drop of saturated KCl solution suitable for reference electrode use. If the pH of this test solution is between 6.0 and 7.0, use it to prepare all standard solutions.

Dry KH_2PO_4 at 110 to 130°C for 2 h before weighing but do not heat unstable hydrated potassium tetroxalate above 60°C nor dry the other specified buffer salts.

Although ACS-grade chemicals generally are satisfactory for preparing buffer solutions, use certified materials available from the National Institute of Standards and Technology when the greatest accuracy is required. For routine analysis, use commercially available buffer tablets, powders, or solutions of tested quality. In preparing buffer solutions from solid salts, insure complete solution.

As a rule, select and prepare buffer solutions classed as primary standards in Table 4500-H+:I; reserve secondary standards for extreme situations encountered in wastewater measurements. Consult Table 4500-H+:II for accepted pH of standard buffer solutions at temperatures other than 25°C. In routine use, store buffer solutions and samples in polyethylene bottles. Replace buffer solutions every 4 weeks.

b. Saturated potassium hydrogen tartrate solution: Shake vigorously an excess (5 to 10 g) of finely crystalline $\text{KHC}_4\text{H}_4\text{O}_6$ with 100 to 300 mL distilled water at 25°C in a glass-stoppered bottle. Separate clear solution from undissolved material by decantation or filtration. Preserve for

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2 months or more by adding one thymol crystal (8 mm diam) per 200 mL solution.

c. Saturated calcium hydroxide solution: Calcine a well-washed, low-alkali grade CaCO_3 in a platinum dish by igniting for 1 h at 1000°C . Cool, hydrate by slowly adding distilled water with stirring, and heat to boiling. Cool, filter, and collect solid Ca(OH)_2 on a fritted glass filter of medium porosity. Dry at 110°C , cool, and pulverize to uniformly fine granules. Vigorously shake an excess of fine granules with distilled water in a stoppered polyethylene bottle. Let temperature come to 25°C after mixing. Filter supernatant under suction through a sintered glass filter of medium porosity and use filtrate as the buffer solution. Discard buffer solution when atmospheric CO_2 causes turbidity to appear.

d. Auxiliary solutions: 0.1N NaOH, 0.1N HCl, 5N HCl (dilute five volumes 6N HCl with one volume distilled water), and acid potassium fluoride solution (dissolve 2 g KF in 2 mL conc H_2SO_4 and dilute to 100 mL with distilled water).

4. Procedure

a. Instrument calibration: In each case follow manufacturer's instructions for pH meter and for storage and preparation of electrodes for use. Recommended solutions for short-term storage of electrodes vary with type of electrode and manufacturer, but generally have a conductivity greater than $4000\ \mu\text{mhos/cm}$. Tap water is a better substitute than distilled water, but pH 4 buffer is best for the single glass electrode and saturated KCl is preferred for a calomel and Ag/AgCl reference electrode. Saturated KCl is the preferred solution for a combination electrode. Keep electrodes wet by returning them to storage solution whenever pH meter is not in use.

Before use, remove electrodes from storage solution, rinse, blot dry with a soft tissue, place in initial buffer solution, and set the isopotential point (§ 2a above). Select a second buffer within 2 pH units of sample pH and bring sample and buffer to same temperature, which may be the room temperature, a fixed temperature such as 25°C , or the temperature of a fresh sample. Remove electrodes from first buffer, rinse thoroughly with distilled water, blot dry, and immerse in second buffer. Record temperature of measurement and adjust temperature dial on meter so that meter indicates pH value of buffer at test temperature (this is a slope adjustment).

Use the pH value listed in the tables for the buffer used at the test temperature. Remove electrodes from second buffer, rinse thoroughly with distilled water and dry electrodes as indicated above. Immerse in a third buffer below pH 10, approximately 3 pH units different from the second; the reading should be within 0.1 unit for the pH of the third buffer. If the meter response shows a difference greater than 0.1 pH unit from expected value, look for trouble with the electrodes or potentiometer (see § 5a and § 5b below).

The purpose of standardization is to adjust the response of the glass electrode to the instrument. When only occasional pH measurements are made standardize instrument before each measurement. When frequent measurements are made and the instrument is stable, standardize less frequently. If sample pH values vary widely, standardize for each sample with a buffer having a pH within 1 to 2 pH units of the sample.

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b. Sample analysis: Establish equilibrium between electrodes and sample by stirring sample to insure homogeneity; stir gently to minimize carbon dioxide entrainment. For buffered samples or those of high ionic strength, condition electrodes after cleaning by dipping them into sample for 1 min. Blot dry, immerse in a fresh portion of the same sample, and read pH.

With dilute, poorly buffered solutions, equilibrate electrodes by immersing in three or four successive portions of sample. Take a fresh sample to measure pH.

5. Trouble Shooting

a. Potentiometer: To locate trouble source disconnect electrodes and, using a short-circuit strap, connect reference electrode terminal to glass electrode terminal. Observe change in pH when instrument calibration knob is adjusted. If potentiometer is operating properly, it will respond rapidly and evenly to changes in calibration over a wide scale range. A faulty potentiometer will fail to respond, will react erratically, or will show a drift upon adjustment. Switch to the millivolt scale on which the meter should read zero. If inexperienced, do not attempt potentiometer repair other than maintenance as described in instrument manual.

b. Electrodes: If potentiometer is functioning properly, look for the instrument fault in the electrode pair. Substitute one electrode at a time and cross-check with two buffers that are about 4 pH units apart. A deviation greater than 0.1 pH unit indicates a faulty electrode. Glass electrodes fail because of scratches, deterioration, or accumulation of debris on the glass surface. Rejuvenate electrode by alternately immersing it three times each in 0.1N HCl and 0.1N NaOH. If this fails, immerse tip in KF solution for 30 s. After rejuvenation, soak in pH 7.0 buffer overnight. Rinse and store in pH 7.0 buffer. Rinse again with distilled water before use. Protein coatings can be removed by soaking glass electrodes in a 10% pepsin solution adjusted to pH 1 to 2.

To check reference electrode, oppose the emf of a questionable reference electrode against another one of the same type that is known to be good. Using an adapter, plug good reference electrode into glass electrode jack of potentiometer; then plug questioned electrode into reference electrode jack. Set meter to read millivolts and take readings with both electrodes immersed in the same electrolyte (KCl) solution and then in the same buffer solution. The millivolt readings should be 0 ± 5 mV for both solutions. If different electrodes are used, i.e., silver: silver-chloride against calomel or vice versa, the reading will be 44 ± 5 mV for a good reference electrode.

Reference electrode troubles generally are traceable to a clogged junction. Interruption of the continuous trickle of electrolyte through the junction causes increase in response time and drift in reading. Clear a clogged junction by applying suction to the tip or by boiling tip in distilled water until the electrolyte flows freely when suction is applied to tip or pressure is applied to the fill hole. Replaceable junctions are available commercially.

6. Precision and Bias

By careful use of a laboratory pH meter with good electrodes, a precision of ± 0.02 pH unit and an accuracy of ± 0.05 pH unit can be achieved. However, ± 0.1 pH unit represents the limit of

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accuracy under normal conditions, especially for measurement of water and poorly buffered solutions. For this reason, report pH values to the nearest 0.1 pH unit. A synthetic sample of a Clark and Lubs buffer solution of pH 7.3 was analyzed electrometrically by 30 laboratories with a standard deviation of ± 0.13 pH unit.

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4500-I IODINE*#(45)

4500-I A. Introduction

1. Uses and Forms

Elemental iodine is not a natural constituent of natural waters. Iodine may be added to potable and swimming pool waters as a disinfectant. For wastewaters, iodine has had limited application. Use of iodine generally is restricted to personal or remote water supplies where ease of application, storage stability, and an inertness toward organic matter are important considerations. Some swimming pool waters are treated with iodine to lessen eye burn among swimmers and to provide a stable disinfectant residual less affected by adverse environmental conditions.

Iodine is applied in the elemental form or produced in situ by the simultaneous addition of an iodide salt and a suitable oxidant. In the latter case, an excess of iodide may be maintained to serve as a reservoir for iodine production; the determination of iodide is desirable for disinfectant control (see Iodide, Section 4500-I).

Elemental I_2 can undergo hydrolysis to form hypoiodous acid (HOI), which can dissociate to form hypoiodite (OI^-) under strongly basic conditions. Hypoiodous acid/hypoiodite ion may further disproportionate to form iodate. In the presence of excess iodide, iodine may react with iodide to form tri-iodide ion (I_3^-). The rate and the extent to which these reactions may occur depend on pH and the concentration of iodide in the solution. Basic conditions favor formation of hypoiodite and iodate. Acidic conditions and the presence of iodide favor formation of iodine and tri-iodide ion. Thus, the relative concentrations of these iodine species in the resulting solution can be quite variable. Hypoiodous acid/hypoiodite also can act as an iodinating agent, reacting with organic compounds to form iodinated organic compounds. Elemental I_2 , hypoiodous acid, hypoiodite ion, and tri-iodide ion are considered active iodine. There is no generally accepted method for the determination of each of these species individually. Most analytical methods use the oxidizing power of all forms of active iodine for its determination and the results usually are expressed as an equivalent concentration of elemental iodine. The effects of iodate or dissolved organic iodine on these methods have not been thoroughly investigated.

2. Selection of Method

For potable and swimming pool waters treated with elemental iodine, both the amperometric titration and leuco crystal violet colorimetric methods give acceptable results. However, oxidized forms of manganese interfere with the leuco crystal violet method. Where the iodide and chloride ion concentrations are above 50 mg/L and 200 mg/L, respectively, interference in color production may occur in the leuco crystal violet method and the amperometric method is

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preferred. However, because of the extreme sensitivity of the leuco crystal violet method, this interference may be eliminated by sample dilution to obtain halogen ion concentrations less than 50 mg/L.

For wastewaters or highly polluted waters, organic constituents normally do not interfere with either the amperometric or leuco crystal violet procedures. Determine which of the methods yields the more acceptable results, because specific substances present in these waters may interfere in one method but not in the other. Certain metallic cations such as copper and silver interfere in the amperometric titration procedure. Iodate, which is a naturally occurring species of iodine in marine waters, will also interfere in the amperometric titration by reacting with excess iodide under acidic conditions to form I_2 and/or I_3^- . The rate of the reaction is most pH-dependent between pH 3 and 5. Thus, the magnitude of this interference may depend on the concentration of iodate present and the analytical conditions. The leuco crystal violet method is relatively free of interference from these and other cations and anions with the exceptions noted previously.

For waters containing iodine coexisting with free chlorine, combined chlorine, or other excess oxidants, of the methods described only the leuco crystal violet method can determine iodine specifically. This condition occurs in the in-situ production of iodine by the reaction of iodide and excess oxidant. Under these conditions, the amperometric method would continue to titrate the iodine produced in a cyclic reaction until exhaustion of the oxidant.

4500-I B. Leuco Crystal Violet Method

1. General Discussion

The leuco crystal violet method determines aqueous iodine present as elemental iodine and hypiodous acid. Excess common oxidants do not interfere. While the method utilizes the sum of the oxidative power of all forms of active iodine residuals, the results are expressed as the equivalent concentration of iodine. The method also is capable of determining the sum of iodine and free iodide concentrations; the free iodide concentration can be determined by difference (see Iodide, Section 4500-I⁻).

a. Principle: Mercuric chloride added to aqueous elemental iodine solutions causes essentially complete hydrolysis of iodine and the stoichiometric production of hypiodous acid. The compound 4,4',4''-methylidynetris (*N,N*-dimethylaniline), also known by the common name of leuco crystal violet, reacts instantaneously with the hypiodous acid to form crystal violet dye. The absorbance of this dye is highly pH-dependent. The maximum absorbance is produced in the pH range of 3.5 to 4.0 and is measured at a wavelength of 592 nm. Below a pH of 3.5, the absorbance drops precipitously. Above a pH of about 4.7, the excess leuco crystal violet in the sample precipitates and masks the absorbance of the crystal violet dye. Accurate pH control is essential to maximize precision. The absorbance follows Beer's law over a wide range of iodine concentrations and the developed color is stable for several hours.

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In the presence of certain excess oxidants such as free chlorine or chloramines, the iodine residual will exist exclusively in the form of hypoiodous acid. The leuco crystal violet is relatively insensitive to the combined forms of chlorine while any free chlorine is converted to chloramine by reaction with an ammonium salt incorporated in the test reagents. All the hypoiodous acid is determined. As hypoiodous acid, the weight concentration value found, expressed as an equivalent elemental I_2 concentration, is equal to twice that of an elemental I_2 solution of the same weight concentration.

b. Interference: Oxidized forms of manganese interfere by oxidizing the indicator to crystal violet dye and yield apparent high iodine concentrations.

Iodide and chloride ion concentrations above 50 mg/L and 200 mg/L, respectively, interfere by inhibiting full color production. Dilute the sample to eliminate this interference.

Combined chlorine residuals normally do not interfere provided that the test is completed within 5 min after adding the indicator solution. Eliminate interference from free chlorine by adding an ammonium salt buffer to form combined chlorine.

c. Minimum detectable concentration: 10 $\mu\text{g I as } I_2/\text{L}$.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Filter photometer*, with a light path of 1 cm or longer, equipped with an orange filter having maximum transmittance near 592 nm.

2) *Spectrophotometer*, for use at 592 nm, with a light path of 1 cm or longer.

b. Volumetric flasks, 100-mL, with plastic caps or ground-glass stoppers.

c. Glassware: Completely remove reducing substances from glassware or plastic containers, including containers for storage of reagent solutions (see Section 4500-Cl.D.2d).

3. Reagents

a. Iodine-demand-free water: See Section 4500-I⁻.B.3a. Prepare all stock iodine and reagent solutions with iodine-demand-free water.

b. Stock iodine solution: Prepare a saturated iodine solution by dissolving 20 g elemental iodine in 300 mL water. Let stand several hours. Decant iodine solution and dilute 170 mL to 2000 mL. Standardize solution by titrating with standard sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) titrant as described in Section 4500-Cl.B.3b and *c* or amperometrically as in Section 4500-I.C.

Calculate iodine concentration:

$$\text{mg I as } I_2/\text{mL} = \text{normality of iodine solution} \times 126.9$$

Prepare a working solution of 10 $\mu\text{g I as } I_2/\text{mL}$ by appropriate dilution of the standardized

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stock solution.

c. *Citric buffer solution*, pH 3.8: See Section 4500-I⁻.B.3c.

d. *Leuco crystal violet indicator*: See Section 4500-I⁻.B.3d.

e. *Sodium thiosulfate solution*: See Section 4500-I⁻.B.3 f.

4. Procedure

a. *Preparation of temporary iodine standards*: For greater accuracy, standardize working solution immediately before use by the amperometric titration method (Method C). Prepare standards in the range of 0.1 to 6.0 mg I as I₂/L by adding 1 to 60 mL working solution to 100 mL glass-stoppered volumetric flasks, in increments of 1 mL or larger. Adjust these volumes if the measured iodine concentration of working solution varies by 5% or more from 10 µg I as I₂/mL.

Measure 50.0 mL of each diluted iodine working solution into a 100-mL glass-stoppered volumetric flask. Add 1.0 mL citric buffer solution, gently swirl to mix, and let stand for at least 30 s. Add 1.0 mL leuco crystal violet indicator and swirl to develop color. Dilute to 100 mL and mix.

b. *Photometric calibration*: Transfer colored temporary standards of known iodine concentrations to cells of 1-cm light path and read absorbance in a photometer or spectrophotometer at a wavelength of 592 nm against a distilled water reference. Plot absorbance values against iodine concentrations to construct a curve that follows Beer's law.

c. *Color development of iodine sample*: Measure 50.0 mL sample into a 100-mL volumetric flask and treat as described for preparation of temporary iodine standards, ¶ 4a. Match test sample visually with temporary standards or read absorbance photometrically and refer to standard calibration curve for the iodine equivalent.

d. *Samples containing >6.0mg I as I₂/L*: Place approximately 25mL water in a 100-mL volumetric flask. Add 1.0mL citric buffer solution and a measured volume of 25mL or less of sample. Mix and let stand for at least 30s. Add 1.0mL leuco crystal violet indicator, mix, and dilute to mark. Match visually with standards or read absorbance photometrically and compare with calibration curve from which the initial iodine is obtained by applying the dilution factor. Select one of the following sample volumes to remain within optimum iodine range:

Iodine mg/L	Sample Volume Required mL
6.0–12.0	25.0
12.0–30	10.0
30–60	5.0

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e. Samples containing both chlorine and iodine: For samples containing free or combined chlorine and iodine, follow procedure given in ¶ 4c or *d* above but read absorbance within 5 min after adding leuco crystal violet indicator.

f. Compensation for turbidity and color: Compensate for natural color or turbidity by adding 5 mL $\text{Na}_2\text{S}_2\text{O}_3$ solution to a 50-mL sample. Add reagents to sample as described previously and use as blank to set zero absorbance on the photometer. Measure all samples in relation to this blank and, from calibration curve, determine concentrations of iodine.

5. Bibliography

BLACK, A.P. & G.P. WHITTLE. 1967. New methods for the colorimetric determination of halogen residuals. Part I. Iodine, iodide, and iodate. *J. Amer. Water Works Assoc.* 59:471.

4500-I C. Amperometric Titration Method

1. General Discussion

The amperometric titration method for iodine is a modification of the amperometric method for residual chlorine (see Section 4500-Cl.D). Iodine residuals over 7 mg/L are best measured with smaller samples or by dilution. In most cases the titration results represent free iodine because combined iodine rarely is encountered.

a. Principle: The principle of the amperometric method as described for the determination of total residual chlorine is applicable to the determination of residual iodine. Iodine is determined using buffer solution, pH 4.0, and potassium iodide (KI) solution. Maintain pH at 4.0 because at pH values less than 3.5 substances such as oxidized forms of manganese interfere, while at pH values greater than 4.5, the reaction is not quantitative. Adding KI improves the sharpness of the end point.

b. Interference: Free chlorine and the interferences described in Section 4500-Cl.D.1b also interfere in the iodine determination.

2. Apparatus

See Section 4500-Cl.D.2a through *d*.

3. Reagents

With the exception of phosphate buffer solution, pH 7.0, all reagents listed for the determination of residual chlorine in Section 4500-Cl.D.3 are required. Standardized phenylarsine oxide solution (1 mL = 1 mg chlorine/L for a 200-mL sample) is equivalent to 3.58 mg I as I_2 /mL for a 200-mL sample.

4. Procedure

a. Sample volume: Select a sample volume that will require no more than 2 mL phenylarsine

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oxide titrant. For iodine concentrations of 7 mg/L or less, take a 200-mL volume; for iodine levels above 7 mg/L, use 100 mL or proportionately less diluted to 200 mL with water.

b. Free iodine: To the sample add 1 mL KI solution and 1 mL acetate buffer, pH 4.0 solution. Titrate with phenylarsine oxide titrant to the end point described in Section 4500-Cl.D.4.

5. Calculation

Calculate the iodine concentration by the following equation:

$$\text{mg I as I}_2/\text{L} = \frac{A \times 3.58 \times 200}{\text{mL sample}}$$

where:

A = mL phenylarsine oxide titration to the end point.

6. Bibliography

MARKS, H.C. & J.R. GLASS. 1942. A new method of determining residual chlorine. *J. Amer. Water Works Assoc.* 34:1227.

4500-I⁻ IODIDE*#(46)

4500-I⁻ A. Introduction

1. Occurrence

Iodide is found in natural waters at concentrations ranging from 40 µg I⁻/L in coastal surface seawater to <1 µg I⁻/L in deep ocean water and fresh water. Higher concentrations may be found in brines, certain industrial wastes, and waters treated with iodine. Iodide is thermodynamically unstable relative to iodate in oxygenated waters.

2. Selection of Method

The leuco crystal violet method (B) is applicable to iodide concentrations of 50 to 6000 µg/L. The catalytic reduction method (C) is applicable to iodide concentrations of 80 µg I⁻/L or less. The voltammetric method (D) is the most sensitive method. It can be used for samples with iodide concentrations of 0.13 to 10.2 µg I⁻/L. It is also species-specific. It is insensitive to iodate, iodine, and most organic iodine compounds. It requires minimal sample manipulation, aside from an occasional dilution for samples with high concentrations of iodide. Thus, the concentrations of iodide in many types of water samples may be determined directly with the voltammetric method.

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The choice of method depends on the sample and concentration to be determined. The high chloride concentrations of brines, seawater, and many estuarine waters will interfere with color development in the leuco crystal violet method. In the presence of iodine, the leuco crystal violet method gives the sum of iodine and iodide. Iodide may be determined by the difference after concentration of iodine has been estimated independently (see Section 4500-I). In the catalytic reduction method, As(III), under acidic conditions, is a strong reducing agent and will reduce the oxidized forms of iodine to iodide. Thus, this method measures not only iodide, but also the sum of all the inorganic iodine species including iodide, iodate, hypoiodous acid, hypoiodite ion, and elemental iodine. Because iodate is the thermodynamically stable form of dissolved iodine in oxygenated natural waters and is frequently the dominant species of dissolved iodine, the catalytic reduction method is likely to overestimate the concentration of iodide. This method works well only under exactly reproducible conditions.

4500-I⁻ B. Leuco Crystal Violet Method

1. General Discussion

a. *Principle:* Iodide is selectively oxidized to iodine by the addition of potassium peroxymonosulfate, KHSO_5 . The iodine produced reacts instantaneously with the colorless indicator reagent containing 4,4',4''-methylidynetris (*N,N*-dimethylaniline), also known as leuco crystal violet, to produce the highly colored crystal violet dye. The developed color is sufficiently stable for the determination of an absorbance value and adheres to Beer's law over a wide range of iodine concentrations. Absorbance is highly pH-dependent, and must be measured within the pH range of 3.5 to 4.0 at a wavelength of 592 nm. Accurate control of pH is essential for maximum precision. (See Section 4500-I.B.1a.) Follow the general principles for quality control (Section 4020).

b. *Interference:* Chloride concentrations greater than 200 mg/ L may interfere with color development. Reduce these interferences by diluting sample to contain less than 200 mg Cl^-/L .

2. Apparatus

a. *Colorimetric equipment:* One of the following is required:

1) *Filter photometer*, providing a light path of 1 cm or longer, equipped with an orange filter having maximum transmittance near 592 nm.

2) *Spectrophotometer*, for use at 592 nm, providing a light path of 1 cm or longer.

b. *Volumetric flasks:* 100-mL with plastic caps or ground-glass stoppers.

c. *Glassware:* Completely remove any reducing substances from all glassware or plastic containers, including containers for storing reagent solutions (see Section 4500-Cl.D.2d).

3. Reagents

a. *Iodine-demand-free water:* Prepare a 1-m ion-exchange column of 2.5 to 5 cm diam,

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containing strongly acid cation and strong basic anion exchange resins. If a commercial analytical-grade mixed-bed resin is used, verify that compounds that react with iodine are removed. Pass distilled water at a slow rate through the resin bed and collect in clean container that will protect the treated water from undue exposure to the atmosphere.

Prepare all stock iodide and reagent solutions with iodine-demand-free water.

b. *Stock iodide solution*: Dissolve 1.3081 g KI in water and dilute to 1000 mL; 1 mL = 1 mg I⁻.

c. *Citric buffer solution*, pH 3.8:

1) *Citric acid*: Dissolve 192.2 g C₆H₈O₇ or 210.2 g C₆H₈O₇·H₂O and dilute to 1 L with water.

2) *Ammonium hydroxide, 2N*: Add 131 mL conc NH₄OH to about 700 mL water and dilute to 1 L. Store in a polyethylene bottle.

3) *Final buffer solution*: Slowly add, with mixing, 350 mL 2N NH₄OH solution to 670 mL citric acid. Add 80 g ammonium dihydrogen phosphate (NH₄H₂PO₄) and stir to dissolve.

d. *Leuco crystal violet indicator*: Measure 200 mL water and 3.2 mL conc sulfuric acid (H₂SO₄) into a brown glass container of at least 1-L capacity. Introduce a magnetic stirring bar and mix at moderate speed. Add 1.5 g 4,4',4''-methylidynetris (*N,N*-dimethylaniline)*†(47) and with a small amount of water wash down any reagent adhering to neck or sides of container. Mix until dissolved.

To 800 mL water, add 2.5 g mercuric chloride (HgCl₂) and stir to dissolve. With mixing, add HgCl₂ solution to leuco crystal violet solution. For maximum stability, adjust pH of final solution to 1.5 or less, adding, if necessary, conc H₂SO₄ dropwise. Store in a brown glass bottle away from direct sunlight. Discard after 6 months. Do not use a rubber stopper.

e. *Potassium peroxymonosulfate solution*: Obtain KHSO₅ as a commercial product,†‡(48) which is a stable powdered mixture containing 42.8% KHSO₅ by weight and a mixture of KHSO₄ and K₂SO₄. Dissolve 1.5 g powder in water and dilute to 1 L.

f. *Sodium thiosulfate solution*: Dissolve 5.0 g Na₂S₂O₃·H₂O in water and dilute to 1 L.

4. Procedure

a. *Preparation of temporary iodine standards*: Add suitable portions of stock iodide solution, or of dilutions of stock iodide solution, to water to prepare a series of 0.1 to 6.0 mg I⁻/L in increments of 0.1 mg/L or larger.

Measure 50.0 mL dilute KI standard solution into a 100-mL glass-stoppered volumetric flask. Add 1.0 mL citric buffer and 0.5 mL KHSO₅ solution. Swirl to mix and let stand approximately 1 min. Add 1.0 mL leuco crystal violet indicator, mix, and dilute to 100 mL. For best results, read absorbance as described below within 5 min after adding indicator solution.

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b. Photometric calibration: Transfer colored temporary standards of known iodide concentrations to cells of 1-cm light path and read absorbance in a photometer or spectrophotometer at a wavelength of 592 nm against a water reference. Plot absorbance values against iodide concentrations to construct a curve that follows Beer's law.

c. Color development of sample: Measure a 50.0-mL sample into a 100-mL volumetric flask and treat as described for preparation of temporary iodine standards, ¶ 4a. Read absorbance photometrically and refer to standard calibration curve for iodide equivalent.

d. Samples containing >6.0mg I⁻/L: Place approximately 25mL water in a 100-mL volumetric flask. Add 1.0mL citric buffer and a measured volume of 25mL or less of sample. Add 0.5mL KHSO₅ solution. Swirl to mix and let stand approximately 1min. Add 1.0mL leuco crystal violet indicator, mix, and dilute to 100mL.

Read absorbance photometrically and compare with calibration curve from which the initial iodide concentration is obtained by applying the dilution factor. Select one of the following sample volumes to remain within the optimum iodide range.

Iodide mg/L	Sample Volume Required mL
6.0–12.0	25.0
12.0–30	10.0
30–60	5.0

e. Determination of iodide in the presence of iodine: On separate samples determine (1) total iodide and iodine, and (2) iodine. The iodide concentration is the difference between the iodine determined and the total iodine-iodide obtained. Determine iodine by not adding KHSO₅ solution in the iodide method and by comparing the absorbance value to the calibration curve developed for iodide.

f. Compensation for turbidity and color: Compensate for natural color or turbidity by adding 5 mL Na₂S₂O₃ solution to a 50-mL sample. Add reagents to sample as described previously and use as the blank to set zero absorbance on photometer. Measure all samples in relation to this blank and, from the calibration curve, determine concentrations of iodide or total iodine-iodide.

5. Bibliography

BLACK, A.P. & G.P. WHITTLE. 1967. New methods for the colorimetric determination of halogen residuals. Part I. Iodine, iodide, and iodate. *J. Amer. Water Works Assoc.* 59:471.

4500-I⁻ C. Catalytic Reduction Method

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1. General Discussion

a. *Principle:* Iodide can be determined by using its ability to catalyze the reduction of ceric ions by arsenious acid. The effect is nonlinearly proportional to the amount of iodide present. The reaction is stopped after a specific time interval by the addition of ferrous ammonium sulfate. The resulting ferric ions are directly proportional to the remaining ceric ions and develop a relatively stable color complex with potassium thiocyanate.

Pretreatment by digestion with chromic acid and distillation is necessary to estimate the nonsusceptible bound forms of iodine.

b. *Interferences:* The formation of noncatalytic forms of iodine and the inhibitory effects of silver and mercury are reduced by adding an excess of sodium chloride (NaCl) that sensitizes the reaction. Iodate, hypoiodous acid/hypoiodite ion, and elemental iodine interfere. Under acidic conditions, As(III) may reduce these forms of inorganic iodine to iodide and include them as iodide in the subsequent detection of iodide.

2. Apparatus

a. *Water bath*, capable of temperature control to $30 \pm 0.5^{\circ}\text{C}$.

b. *Colorimetric equipment:* One of the following is required:

1) *Spectrophotometer*, for use at wavelengths of 510 or 525 nm and providing a light path of 1 cm.

2) *Filter photometer*, providing a light path of 1 cm and equipped with a green filter having maximum transmittance near 525 nm.

c. *Test tubes*, 2×15 cm.

d. *Stopwatch*.

3. Reagents

Store all stock solutions in tightly stoppered containers in the dark. Prepare all reagent solutions in distilled water.

a. *Distilled water*, containing less than $0.3 \mu\text{g}$ total I/L.

b. *Sodium chloride solution:* Dissolve 200.0 g NaCl in water and dilute to 1 L. Recrystallize the NaCl if an interfering amount of iodine is present, using a water-ethanol mixture.

c. *Arsenious acid:* Dissolve 4.946 g As_2O_3 in water, add 0.20 mL conc H_2SO_4 , and dilute to 1000 mL.

d. *Sulfuric acid*, H_2SO_4 , conc.

e. *Ceric ammonium sulfate:* Dissolve 13.38 g $\text{Ce}(\text{NH}_4)_4(\text{SO}_4)_4 \cdot 4\text{H}_2\text{O}$ in water, add 44 mL conc H_2SO_4 , and make up to 1 L.

f. *Ferrous ammonium sulfate reagent:* Dissolve 1.50 g $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ in 100 mL

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distilled water containing 0.6 mL conc H_2SO_4 . Prepare daily.

g. *Potassium thiocyanate solution*: Dissolve 4.00 g KSCN in 100 mL water.

h. *Stock iodide solution*: Dissolve 261.6 mg anhydrous KI in water and dilute to 1000 mL; 1.00 mL = 200 $\mu\text{g I}^-$.

i. *Intermediate iodide solution*: Dilute 20.00 mL stock iodide solution to 1000 mL with water; 1.00 mL = 4.00 $\mu\text{g I}^-$.

j. *Standard iodide solution*: Dilute 25.00 mL intermediate iodide solution to 1000 mL with water; 1.00 mL = 0.100 $\mu\text{g I}^-$.

4. Procedure

a. *Sample size*: Add 10.00 mL sample, or a portion made up to 10.00 mL with water, to a 2- $\times$ 15-cm test tube. If possible, keep iodide content in the range 0.2 to 0.6 μg . Use thoroughly clean glassware and apparatus.

b. *Color measurement*: Add reagents in the following order: 1.00 mL NaCl solution, 0.50 mL As_2O_3 solution, and 0.50 mL conc H_2SO_4 .

Place reaction mixture and ceric ammonium sulfate solution in 30°C water bath and let come to temperature equilibrium. Add 1.0 mL ceric ammonium sulfate solution, mix by inversion, and start stopwatch to time reaction. Use an inert clean test tube stopper when mixing. After 15 ± 0.1 min remove sample from water bath and add immediately 1.00 mL ferrous ammonium sulfate reagent with mixing, whereupon the yellow ceric ion color should disappear. Then add, with mixing, 1.00 mL KSCN solution. Replace sample in water bath. Within 1 h after adding thiocyanate read absorbance in a photometric instrument. Maintain temperature of solution and cell compartment at $30 \pm 0.5^\circ\text{C}$ until absorbance is determined. If several samples are run, start reactions at 1-min intervals to allow time for additions of ferrous ammonium sulfate and thiocyanate. (If temperature control of cell compartment is not possible, let final solution come to room temperature and measure absorbance with cell compartment at room temperature.)

c. *Calibration standards*: Treat standards containing 0, 0.2, 0.4, 0.6, and 0.8 $\mu\text{g I}^-/10.00$ mL of solution as in ¶ 4b above. Run with each set of samples to establish a calibration curve.

5. Calculation

$$\text{mg I}^-/\text{L} = \frac{\mu\text{g I (in 15 mL final volume)}}{\text{mL sample}}$$

6. Precision and Bias

Results obtained by this method are reproducible on samples of Los Angeles source waters, and have been reported to be accurate to $\pm 0.3 \mu\text{g I}^-/\text{L}$ on samples of Yugoslavian water

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containing from 0 to 14.0 $\mu\text{g I}^-/\text{L}$. Follow general principles for quality control (Section 4020).

7. Bibliography

ROGINA, B. & M. DUBRAVCIC. 1953. Microdetermination of iodides by arresting the catalytic reduction of ceric ions. *Analyst* 78:594.

DUBRAVCIC, M. 1955. Determination of iodine in natural waters (sodium chloride as a reagent in the catalytic reduction of ceric ions). *Analyst* 80:295.

4500- I^- D. Voltammetric Method

1. General Discussion

a. *Principle:* Iodide is deposited onto the surface of a static mercury drop electrode (SMDE) as mercurous iodide under an applied potential for a specified period of time. The deposited mercurous iodide is reduced by a cathodic potential scan. This reaction gives rise to a current peak at about -0.33 V relative to the saturated calomel electrode. The height of the current peak is directly proportional to the concentration of iodide in solution, which is quantified by the method of internal standard additions.

b. *Interferences:* Sulfide can interfere. Remove it as hydrogen sulfide by acidifying the sample and then purging it with air. Adjust pH of sample back to about pH 8 before analysis.

2. Apparatus

a. *Voltammetric analyzer system,* consisting of a potentiostat, static mercury drop electrode (SMDE), stirrer, and plotter, that can be operated in the cathodic stripping square wave voltammetry-SMDE mode with adjustable deposition potential, deposition time, equilibration time, scan rate, scan range, scan increment, pulse height, frequency, and drop size.

A saturated calomel electrode is used as the reference electrode through a salt bridge.

b. *Glassware:* Wash glassware and other surfaces contacting the sample or reagents with 10% (v/v) HCl (low in iodide); thoroughly rinse with reagent water (see Section 1080) before use.

3. Reagents

Use chemicals low in iodide whenever available.

a. *Oxygen-free water:* Remove oxygen in reagent water (see Section 1080) by bubbling it with argon gas while boiling it for 20 min in an erlenmeyer flask. Let water cool while argon bubbling continues. Tighten stopper flask and store water under nitrogen. Prepare water immediately before use.

b. *Alkaline pyrogallol solution:* Dissolve 30 g pyrogallol in 200 mL oxygen-free water. Dissolve 120 g potassium hydroxide (KOH) in 400 mL oxygen-free water. Mix 300 mL KOH solution with 100 mL pyrogallol solution.

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c. *Sodium sulfite solution, 1M*: Dissolve 1.26 g sodium sulfite, Na_2SO_3 , in oxygen-free water and dilute to 10 mL.

d. *Sodium sulfite solution, 0.1M*: Dilute 5.0 mL 1M sodium sulfite solution to 50 mL. Prepare fresh daily.

e. *Oxygen-free argon gas*: Bubble argon gas (at least 99.99% pure) through a series of three traps containing, respectively, alkaline pyrogallol solution, 0.1M sodium sulfite solution, and oxygen-free water.

f. *Standard iodide solution*: Dry several grams potassium iodide, KI, in an oven at 80°C overnight. Dissolve 1.660 g dried KI in reagent water (see Section 1080) and dilute to 500 mL. Dilute 5 mL solution to 500 mL, and dilute 5 mL of the latter solution to 500 mL.

g. *Polyethylene glycol p-isooctylphenyl ether (PEG-IOPE) solution, 0.2%*: Dilute 0.2 mL commercially available reagent*(49) to 100 mL in reagent water (see Section 1080).

4. Procedure

a. *Sample measurement*: Transfer 10 mL sample, 0.05 mL PEG-IOPE solution, and 0.2 mL 1M Na_2SO_3 solution (which also acts as the supporting electrolyte in fresh-water samples) to polarographic cell containing a magnetic stirrer. Purge solution with oxygen-free argon gas for 1 min. Set electrode at SMDE mode. Record a voltammogram in the cathodic stripping square wave voltammetry mode under the following conditions: deposition potential, -0.15 V; deposition time, 60 s; equilibration time, 5 s; scan rate, 200 mV/s; scan range, 0.15 to -0.6 V; scan increment, 2 mV; pulse height, 20 mV; frequency, 100 Hz; and the largest drop size. Measure magnitude of current peak above baseline at center of peak at an applied potential of about -0.33 V relative to saturated calomel electrode in the voltammogram.

b. *Internal standard additions*: Add 0.1 mL 2 μM standard KI solution to the cell. Purge solution with oxygen-free argon gas for 0.5 min. Record a voltammogram under conditions described in ¶ 4a and again determine magnitude of current peak. Repeat procedure twice, for a total of three additions.

c. *Blank determination*: Determine method reagent blank by treating reagent water as a sample.

5. Calculation

For the j th addition of the standard KI ($j = 0, 1, 2, 3$), compute the following variables:

$$Y_j = I_j (V_x + jV_s + V_c)$$

$$X_j = jV_s C_s$$

where:

I_j = height of j th peak, nA,

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V_x = sample volume, mL,

V_s = volume of standard KI added during each internal addition, mL,

V_c = total volume of PEG-IOPE solution and sodium sulfite added during analysis, mL, and

C_s = concentration of iodide in standard KI solution, nM.

Determine slope, B , and intercept, A , of line relating Y_j to X_j by linear least squares method.

Calculate concentration of iodide in sample as:

$$C_x = \frac{A}{B \times V_x}$$

where:

C_x = concentration of iodide, nM, and other terms are as defined above.

If there is a reagent blank, subtract the reagent blank from C_x to get true concentration in sample.

Multiply C_x (or blank-corrected C_x) by 0.1269 to obtain concentration in $\mu\text{g/L}$.

6. Precision

In one laboratory, using seawater samples with a concentration of iodide of about $6 \mu\text{g I/L}$, the precision was about $\pm 5\%$. Follow general principles of quality control as in Section 4020.

7. Bibliography

LUTHER, G.W., III, C.B. SWARTZ & W.J. ULLMAN. 1988. Direct determination of iodide in seawater by cathodic stripping square wave voltammetry. *Anal. Chem.* 60:1721.

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4500-IO₃⁻ IODATE*#(50)

4500-IO₃⁻ A. Introduction

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1. Occurrence

Iodate is found in natural waters at concentrations ranging from 60 $\mu\text{g I/L}$ in deep ocean water to undetectable (3 $\mu\text{g I/L}$) in estuarine water and fresh water. Iodate is the thermodynamically stable form of dissolved inorganic iodine in waters containing dissolved oxygen; it is absent in anoxic waters.

2. Selection of Method

The differential pulse polarographic method is species-specific and highly sensitive. It is applicable to iodate concentrations of 3 to at least 130 $\mu\text{g I/L}$ and can determine iodate in the presence of other iodine species such as iodide and organic iodine. It can be used for the direct determination of iodate in many types of water samples.

3. Sampling and Storage

Collect representative samples in clean glass or plastic bottles. Clean sample bottles with 10% (v/v) hydrochloric acid (low in iodate) and thoroughly rinse them with reagent water (see Section 1080) before use. Most samples can be analyzed directly without further treatment. Highly turbid samples may be filtered through glass fiber filters before analysis. For storage of up to 2 d, refrigerate sample at 4°C. For longer storage, freeze sample and store at or below -5°C. Frozen samples can be stored for at least 1 month.

4500- IO_3^- B. Polarographic Method

1. General Discussion

a. Principle: Under mildly basic conditions, iodate is reduced to iodide at a dropping mercury electrode by a cathodic potential scan. This reaction gives rise to a current peak centered around -1.1 V relative to the saturated calomel electrode. The height of the current peak is directly proportional to the concentration of iodate, which is quantified by the method of standard additions.

b. Interferences: Dissolved oxygen and zinc interfere. Remove dissolved oxygen by bubbling oxygen-free argon gas through sample and by reacting oxygen with added sodium sulfite. Remove interference from zinc by complexing with EDTA (ethylene diaminetetraacetate).

2. Apparatus

a. Polarographic analyzer system: A polarographic analyzer system, consisting of a potentiostat, a static mercury drop electrode (SMDE), a stirrer, and a plotter, that can be operated in the difference pulse polarography-SMDE mode with adjustable drop time, scan increment, pulse height, scan range, and drop size.

Use a saturated calomel electrode as the reference electrode.

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b. *Glassware*: Acid-wash glassware and other surfaces contacting sample or reagents with 10% (v/v) HCl (low in iodate); thoroughly rinse with reagent water (see Section 1080) before use.

3. Reagents

Use chemicals low in iodate whenever available.

a. *Oxygen-free water*: See Section 4500-I⁻.D.3a.

b. *Alkaline pyrogallol solution*: See Section 4500-I⁻.D.3b.

c. *Oxygen-free argon gas*: See Section 4500-I⁻.D.3e.

d. *Sodium sulfite solution*: See Section 4500-I⁻.D.3c.

e. *Standard iodate solution, 25 μ M*: Dry several grams potassium iodate, KIO₃, in an oven at 80°C overnight. Dissolve 1.070 g dried KIO₃ in reagent water (see Section 1080) and dilute to 1000 mL. Dilute 5 mL of this solution to 1000 mL.

f. *Na₂EDTA solution, 0.1M*: Dissolve 3.722 g Na₂EDTA·2H₂O (disodium ethylenediaminetetraacetate) in reagent water (see Section 1080) and dilute to 100 mL.

g. *Supporting electrolyte*: Dissolve 54.8 g sodium chloride, 0.30 g potassium bromide, and 1.05 g sodium bicarbonate in reagent water (see Section 1080) to form a final volume of 250 mL.

4. Procedure

a. *Sample measurement*: Transfer 5 mL sample and 0.5 mL supporting electrolyte to polarographic cell containing a magnetic stirrer. Check pH of solution to make sure it is about 8. (For marine waters with salinities above 15, the supporting electrolyte is not needed.) Remove dissolved oxygen by bubbling sample rigorously with oxygen-free argon gas for 0.5 min with stirring. Add 0.1 mL 1M sodium sulfite solution to sample and purge, with stirring, with oxygen-free argon gas for one additional minute. Add 0.01 mL 0.1M disodium EDTA and purge, with stirring, with oxygen-free argon for another 0.5 min. Set electrode in the SMDE mode. Record a polarogram in the differential pulse polarography mode under the following conditions: drop time, 1 s; scan increment, 6 mV; pulse height, 0.06 V; and scan range, -0.65 to -1.35 V. Use a medium drop size that allows mercury droplets to be formed and dislodged from the dropping mercury electrode at a steady and consistent rate. Measure height of current peak above base line at an applied potential of about -1.1 V relative to the saturated calomel electrode.

b. *Internal standard additions*: Add 0.05 mL 25 μ M standard iodate solution to cell. Purge solution, with stirring, with oxygen-free argon gas for 0.5 min.

Record a polarogram under conditions described in ¶ 4a and determine height of current peak again. Repeat this procedure two additional times.

c. *Blank determination*: Determine method reagent blank by treating reagent water as a sample.

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5. Calculation

Follow calculations given in Section 4500-I⁻.D.5, with substitution of appropriate compounds in the definitions of terms.

6. Precision

In one laboratory, analyzing seawater samples with a concentration of iodate of 60 µg I/L, the precision was about ±3%. Follow general principles for quality control (see Section 4020).

7. Bibliography

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4500-N NITROGEN*(51)

4500-N A. Introduction

In waters and wastewaters the forms of nitrogen of greatest interest are, in order of decreasing oxidation state, nitrate, nitrite, ammonia, and organic nitrogen. All these forms of nitrogen, as well as nitrogen gas (N₂), are biochemically interconvertible and are components of the nitrogen cycle. They are of interest for many reasons.

Organic nitrogen is defined functionally as organically bound nitrogen in the trinegative oxidation state. It does not include all organic nitrogen compounds. Analytically, organic nitrogen and ammonia can be determined together and have been referred to as “kjeldahl nitrogen,” a term that reflects the technique used in their determination. Organic nitrogen includes such natural materials as proteins and peptides, nucleic acids and urea, and numerous synthetic organic materials. Typical organic nitrogen concentrations vary from a few hundred micrograms per liter in some lakes to more than 20 mg/L in raw sewage.

Total oxidized nitrogen is the sum of nitrate and nitrite nitrogen. Nitrate generally occurs in trace quantities in surface water but may attain high levels in some groundwater. In excessive amounts, it contributes to the illness known as methemoglobinemia in infants. A limit of 10 mg nitrate as nitrogen/L has been imposed on drinking water to prevent this disorder. Nitrate is found only in small amounts in fresh domestic wastewater but in the effluent of nitrifying biological treatment plants nitrate may be found in concentrations of up to 30 mg nitrate as

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nitrogen/ L. It is an essential nutrient for many photosynthetic autotrophs and in some cases has been identified as the growth-limiting nutrient.

Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate and in the reduction of nitrate. Such oxidation and reduction may occur in wastewater treatment plants, water distribution systems, and natural waters. Nitrite can enter a water supply system through its use as a corrosion inhibitor in industrial process water. Nitrite is the actual etiologic agent of methemoglobinemia. Nitrous acid, which is formed from nitrite in acidic solution, can react with secondary amines ($RR'NH$) to form nitrosamines ($RR'N-NO$), many of which are known to be carcinogens. The toxicologic significance of nitrosation reactions in vivo and in the natural environment is the subject of much current concern and research.

Ammonia is present naturally in surface and wastewaters. Its concentration generally is low in groundwaters because it adsorbs to soil particles and clays and is not leached readily from soils. It is produced largely by deamination of organic nitrogen-containing compounds and by hydrolysis of urea. At some water treatment plants ammonia is added to react with chlorine to form a combined chlorine residual. Ammonia concentrations encountered in water vary from less than 10 μg ammonia nitrogen/L in some natural surface and groundwaters to more than 30 mg/L in some wastewaters.

In this manual, organic nitrogen is referred to and reported as organic N, nitrate nitrogen as NO_3^- -N, nitrite nitrogen as NO_2^- -N, and ammonia nitrogen as NH_3 -N.

Total nitrogen can be determined through oxidative digestion of all digestible nitrogen forms to nitrate, followed by quantitation of the nitrate. Two procedures, one using a persulfate/UV digestion (Section 4500-N.B), and the other using persulfate digestion (Section 4500-N.C) are presented. The procedures give good results for total nitrogen, composed of organic nitrogen (including some aromatic nitrogen-containing compounds), ammonia, nitrite, and nitrate. Molecular nitrogen is not determined and recovery of some industrial nitrogen-containing compounds is low.

Chloride ions do not interfere with persulfate oxidation, but the rate of reduction of nitrate to nitrite (during subsequent nitrate analysis by cadmium reduction) is significantly decreased by chlorides. Ammonium and nitrate ions adsorbed on suspended pure clay or silt particles should give a quantitative yield from persulfate digestion. If suspended matter remains after digestion, remove it before the reduction step.

If suspended organic matter is dissolved by the persulfate digestion reagent, yields comparable to those from true solutions are obtained; if it is not dissolved, the results are unreliable and probably reflect a negative interference. The persulfate method is not effective in wastes with high organic loadings. Dilute such samples and re-analyze until results from two dilutions agree.

4500-N B. In-Line UV/Persulfate Digestion and Oxidation with Flow Injection Analysis (PROPOSED)

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1. General Discussion

a. *Principle:* Nitrogen compounds are digested and oxidized in-line to nitrate by use of heated alkaline persulfate and ultraviolet radiation. The digested sample is injected onto the manifold where its nitrate is reduced to nitrite by a cadmium granule column. The nitrite then is determined by diazotization with sulfanilamide under acidic conditions to form a diazonium ion. The diazonium ion is coupled with *N*-(1-naphthyl)ethylenediamine dihydrochloride. The resulting pink dye absorbs at 540 nm and is proportional to total nitrogen.

This method recovers nearly all forms of organic and inorganic nitrogen, reduced and oxidized, including ammonia, nitrate, and nitrite. It differs from the total kjeldahl nitrogen method described in Section 4500-N_{org}.D, which does not recover the oxidized forms of nitrogen. This method recovers nitrogen components of biological origin such as amino acids, proteins, and peptides as ammonia, but may not recover the nitrogenous compounds of some industrial wastes such as amines, nitro-compounds, hydrazones, oximes, semicarbazones, and some refractory tertiary amines.

See Section 4500-N.A for a discussion of the various forms of nitrogen found in waters and wastewaters, Section 4500-N_{org}.A and Section 4500-N_{org}.B for a discussion of total nitrogen methods, and Section 4130, Flow Injection Analysis (FIA). Also see Section 4500-N.C for a similar, batch total nitrogen method that uses only persulfate.

b. *Interferences:* Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

2. Apparatus

Flow injection analysis equipment consisting of:

a. *FIA injection valve* with sample loop or equivalent.

b. *Multichannel proportioning pump.*

c. *FIA manifold* (Figure 4500-N:1) with tubing heater, in-line ultraviolet digestion fluidics including a debubbler consisting of a gas-permeable TFE membrane and its holder, and flow cell. In Figure 4500-N:1, relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE. The block marked “UV” should consist of TFE tubing irradiated by a mercury discharge ultraviolet lamp emitting radiation at 254 nm.

d. *Absorbance detector*, 540 nm, 10-nm bandpass.

e. *Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and for all solutions. To prevent bubble formation, degas carrier and all reagents with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing

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reagents by weight/weight, use weight/volume.

a. Borate solution, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$: In a 1-L volumetric flask dissolve 38.0 g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ and 3.0 g sodium hydroxide, NaOH, in approximately 900 mL water, using a magnetic stirring bar. Gentle heating will speed dissolution. Adjust to pH 9.0 with NaOH or conc hydrochloric acid (HCl). Dilute to mark and invert to mix.

b. Persulfate solution, $\text{K}_2\text{S}_2\text{O}_8$: Potassium persulfate solid reagent usually contains nitrogen contamination. Higher contamination levels result in larger blank peaks.

To a tared 1-L container, add 975 g water and 49 g $\text{K}_2\text{S}_2\text{O}_8$. Add a magnetic stirring bar, dissolve persulfate, and dilute to mark. Invert to mix.

c. Ammonium chloride buffer: CAUTION: *Fumes. Use a hood.* To a 1-L volumetric flask add 500 mL water, 105 mL conc HCl, and 95 mL conc ammonium hydroxide, NH_4OH . Dissolve, dilute to mark, and invert to mix. Adjust to pH 8.5 with 1N HCl or 1N NaOH solution.

d. Sulfanilamide color reagent: To a tared, dark, 1-L container add 876 g water, 170 g 85% phosphoric acid, H_3PO_4 , 40.0 g sulfanilamide, and 1.0 g *N*-(1-naphthyl)ethylenediamine dihydrochloride (NED). Shake to wet solids and stir for 30 min to dissolve. Store in a dark bottle and discard when solution turns dark pink.

e. Cadmium column: See Section 4500- NO_3^- .I.3c, d, and *e*.

f. Stock nitrate standard, 1000 mg N/L: In a 1-L volumetric flask dissolve 7.221 g potassium nitrate, KNO_3 (dried at 60°C for 1 h), or 4.93 g sodium nitrite, NaNO_2 , in about 800 mL water. Dilute to mark and invert to mix. When refrigerated the standard may be stored for up to 3 months.

g. Standard solutions: Prepare nitrate standards in the desired concentration range, using stock nitrate standards (¶ 3 f), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-N:1 and follow method supplied by manufacturer, or laboratory standard operating procedure for this method.

Carry both standards and samples through this procedure. If samples have been preserved with sulfuric acid, preserve standards similarly. Samples may be homogenized. Turbid samples may be filtered, since digestion effectiveness on nitrogen-containing particles is unknown; however, organic nitrogen may be lost in the filtration.

5. Calculation

Prepare standard curves by plotting absorbance of standards processed through the manifold versus nitrogen concentration. The calibration curve is linear.

Verify digestion efficiency by determining urea, glutamic acid, or nicotinic acid standards (Section 4500-N.C.3d) at regular intervals. In the concentration range of the method, the

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recovery of these compounds should be >95%.

6. Quality Control

See Section 4130B.

7. Precision and Bias

a. MDL: Using a 70- μ L sample loop and a published MDL method,¹ analysts ran 21 replicates of a 0.20-mg N/L standard. These gave a mean of 0.18 mg N/L, a standard deviation of 0.008 mg N/L, and MDL of 0.020 mg N/L.

b. Precision study: Ten injections each of a 4.00-mg N/L standard and of a 10.0-mg N/L standard both gave a relative standard deviation of 0.6%.

c. Recovery of total nitrogen: Table 4500-N:I shows recoveries for various nitrogen compounds determined at 10 mg N/L and 4.0 mg N/L. All compounds were determined in triplicate.

d. Ammonia recoveries from wastewater treatment plant effluent with known additions: To a sample of wastewater treatment plant effluent, ammonium chloride was added at two concentrations, 2.50 and 5.00 mg N/L, and analyses were made in triplicate to give mean recoveries of 96% and 95%, respectively. A sample with no additions also was diluted twofold in triplicate to give a mean recovery of 99%.

8. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430, October 26, 1984.

4500-N C. Persulfate Method

1. General Discussion

The persulfate method determines total nitrogen by oxidation of all nitrogenous compounds to nitrate. Should ammonia, nitrate, and nitrite be determined individually, “organic nitrogen” can be obtained by difference.

a. Principle: Alkaline oxidation at 100 to 110°C converts organic and inorganic nitrogen to nitrate. Total nitrogen is determined by analyzing the nitrate in the digestate.

b. Selection of nitrate measurement method: Automated or manual cadmium reduction may be used to determine total nitrogen levels below 2.9 mg N/L. Results summarized in Table 4500-N:II were obtained using automated cadmium reduction.

2. Apparatus

- a. Autoclave, or hotplate and pressure cooker* capable of developing 100 to 110°C for 30

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min.

b. *Glass culture tubes:*†#(52) 30-mL screw-capped (polypropylene linerless caps), 20 mm OD × 150 mm long. Clean before initial use by autoclaving with digestion reagent.

c. *Apparatus for nitrate determination:* See Section 4500-NO₃⁻.E or Section 4500-NO₃⁻.F.

d. *Automated analytical equipment:* An example of the continuous-flow analytical instrument consists of components shown in Figure 4500-NO₃⁻:2.

3. Reagents

a. *Ammonia-free and nitrate-free water:* Prepare by ion exchange or distillation methods as directed in Section 4500-NH₃.B.3a and Section 4500-NO₃⁻.B.3a.

b. *Stock nitrate solution:* Prepare as directed in Section 4500-NO₃⁻.B.3b.

c. *Intermediate nitrate solution:* Prepare as directed in Section 4500-NO₃⁻.B.3c.

d. *Stock glutamic acid solution:* Dry glutamic acid, C₃H₅NH₂(COOH)₂, in an oven at 105°C for 24 h. Dissolve 1.051 g in water and dilute to 1000 mL; 1.00 mL = 100 µg N. Preserve with 2 mL CHCl₃/L.

e. *Intermediate glutamic acid solution:* Dilute 100 mL stock glutamic acid solution to 1000 mL with water; 1.00 mL = 10.0 µg N. Preserve with 2 mL CHCl₃/L.

gc

f. *Digestion reagent:* Dissolve 20.1 g low nitrogen (<0.001% N) potassium persulfate, K₂S₂O₈, and 3.0 g NaOH in water and dilute to 1000 mL just before use.

g. *Borate buffer solution:* Dissolve 61.8 g boric acid, H₃BO₃, and 8.0 g NaOH in water and dilute to 1000 mL.

h. *Copper sulfate solution:* Dissolve 2.0 g CuSO₄·5H₂O in 90 mL water and dilute to 100 mL.

i. *Ammonium chloride solution:* Dissolve 10.0 g NH₄Cl in 1 L water. Adjust to pH 8.5 by adding three or four NaOH pellets as necessary or NaOH solution before bringing to volume. This reagent is stable for 2 weeks when refrigerated.

j. *Color reagent:* Combine 1500 mL water, 200.0 mL conc phosphoric acid, H₃PO₄, 20.0 g sulfanilamide, and 1.0 g N-(1-naphthyl)-ethylenediamine dihydrochloride. Dilute to 2000 mL. Add 2.0 mL polyoxyethylene 23 lauryl ether.†#(53) Store at 4°C in the dark. Prepare fresh reagent every 6 weeks. Alternatively, prepare proportionally smaller volumes to minimize waste.

4. Procedure

a. *Calibration curve:* Prepare NO₃⁻ calibration standards in the range 0 to 2.9 mg NO₃⁻-N/L by diluting to 100 mL the following volumes of intermediate nitrate solution: 0, 1.00, 2.00, 4.00 .

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.. 29.0 mL. Treat standards in the same manner as samples.

b. *Digestion check standard:* Prepare glutamic acid digestion check standard of 2.9 mg N/L by diluting, to 100 mL, a 29.0-mL volume of intermediate glutamic acid solution. Treat digestion check standard in the same manner as samples.

c. *Digestion:* Samples preserved with acid cannot be analyzed by this method. To a culture tube, add 10.0 mL sample or standard or a portion diluted to 10.0 mL. Add 5.0 mL digestion reagent. Cap tightly. Mix by inverting twice. Heat for 30 min in an autoclave or pressure cooker at 100 to 110°C. Slowly cool to room temperature. Add 1.0 mL borate buffer solution. Mix by inverting at least twice.

d. *Blank:* Carry a reagent blank through all steps of the procedure and apply necessary corrections to the results.

e. *Nitrate measurement:* Determine nitrate by cadmium reduction. Set up manifold as shown in Figure 4500-NO₃⁻:2, but use reagents specified in Section 4500-N_{org}:C.3.

5. Calculation

Prepare the standard curve by plotting the absorbances or peak heights of the nitrate calibration standards carried through the digestion procedure against their nitrogen concentrations. Compute organic N sample concentration by comparing sample absorbance or peak height with the standard curve.

6. Precision and Bias

See Table 4500-N:II.

7. Bibliography

- D'ELIA, C.F., P.A. STEUDLER & N. CORWIN. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnol. Oceanogr.* 22:760.
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4500-NH₃ NITROGEN (AMMONIA)*#(54)

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4500-NH₃ A. Introduction

1. Selection of Method

The two major factors that influence selection of the method to determine ammonia are concentration and presence of interferences. In general, direct manual determination of low concentrations of ammonia is confined to drinking waters, clean surface or groundwater, and good-quality nitrified wastewater effluent. In other instances, and where interferences are present or greater precision is necessary, a preliminary distillation step (B) is required.

A titrimetric method (C), an ammonia-selective electrode method (D), an ammonia-selective electrode method using known addition (E), a phenate method (F), and two automated versions of the phenate method (G and H) are presented. Methods D, E, F, G, and H may be used either with or without sample distillation. The data presented in Table 4500-NH₃:I and Table 4500-NH₃:III should be helpful in selecting the appropriate method of analysis.

Nesslerization has been dropped as a standard method, although it has been considered a classic water quality measurement for more than a century. The use of mercury in this test warrants its deletion because of the disposal problems.

The distillation and titration procedure is used especially for NH₃-N concentrations greater than 5 mg/L. Use boric acid as the absorbent following distillation if the distillate is to be titrated.

The ammonia-selective electrode method is applicable over the range from 0.03 to 1400 mg NH₃-N/L.

The manual phenate method is applicable to both fresh water and seawater and is linear to 0.6 mg NH₃-N/L. Distill into sulfuric acid (H₂SO₄) absorbent for the phenate method when interferences are present.

The automated phenate method is applicable over the range of 0.02 to 2.0 mg NH₃-N/L.

2. Interferences

Glycine, urea, glutamic acid, cyanates, and acetamide hydrolyze very slowly in solution on standing but, of these, only urea and cyanates will hydrolyze on distillation at pH of 9.5. Hydrolysis amounts to about 7% at this pH for urea and about 5% for cyanates. Volatile alkaline compounds such as hydrazine and amines will influence titrimetric results. Residual chlorine reacts with ammonia; remove by sample pretreatment. If a sample is likely to contain residual chlorine, immediately upon collection, treat with dechlorinating agent as in Section 4500-NH₃.B.3d.

3. Storage of Samples

Most reliable results are obtained on fresh samples. If samples are to be analyzed within 24 h

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of collection, refrigerate unacidified at 4°C. For preservation for up to 28 d, freeze at – 20°C unacidified, or preserve samples by acidifying to pH <2 and storing at 4°C. If acid preservation is used, neutralize samples with NaOH or KOH immediately before making the determination. CAUTION: Although acidification is suitable for certain types of samples, it produces interferences when exchangeable ammonium is present in unfiltered solids.

4. Bibliography

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- SALLEY, B.A., J.G. BRADSHAW & B.J. NEILSON. 1986. Results of Comparative Studies of Presevation Techniques for Nutrient Analysis on Water Samples. Virginia Institute of Marine Science, Gloucester Point.

4500-NH₃ B. Preliminary Distillation Step

1. General Discussion

The sample is buffered at pH 9.5 with a borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds. It is distilled into a solution of boric acid when titration is to be used or into H₂SO₄ when the phenate method is used. The ammonia in the distillate can be determined either colorimetrically by the phenate method or titrimetrically with standard H₂SO₄ and a mixed indicator or a pH meter. The choice between the colorimetric and the acidimetric methods depends on the concentration of ammonia. Ammonia in the distillate also can be determined by the ammonia-selective electrode method, using 0.04N H₂SO₄ to trap the ammonia.

2. Apparatus

a. *Distillation apparatus:* Arrange a borosilicate glass flask of 800- to 2000-mL capacity attached to a vertical condenser so that the outlet tip may be submerged below the surface of the receiving acid solution. Use an all-borosilicate-glass apparatus or one with condensing units constructed of block tin or aluminum tubes.

b. *pH meter.*

3. Reagents

a. *Ammonia-free water:* Prepare by ion-exchange or distillation methods:

1) Ion exchange—Prepare ammonia-free water by passing distilled water through an ion-exchange column containing a strongly acidic cation-exchange resin mixed with a strongly basic anion-exchange resin. Select resins that will remove organic compounds that interfere with the ammonia determination. Some anion-exchange resins tend to release ammonia. If this occurs, prepare ammonia-free water with a strongly acidic cation-exchange resin. Regenerate the column

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according to the manufacturer's instructions. Check ammonia-free water for the possibility of a high blank value.

2) Distillation—Eliminate traces of ammonia in distilled water by adding 0.1 mL conc H_2SO_4 to 1 L distilled water and redistilling. Alternatively, treat distilled water with sufficient bromine or chlorine water to produce a free halogen residual of 2 to 5 mg/L and redistill after standing at least 1 h. Discard the first 100 mL distillate. Check redistilled water for the possibility of a high blank.

It is very difficult to store ammonia-free water in the laboratory without contamination from gaseous ammonia. However, if storage is necessary, store in a tightly stoppered glass container to which is added about 10 g ion-exchange resin (preferably a strongly acidic cation-exchange resin)/L ammonia-free water. For use, let resin settle and decant ammonia-free water. If a high blank value is produced, replace the resin or prepare fresh ammonia-free water.

Use ammonia-free distilled water for preparing all reagents, rinsing, and sample dilution.

b. Borate buffer solution: Add 88 mL 0.1N NaOH solution to 500 mL approximately 0.025M sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7$) solution (9.5 g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$ /L) and dilute to 1 L.

c. Sodium hydroxide, 6N.

d. Dechlorinating reagent: Dissolve 3.5 g sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) in water and dilute to 1 L. Prepare fresh weekly. Use 1 mL reagent to remove 1 mg/L residual chlorine in 500-mL sample.

e. Neutralization agent.

1) Sodium hydroxide, NaOH, 1N.

2) Sulfuric acid, H_2SO_4 , 1N.

f. Absorbent solution, plain boric acid: Dissolve 20 g H_3BO_3 in water and dilute to 1 L.

g. Indicating boric acid solution: See Section 4500-NH₃.C.3a and *b*.

h. Sulfuric acid, 0.04N: Dilute 1.0 mL conc H_2SO_4 to 1 L.

4. Procedure

a. Preparation of equipment: Add 500 mL water and 20 mL borate buffer, adjust pH to 9.5 with 6N NaOH solution, and add to a distillation flask. Add a few glass beads or boiling chips and use this mixture to steam out the distillation apparatus until distillate shows no traces of ammonia.

b. Sample preparation: Use 500 mL dechlorinated sample or a known portion diluted to 500 mL with water. When $\text{NH}_3\text{-N}$ concentration is less than 100 $\mu\text{g/L}$, use a sample volume of 1000 mL. Remove residual chlorine by adding, at the time of collection, dechlorinating agent equivalent to the chlorine residual. If necessary, neutralize to approximately pH 7 with dilute acid or base, using a pH meter.

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Add 25 mL borate buffer solution and adjust to pH 9.5 with 6N NaOH using a pH meter.

c. Distillation: To minimize contamination, leave distillation apparatus assembled after steaming out and until just before starting sample distillation. Disconnect steaming-out flask and immediately transfer sample flask to distillation apparatus. Distill at a rate of 6 to 10 mL/min with the tip of the delivery tube below the surface of acid receiving solution. Collect distillate in a 500-mL erlenmeyer flask containing 50 mL indicating boric acid solution for titrimetric method. Distill ammonia into 50 mL 0.04N H₂SO₄ for the ammonia-selective electrode method and for the phenate method. Collect at least 200 mL distillate. Lower distillation receiver so that the end of the delivery tube is free of contact with the liquid and continue distillation during the last minute or two to cleanse condenser and delivery tube. Dilute to 500 mL with water.

When the phenate method is used for determining NH₃-N, neutralize distillate with 1N NaOH solution.

d. Ammonia determination: Determine ammonia by the titrimetric method (C), the ammonia-selective electrode methods (D and E), or the phenate methods (F and G).

5. Bibliography

- NICHOLS, M.S. & M.E. FOOTE. 1931. Distillation of free ammonia from buffered solutions. *Ind. Eng. Chem., Anal. Ed.* 3:311.
- GRIFFIN, A.E. & N.S. CHAMBERLIN. 1941. Relation of ammonia nitrogen to breakpoint chlorination. *Amer. J. Pub. Health* 31:803.
- PALIN, A.T. 1950. Symposium on the sterilization of water. Chemical aspects of chlorination. *J. Inst. Water Eng.* 4:565.
- TARAS, M.J. 1953. Effect of free residual chlorination of nitrogen compounds in water. *J. Amer. Water Works Assoc.* 45:47.

4500-NH₃ C. Titrimetric Method

1. General Discussion

The titrimetric method is used only on samples that have been carried through preliminary distillation (see Section 4500-NH₃.B). The following table is useful in selecting sample volume for the distillation and titration method.

Ammonia Nitrogen in Sample mg/L	Sample Volume mL

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Ammonia Nitrogen in Sample <i>mg/L</i>	Sample Volume <i>mL</i>
5–10	250
10–20	100
20–50	50.0
50–100	25.0

2. Apparatus

Distillation apparatus: See Section 4500-NH₃.B.2a and Section 4500-NH₃.B.2b.

3. Reagents

Use ammonia-free water in making all reagents and dilutions.

a. Mixed indicator solution: Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol. Combine solutions. Prepare monthly.

b. Indicating boric acid solution: Dissolve 20 g H₃BO₃ in water, add 10 mL mixed indicator solution, and dilute to 1 L. Prepare monthly.

c. Standard sulfuric acid titrant, 0.02N: Prepare and standardize as directed in Alkalinity, Section 2320B.3c. For greatest accuracy, standardize titrant against an amount of Na₂CO₃ that has been incorporated in the indicating boric acid solution to reproduce the actual conditions of sample titration; 1.00 mL = 14 × normality × 1000 µg N. (For 0.02N, 1.00 mL = 280 µg N.)

4. Procedure

a. Proceed as described in Section 4500-NH₃.B using indicating boric acid solution as absorbent for the distillate.

b. Sludge or sediment samples: Rapidly weigh to within ±1% an amount of wet sample, equivalent to approximately 1 g dry weight, in a weighing bottle or crucible. Wash sample into a 500-mL kjeldahl flask with water and dilute to 250 mL. Proceed as in ¶ 4a but add a piece of paraffin wax to distillation flask and collect only 100 mL distillate.

c. Titrate ammonia in distillate with standard 0.02N H₂SO₄ titrant until indicator turns a pale lavender.

d. Blank: Carry a blank through all steps of the procedure and apply the necessary correction to the results.

5. Calculation

a. Liquid samples:

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$$\text{mg NH}_3\text{-N/L} = \frac{(A - B) \times 280}{\text{mL sample}}$$

b. Sludge or sediment samples:

$$\text{mg NH}_3\text{-N/kg} = \frac{(A - B) \times 280}{\text{g dry wt sample}}$$

where:

A = volume of H₂SO₄ titrated for sample, mL, and

B = volume of H₂SO₄ titrated for blank, mL.

6. Precision and Bias

Three synthetic samples containing ammonia and other constituents dissolved in distilled water were distilled and analyzed by titration.

Sample 1 contained 200 µg NH₃-N/L, 10 mg Cl⁻/L, 1.0 mg NO₃⁻-N/L, 1.5 mg organic N/L, 10.0 mg PO₄³⁻/L, and 5.0 mg silica/L. The relative standard deviation and relative error for the 21 participating laboratories were 69.8% and 20%, respectively.

Sample 2 contained 800 µg NH₃-N/L, 200 mg Cl⁻/L, 1.0 mg NO₃⁻-N/L, 0.8 mg organic N/L, 5.0 mg PO₄³⁻/L, and 15.0 mg silica/L. The relative standard deviation and relative error for the 20 participating laboratories were 28.6% and 5%, respectively.

Sample 3 contained 1500 µg NH₃-N/L, 400 mg Cl⁻/L, 1.0 mg NO₃⁻-N/L, 0.2 mg organic N/L, 0.5 mg PO₄³⁻/L, and 30.0 mg silica/L. The relative standard deviation and relative error for the 21 participating laboratories were 21.6%, and 2.6%, respectively.

7. Bibliography

MEEKER, E.W. & E.C. WAGNER. 1933. Titration of ammonia in the presence of boric acid. *Ind. Eng. Chem., Anal. Ed.* 5:396.

WAGNER, E.C. 1940. Titration of ammonia in the presence of boric acid. *Ind. Eng. Chem., Anal. Ed.* 12:711.

4500-NH₃ D. Ammonia-Selective Electrode Method

1. General Discussion

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a. Principle: The ammonia-selective electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an electrode internal solution of ammonium chloride. Dissolved ammonia ($\text{NH}_{3(\text{aq})}$ and NH_4^+) is converted to $\text{NH}_{3(\text{aq})}$ by raising pH to above 11 with a strong base. $\text{NH}_{3(\text{aq})}$ diffuses through the membrane and changes the internal solution pH that is sensed by a pH electrode. The fixed level of chloride in the internal solution is sensed by a chloride ion-selective electrode that serves as the reference electrode. Potentiometric measurements are made with a pH meter having an expanded millivolt scale or with a specific ion meter.

b. Scope and application: This method is applicable to the measurement of 0.03 to 1400 mg $\text{NH}_3\text{-N/L}$ in potable and surface waters and domestic and industrial wastes. High concentrations of dissolved ions affect the measurement, but color and turbidity do not. Sample distillation is unnecessary. Use standard solutions and samples that have the same temperature and contain about the same total level of dissolved species. The ammonia-selective electrode responds slowly below 1 mg $\text{NH}_3\text{-N/L}$; hence, use longer times of electrode immersion (2 to 3 min) to obtain stable readings.

c. Interference: Amines are a positive interference. This may be enhanced by acidification. Mercury and silver interfere by complexing with ammonia, unless the NaOH/EDTA solution (3c) is used.

d. Sample preservation: Refrigerate at 4°C for samples to be analyzed within 24 h. Preserve samples high in organic and nitrogenous matter, and any other samples for longer storage, by lowering pH to 2 or less with conc H_2SO_4 .

2. Apparatus

a. Electrometer: A pH meter with expanded millivolt scale capable of 0.1 mV resolution between -700 mV and $+700\text{ mV}$ or a specific ion meter.

b. Ammonia-selective electrode.#(55)*

c. Magnetic stirrer, thermally insulated, with TFE-coated stirring bar.

3. Reagents

a. Ammonia-free water: See Section 4500- NH_3 .B.3a. Use for making all reagents.

b. Sodium hydroxide, 10N.

c. NaOH/EDTA solution, 10N: Dissolve 400 g NaOH in 800 mL water. Add 45.2 g ethylenediaminetetraacetic acid, tetrasodium salt, tetrahydrate ($\text{Na}_4\text{EDTA}\cdot 4\text{H}_2\text{O}$) and stir to dissolve. Cool and dilute to 1000 mL.

d. Stock ammonium chloride solution: Dissolve 3.819 g anhydrous NH_4Cl (dried at 100°C) in water, and dilute to 1000 mL; $1.00\text{ mL} = 1.00\text{ mg N} = 1.22\text{ mg NH}_3$.

e. Standard ammonium chloride solutions: See ¶ 4a below.

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4. Procedure

a. Preparation of standards: Prepare a series of standard solutions covering the concentrations of 1000, 100, 10, 1, and 0.1 mg NH₃-N/L by making decimal dilutions of stock NH₄Cl solution with water.

b. Electrometer calibration: Place 100 mL of each standard solution in a 150-mL beaker. Immerse electrode in standard of lowest concentration and mix with a magnetic stirrer. Limit stirring speed to minimize possible loss of ammonia from the solution. Maintain the same stirring rate and a temperature of about 25°C throughout calibration and testing procedures. Add a sufficient volume of 10N NaOH solution (1 mL usually is sufficient) to raise pH above 11. If the presence of silver or mercury is possible, use NaOH/EDTA solution in place of NaOH solution. If it is necessary to add more than 1 mL of either NaOH or NaOH/ EDTA solution, note volume used, because it is required for subsequent calculations. Keep electrode in solution until a stable millivolt reading is obtained. Do not add NaOH solution before immersing electrode, because ammonia may be lost from a basic solution. Repeat procedure with remaining standards, proceeding from lowest to highest concentration. Wait until the reading has stabilized (at least 2 to 3 min) before recording millivolts for standards and samples containing ≤ 1 mg NH₃-N/L.

c. Preparation of standard curve: Using semilogarithmic graph paper, plot ammonia concentration in milligrams NH₃-N per liter on the log axis vs. potential in millivolts on the linear axis starting with the lowest concentration at the bottom of the scale. If the electrode is functioning properly a tenfold change of NH₃-N concentration produces a potential change of about 59 mV.

d. Calibration of specific ion meter: Refer to manufacturer's instructions and proceed as in ¶s 4a and b.

e. Measurement of samples: Dilute if necessary to bring NH₃-N concentration to within calibration curve range. Place 100 mL sample in 150-mL beaker and follow procedure in ¶ 4b above. Record volume of 10N NaOH added. Read NH₃-N concentration from standard curve.

5. Calculation

$$\text{mg NH}_3\text{-N/L} = A \times B \times \left[\frac{100 + D}{100 + C} \right]$$

where:

A = dilution factor,

B = concentration of NH₃-N/L, mg/L, from calibration curve,

C = volume of 10N NaOH added to calibration standards, mL, and

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D = volume of 10N NaOH added to sample, mL.

6. Precision and Bias

For the ammonia-selective electrode in a single laboratory using surface water samples at concentrations of 1.00, 0.77, 0.19, and 0.13 mg $\text{NH}_3\text{-N/L}$, standard deviations were ± 0.038 , ± 0.017 , ± 0.007 , and ± 0.003 , respectively. In a single laboratory using surface water samples at concentrations of 0.10 and 0.13 mg $\text{NH}_3\text{-N/L}$, recoveries were 96% and 91%, respectively. The results of an interlaboratory study involving 12 laboratories using the ammonia-selective electrode on distilled water and effluents are summarized in Table 4500-NH₃:I.

7. Bibliography

- BANWART, W.L., J.M. BREMNER & M.A. TABATABAI. 1972. Determination of ammonium in soil extracts and water samples by an ammonia electrode. *Comm. Soil Sci. Plant Anal.* 3:449.
- MIDGLEY, C. & K. TERRANCE. 1972. The determination of ammonia in condensed steam and boiler feed-water with a potentiometric ammonia probe. *Analyst* 97:626.
- BOOTH, R.L. & R.F. THOMAS. 1973. Selective electrode determination of ammonia in water and wastes. *Environ. Sci. Technol.* 7:523.
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- AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1979. Method 1426-79. American Soc. Testing & Materials, Philadelphia, Pa.

4500-NH₃ E. Ammonia-Selective Electrode Method Using Known Addition

1. General Discussion

a. Principle: When a linear relationship exists between concentration and response, known addition is convenient for measuring occasional samples because no calibration is needed. Because an accurate measurement requires that the concentration at least double as a result of the addition, sample concentration must be known within a factor of three. Total concentration of ammonia can be measured in the absence of complexing agents down to 0.8 mg $\text{NH}_3\text{-N/L}$ or in the presence of a large excess (50 to 100 times) of complexing agent. Known addition is a convenient check on the results of direct measurement.

b. See Section 4500-NH₃.D.1 for further discussion.

2. Apparatus

Use apparatus specified in Section 4500-NH₃.D.2.

3. Reagents

Use reagents specified in Section 4500-NH₃.D.3.

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Add standard ammonium chloride solution approximately 10 times as concentrated as samples being measured.

4. Procedure

- a. Dilute 1000 mg/L stock solution to make a standard solution about 10 times as concentrated as the sample concentrate.
- b. Add 1 mL 10N NaOH to each 100 mL sample and immediately immerse electrode. When checking a direct measurement, leave electrode in 100 mL of sample solution. Use magnetic stirring throughout. Measure mV reading and record as E_1 .
- c. Pipet 10 mL of standard solution into sample. Thoroughly stir and immediately record new mV reading as E_2 .

5. Calculation

- a. $\Delta E = E_1 - E_2$.
- b. From Table 4500-NH₃:II find the concentration ratio, Q , corresponding to change in potential, ΔE . To determine original total sample concentration, multiply Q by the concentration of the added standard:

$$C_o = Q C_s$$

where:

- C_o = total sample concentration, mg/L,
 Q = reading from known-addition table, and
 C_s = concentration of added standard, mg/L.

- c. To check a direct measurement, compare results of the two methods. If they agree within $\pm 4\%$, the measurements probably are good. If the known-addition result is much larger than the direct measurement, the sample may contain complexing agents.

6. Precision and Bias

In 38 water samples analyzed by both the phenate and the known-addition ammonia-selective electrode method, the electrode method yielded a mean recovery of 102% of the values obtained by the phenate method when the NH₃-N concentrations varied between 0.30 and 0.78 mg/L. In 57 wastewater samples similarly compared, the electrode method yielded a mean recovery of 108% of the values obtained by the phenate method using distillation when the NH₃-N concentrations varied between 10.2 and 34.7 mg N/L. In 20 instances in which two to four replicates of these samples were analyzed, the mean standard deviation was 1.32 mg N/L. In three measurements at a sewer outfall, distillation did not change statistically the value obtained by the electrode method. In 12 studies using standards in the 2.5- to 30-mg N/L range, average

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recovery by the phenate method was 97% and by the electrode method 101%.

4500-NH₃ F. Phenate Method

1. General Discussion

a. Principle: An intensely blue compound, indophenol, is formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by sodium nitroprusside.

b. Interferences: Complexing magnesium and calcium with citrate eliminates interference produced by precipitation of these ions at high pH. There is no interference from other trivalent forms of nitrogen. Remove interfering turbidity by distillation or filtration. If hydrogen sulfide is present, remove by acidifying samples to pH 3 with dilute HCl and aerating vigorously until sulfide odor no longer can be detected.

2. Apparatus

Spectrophotometer for use at 640 nm with a light path of 1 cm or greater.

3. Reagents

a. Phenol solution: Mix 11.1 mL liquified phenol ($\geq 89\%$) with 95% v/v ethyl alcohol to a final volume of 100 mL. Prepare weekly. *CAUTION: Wear gloves and eye protection when handling phenol; use good ventilation to minimize all personnel exposure to this toxic volatile substance.*

b. Sodium nitroprusside, 0.5% w/v: Dissolve 0.5 g sodium nitroprusside in 100 mL deionized water. Store in amber bottle for up to 1 month.

c. Alkaline citrate: Dissolve 200 g trisodium citrate and 10 g sodium hydroxide in deionized water. Dilute to 1000 mL.

d. Sodium hypochlorite, commercial solution, about 5%. This solution slowly decomposes once the seal on the bottle cap is broken. Replace about every 2 months.

e. Oxidizing solution: Mix 100 mL alkaline citrate solution with 25 mL sodium hypochlorite. Prepare fresh daily.

f. Stock ammonium solution: See Section 4500-NH₃.D.3d.

g. Standard ammonium solution: Use stock ammonium solution and water to prepare a calibration curve in a range appropriate for the concentrations of the samples.

4. Procedure

To a 25-mL sample in a 50-mL erlenmeyer flask, add, with thorough mixing after each addition, 1 mL phenol solution, 1 mL sodium nitroprusside solution, and 2.5 mL oxidizing solution. Cover samples with plastic wrap or paraffin wrapper film. Let color develop at room temperature (22 to 27°C) in subdued light for at least 1 h. Color is stable for 24 h. Measure absorbance at 640 nm. Prepare a blank and at least two other standards by diluting stock

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ammonia solution into the sample concentration range. Treat standards the same as samples.

5. Calculations

Prepare a standard curve by plotting absorbance readings of standards against ammonia concentrations of standards. Compute sample concentration by comparing sample absorbance with the standard curve.

6. Precision and Bias

For the manual phenate method, reagent water solutions of ammonium sulfate were prepared and analyzed by two analysts in each of three laboratories. Results are summarized in Table 4500-NH₃:III.

7. Bibliography

- SOLORZANO, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol. Oceanogr.* 14:799.
- PARSONS, T.R., Y. MAITA & C.M. LALLI. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Elmsford, N.Y.

4500-NH₃ G. Automated Phenate Method

1. General Discussion

a. Principle: Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.

b. Interferences: Seawater contains calcium and magnesium ions in sufficient concentrations to cause precipitation during analysis. Adding EDTA and sodium potassium tartrate reduces the problem. Eliminate any marked variation in acidity or alkalinity among samples because intensity of measured color is pH-dependent. Likewise, insure that pH of wash water and standard ammonia solutions approximates that of sample. For example, if sample has been preserved with 0.8 mL conc H₂SO₄/L, include 0.8 mL conc H₂SO₄/L in wash water and standards. Remove interfering turbidity by filtration. Color in the samples that absorbs in the photometric range used for analysis interferes.

c. Application: Ammonia nitrogen can be determined in potable, surface, and saline waters as well as domestic and industrial wastewaters over a range of 0.02 to 2.0 mg/L when photometric measurement is made at 630 to 660 nm in a 10- to 50-mm tubular flow cell at rates of up to 60 samples/h. Determine higher concentrations by diluting the sample.

2. Apparatus

Automated analytical equipment. An example of the required continuous-flow analytical

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instrument consists of the interchangeable components shown in Figure 4500-NH₃:1.

3. Reagents

a. *Ammonia-free distilled water*: See Section 4500-NH₃.B.3a. Use for preparing all reagents and dilutions.

b. *Sulfuric acid, H₂SO₄, 5N, air scrubber solution*: Carefully add 139 mL conc H₂SO₄ to approximately 500 mL water, cool to room temperature, and dilute to 1 L.

c. *Sodium phenate solution*: In a 1-L erlenmeyer flask, dissolve 93 mL liquid (≥89%) phenol in 500 mL water. In small increments and with agitation, cautiously add 32 g NaOH. Cool flask under running water and dilute to 1 L. CAUTION: *Minimize exposure of personnel to this compound by wearing gloves and eye protection, and using proper ventilation.*

d. *Sodium hypochlorite solution*: Dilute 250 mL bleach solution containing 5.25% NaOCl to 500 mL with water.

e. *EDTA reagent*: Dissolve 50 g disodium ethylenediamine tetraacetate and approximately six pellets NaOH in 1 L water. For salt-water samples where EDTA reagent does not prevent precipitation of cations, use sodium potassium tartrate solution prepared as follows:

Sodium potassium tartrate solution: To 900 mL water add 100 g NaKC₄H₄O₆·4H₂O, two pellets NaOH, and a few boiling chips, and boil gently for 45 min. Cover, cool, and dilute to 1 L. Adjust pH to 5.2 ± 0.05 with H₂SO₄. Let settle overnight in a cool place and filter to remove precipitate. Add 0.5 mL polyoxyethylene 23 lauryl ether*#(56) solution and store in stoppered bottle.

f. *Sodium nitroprusside solution*: Dissolve 0.5 g Na₂(NO)Fe(CN)₅·2H₂O in 1 L water.

g. *Ammonia standard solutions*: See Section 4500-NH₃.D.3c and d. Use standard ammonia solution and water to prepare the calibration curve in the appropriate ammonia concentration range. To analyze saline waters use substitute ocean water of the following composition to prepare calibration standards:

Constituent	Concentration g/L
NaCl	24.53
MgCl ₂	5.20
CaCl ₂	1.16
KCl	0.70
SrCl ₂	0.03
Na ₂ SO ₄	4.09

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Constituent	Concentration g/L
NaHCO ₃	0.20
KBr	0.10
H ₃ BO ₃	0.03
NaF	0.003

Subtract blank background response of substitute seawater from standards before preparing standard curve.

4. Procedure

- Eliminate marked variation in acidity or alkalinity among samples. Adjust pH of wash water and standard ammonia solutions to approximately that of sample.
- Set up manifold and complete system as shown in Figure 4500-NH₃:1.
- Obtain a stable base line with all reagents, feeding wash water through sample line.
- Typically, use a 60/h, 6:1 cam with a common wash.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against NH₃-N concentrations in standards. Compute sample NH₃-N concentration by comparing sample response with standard curve.

6. Precision and Bias

For an automated phenate system in a single laboratory using surface water samples at concentrations of 1.41, 0.77, 0.59, and 0.43 mg NH₃-N/L, the standard deviation was ± 0.005 mg/L, and at concentrations of 0.16 and 1.44 mg NH₃-N/L, recoveries were 107 and 99%, respectively.

7. Bibliography

- HILLER, A. & D. VAN SLYKE. 1933. Determination of ammonia in blood. *J. Biol. Chem.* 102:499.
- FIORE, J. & J.E. O'BRIEN. 1962. Ammonia determination by automatic analysis. *Wastes Eng.* 33:352.
- AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1966. Manual on Industrial Water and Industrial Waste Water, 2nd ed. American Soc. Testing & Materials, Philadelphia, Pa.
- O'CONNOR, B., R. DOBBS, B. VILLIERS & R. DEAN. 1967. Laboratory distillation of municipal waste effluents. *J. Water Pollut. Control Fed.* 39:25.

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4500-NH₃ H. Flow Injection Analysis (PROPOSED)

1. General Discussion

a. *Principle:* A water sample containing ammonia or ammonium cation is injected into an FIA carrier stream to which a complexing buffer, alkaline phenol, and hypochlorite are added. This reaction, the Berthelot reaction, produces the blue indophenol dye. The blue color is intensified by the addition of nitroferricyanide. The resulting peak's absorbance is measured at 630 nm. The peak area is proportional to the concentration of ammonia in the original sample.

Also see Section 4500-NH₃.F and Section 4130, Flow Injection Analysis (FIA).

b. *Interferences:* Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Also see Section 4500-NH₃.A. Some interferents are removed by distillation; see Section 4500-NH₃.B.

2. Apparatus

Flow injection analysis equipment consisting of:

- a. *FIA injection valve* with sample loop or equivalent.
- b. *Multichannel proportioning pump.*
- c. *FIA manifold* (Figure 4500-NH₃:2) with tubing heater and flow cell. In Figure 4500-NH₃:2, relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*#(57)
- d. *Absorbance detector*, 630 nm, 10-nm bandpass.
- e. *Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. To prevent bubble formation, degas carrier and buffer with helium. Use He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. *Buffer:* To a 1-L tared container add 50.0 g disodium ethylenediamine tetraacetate and 5.5 g sodium hydroxide, NaOH. Add 968 mL water. Mix with a magnetic stirrer until dissolved.

b. *Phenolate:* CAUTION: *Wear gloves. Phenol causes severe burns and is rapidly absorbed into the body through the skin.* To a tared 1-L container, add 888 g water. Add 94.2 g 88% liquefied phenol or 83 g crystalline phenol, C₆H₅OH. While stirring, slowly add 32 g NaOH.

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Cool and invert to mix thoroughly. Do not degas.

c. *Hypochlorite*: To a tared 500-mL container add 250 g 5.25% sodium hypochlorite, NaOCl bleach solution†#(58) and 250 g water. Stir or shake to mix.

d. *Nitroprusside*: To a tared 1-L container add 3.50 g sodium nitroprusside (sodium nitroferricyanide), $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}\cdot 2\text{H}_2\text{O}$, and 1000 g water. Invert to mix.

e. *Stock ammonia standard*, 1000 mg N/L: In a 1-L volumetric flask dissolve 3.819 g ammonium chloride, NH_4Cl , that has been dried for 2 h at 110°C , in about 800 mL water. Dilute to mark and invert to mix.

f. *Standard ammonia solutions*: Prepare ammonia standards in desired concentration range, using the stock standard (¶ 3e), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-NH₃:2 and follow method supplied by manufacturer or laboratory standard operating procedure for this method. Follow quality control procedures described in Section 4020.

5. Calculations

Prepare standard curves by plotting the absorbance of standards processed through the manifold versus ammonia concentration. The calibration curve is linear.

6. Precision and Bias

a. *Recovery and relative standard deviation*: The results of single-laboratory studies with various matrices are given in Table 4500-NH₃:IV.

b. *MDL*: A 650-μL sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 0.020-mg N/L standard. These gave a mean of 0.0204 mg N/L, a standard deviation of 0.0007 mg N/L, and an MDL of 0.002 mg N/L.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and Procedure for the Determination of Method Detection Limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-NO₂⁻ NITROGEN (NITRITE)*#(59)

4500-NO₂⁻ A. Introduction

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1. Occurrence and Significance

For a discussion of the chemical characteristics, sources, and effects of nitrite nitrogen, see Section 4500-N.

2. Selection of Method

The colorimetric method (B) is suitable for concentrations of 5 to 1000 $\mu\text{g NO}_2^-$ -N/L (See ¶ B.1a). Nitrite values can be obtained by the automated method given in Section 4500- NO_3^- .E with the Cu-Cd reduction step omitted. Additionally, nitrite nitrogen can be determined by ion chromatography (Section 4110), and by flow injection analysis (see Section 4130 and Section 4500- NO_3^- .I).

4500- NO_2^- B. Colorimetric Method

1. General Discussion

a. *Principle:* Nitrite (NO_2^-) is determined through formation of a reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulfanilamide with *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride). The applicable range of the method for spectrophotometric measurements is 10 to 1000 $\mu\text{g NO}_2^-$ -N/L. Photometric measurements can be made in the range 5 to 50 $\mu\text{g N/L}$ if a 5-cm light path and a green color filter are used. The color system obeys Beer's law up to 180 $\mu\text{g N/L}$ with a 1-cm light path at 543 nm. Higher NO_2^- concentrations can be determined by diluting a sample.

b. *Interferences:* Chemical incompatibility makes it unlikely that NO_2^- , free chlorine, and nitrogen trichloride (NCl_3) will coexist. NCl_3 imparts a false red color when color reagent is added. The following ions interfere because of precipitation under test conditions and should be absent: Sb^{3+} , Au^{3+} , Bi^{3+} , Fe^{3+} , Pb^{2+} , Hg^{2+} , Ag^+ , chloroplatinate (PtCl_6^{2-}), and metavanadate (VO_3^{2-}). Cupric ion may cause low results by catalyzing decomposition of the diazonium salt. Colored ions that alter the color system also should be absent. Remove suspended solids by filtration.

c. *Storage of sample:* Never use acid preservation for samples to be analyzed for NO_2^- . Make the determination promptly on fresh samples to prevent bacterial conversion of NO_2^- to NO_3^- or NH_3 . For short-term preservation for 1 to 2 d, freeze at -20°C or store at 4°C .

2. Apparatus

Colorimetric equipment: One of the following is required:

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- a. *Spectrophotometer*, for use at 543 nm, providing a light path of 1 cm or longer.
- b. *Filter photometer*, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 540 nm.

3. Reagents

a. *Nitrite-free water*: If it is not known that the distilled or demineralized water is free from NO_2^- , use either of the following procedures to prepare nitrite-free water:

1) Add to 1 L distilled water one small crystal each of KMnO_4 and either Ba(OH)_2 or Ca(OH)_2 . Redistill in an all-borosilicate-glass apparatus and discard the initial 50 mL of distillate. Collect the distillate fraction that is free of permanganate; a red color with DPD reagent (Section 4500-Cl.F.2b) indicates the presence of permanganate.

2) Add 1 mL conc H_2SO_4 and 0.2 mL MnSO_4 solution (36.4 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ /100 mL distilled water) to each 1 L distilled water, and make pink with 1 to 3 mL KMnO_4 solution (400 mg KMnO_4 /L distilled water). Redistill as described in the preceding paragraph.

Use nitrite-free water in making all reagents and dilutions.

b. *Color reagent*: To 800 mL water add 100 mL 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1 L with water. Solution is stable for about a month when stored in a dark bottle in refrigerator.

c. *Sodium oxalate*, 0.025M (0.05N): Dissolve 3.350 g $\text{Na}_2\text{C}_2\text{O}_4$, primary standard grade, in water and dilute to 1000 mL.

d. *Ferrous ammonium sulfate*, 0.05M (0.05N): Dissolve 19.607 g $\text{Fe(NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ plus 20 mL conc H_2SO_4 in water and dilute to 1000 mL. Standardize as in Section 5220B.3d.

e. *Stock nitrite solution*: Commercial reagent-grade NaNO_2 assays at less than 99%.

Because NO_2^- is oxidized readily in the presence of moisture, use a fresh bottle of reagent for preparing the stock solution and keep bottles tightly stoppered against the free access of air when not in use. To determine NaNO_2 content, add a known excess of standard 0.01M (0.05N) KMnO_4 solution (see ¶ h below), discharge permanganate color with a known quantity of standard reductant such as 0.025M $\text{Na}_2\text{C}_2\text{O}_4$ or 0.05M $\text{Fe(NH}_4)_2(\text{SO}_4)_2$, and back-titrate with standard permanganate solution.

1) Preparation of stock solution—Dissolve 1.232 g NaNO_2 in water and dilute to 1000 mL; 1.00 mL = 250 μg N. Preserve with 1 mL CHCl_3 .

2) Standardization of stock nitrite solution—Pipet, in order, 50.00 mL standard 0.01M (0.05N) KMnO_4 , 5 mL conc H_2SO_4 , and 50.00 mL stock NO_2^- solution into a glass-stoppered flask or bottle. Submerge pipet tip well below surface of permanganate-acid solution while

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adding stock NO_2^- solution. Shake gently and warm to 70 to 80°C on a hot plate. Discharge permanganate color by adding sufficient 10-mL portions of standard 0.025M $\text{Na}_2\text{C}_2\text{O}_4$. Titrate excess $\text{Na}_2\text{C}_2\text{O}_4$ with 0.01M (0.05N) KMnO_4 to the faint pink end point. Carry a water blank through the entire procedure and make the necessary corrections in the final calculation as shown in the equation below.

If standard 0.05M ferrous ammonium sulfate solution is substituted for $\text{Na}_2\text{C}_2\text{O}_4$, omit heating and extend reaction period between KMnO_4 and Fe^{2+} to 5 min before making final KMnO_4 titration.

Calculate NO_2^- -N content of stock solution by the following equation:

$$A = \frac{[(B \times C) - (D \times E)] \times 7}{F}$$

where:

A = mg NO_2^- -N/mL in stock NaNO_2 solution,

B = total mL standard KMnO_4 used,

C = normality of standard KMnO_4 ,

D = total mL standard reductant added,

E = normality of standard reductant, and

F = mL stock NaNO_2 solution taken for titration.

Each 1.00 mL 0.01M (0.05N) KMnO_4 consumed by the NaNO_2 solution corresponds to 1750 μg NO_2^- -N.

f. Intermediate nitrite solution: Calculate the volume, G , of stock NO_2^- solution required for the intermediate NO_2^- solution from $G = 12.5/A$. Dilute the volume G (approximately 50 mL) to 250 mL with water; 1.00 mL = 50.0 μg N. Prepare daily.

g. Standard nitrite solution: Dilute 10.00 mL intermediate NO_2^- solution to 1000 mL with water; 1.00 mL = 0.500 μg N. Prepare daily.

h. Standard potassium permanganate titrant, 0.01M (0.05N): Dissolve 1.6 g KMnO_4 in 1 L distilled water. Keep in a brown glass-stoppered bottle and age for at least 1 week. Carefully decant or pipet supernate without stirring up any sediment. Standardize this solution frequently by the following procedure:

Weigh to the nearest 0.1 mg several 100- to 200-mg samples of anhydrous $\text{Na}_2\text{C}_2\text{O}_4$ into 400-mL beakers. To each beaker, in turn, add 100 mL distilled water and stir to dissolve. Add 10

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mL 1 + 1 H₂SO₄ and heat rapidly to 90 to 95°C. Titrate rapidly with permanganate solution to be standardized, while stirring, to a slight pink end-point color that persists for at least 1 min. Do not let temperature fall below 85°C. If necessary, warm beaker contents during titration; 100 mg will consume about 6 mL solution. Run a blank on distilled water and H₂SO₄.

$$\text{Normality of KMnO}_4 = \frac{\text{g Na}_2\text{C}_2\text{O}_4}{(A - B) \times 0.33505}$$

where:

A = mL titrant for sample and

B = mL titrant for blank.

Average the results of several titrations.

4. Procedure

a. Removal of suspended solids: If sample contains suspended solids, filter through a 0.45-μm-pore-diam membrane filter.

b. Color development: If sample pH is not between 5 and 9, adjust to that range with 1N HCl or NH₄OH as required. To 50.0 mL sample, or to a portion diluted to 50.0 mL, add 2 mL color reagent and mix.

c. Photometric measurement: Between 10 min and 2 h after adding color reagent to samples and standards, measure absorbance at 543 nm. As a guide use the following light paths for the indicated NO₂⁻-N concentrations:

Light Path Length <i>cm</i>	NO ₂ ⁻ -N μg/L
1	2–25
5	2–6
10	<2

5. Calculation

Prepare a standard curve by plotting absorbance of standards against NO₂⁻-N concentration. Compute sample concentration directly from curve.

6. Precision and Bias

In a single laboratory using wastewater samples at concentrations of 0.04, 0.24, 0.55, and

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1.04 mg $\text{NO}_3^- + \text{NO}_2^-$ -N/L, the standard deviations were ± 0.005 , ± 0.004 , ± 0.005 , and ± 0.01 , respectively. In a single laboratory using wastewater samples at concentrations of 0.24, 0.55, and 1.05 mg $\text{NO}_3^- + \text{NO}_2^-$ -N/L, the recoveries were 100%, 102%, and 100%, respectively.¹

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes. Method 353. 3. U.S. Environmental Protection Agency, Washington, D.C.

8. Bibliography

- BOLTZ, D.F., ed. 1958. Colorimetric Determination of Nonmetals. Interscience Publishers, New York, N.Y.
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4500- NO_2^- C. (Reserved)

4500- NO_3^- NITROGEN (NITRATE)*#(60)

4500- NO_3^- A. Introduction

1. Selection of Method

Determination of nitrate (NO_3^-) is difficult because of the relatively complex procedures required, the high probability that interfering constituents will be present, and the limited concentration ranges of the various techniques.

An ultraviolet (UV) technique (Method B) that measures the absorbance of NO_3^- at 220 nm is suitable for screening uncontaminated water (low in organic matter).

Screen a sample; if necessary, then select a method suitable for its concentration range and probable interferences. Nitrate may be determined by ion chromatography (Section 4110) or capillary ion electrophoresis (Section 4140). Applicable ranges for other methods are: nitrate electrode method (D), 0.14 to 1400 mg NO_3^- -N/L; cadmium reduction method (E), 0.01 to 1.0 mg NO_3^- -N/L; automated cadmium reduction methods (F and I), 0.001 to 10 mg NO_3^- -N/L. For higher NO_3^- -N concentrations, dilute into the range of the selected method.

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Colorimetric methods (B, E) require an optically clear sample. Filter turbid sample through 0.45- μm -pore-diam membrane filter. Test filters for nitrate contamination.

2. Storage of Samples

Start NO_3^- determinations promptly after sampling. If storage is necessary, store for up to 2 d at 4°C; disinfected samples are stable much longer without acid preservation. For longer storage of unchlorinated samples, preserve with 2 mL conc $\text{H}_2\text{SO}_4/\text{L}$ and store at 4°C. NOTE: When sample is preserved with acid, NO_3^- and NO_2^- cannot be determined as individual species.

4500- NO_3^- B. Ultraviolet Spectrophotometric Screening Method

1. General Discussion

a. *Principle:* Use this technique only for screening samples that have low organic matter contents, i.e., uncontaminated natural waters and potable water supplies. The NO_3^- calibration curve follows Beer's law up to 11 mg N/L.

Measurement of UV absorption at 220 nm enables rapid determination of NO_3^- . Because dissolved organic matter also may absorb at 220 nm and NO_3^- does not absorb at 275 nm, a second measurement made at 275 nm may be used to correct the NO_3^- value. The extent of this empirical correction is related to the nature and concentration of organic matter and may vary from one water to another. Consequently, this method is not recommended if a significant correction for organic matter absorbance is required, although it may be useful in monitoring NO_3^- levels within a water body with a constant type of organic matter. Correction factors for organic matter absorbance can be established by the method of additions in combination with analysis of the original NO_3^- content by another method. Sample filtration is intended to remove possible interference from suspended particles. Acidification with 1N HCl is designed to prevent interference from hydroxide or carbonate concentrations up to 1000 mg CaCO_3/L . Chloride has no effect on the determination.

b. *Interference:* Dissolved organic matter, surfactants, NO_2^- , and Cr^{6+} interfere. Various inorganic ions not normally found in natural water, such as chlorite and chlorate, may interfere. Inorganic substances can be compensated for by independent analysis of their concentrations and preparation of individual correction curves. For turbid samples, see ¶ A.1.

2. Apparatus

Spectrophotometer, for use at 220 nm and 275 nm with matched silica cells of 1-cm or longer light path.

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3. Reagents

a. *Nitrate-free water*: Use redistilled or distilled, deionized water of highest purity to prepare all solutions and dilutions.

b. *Stock nitrate solution*: Dry potassium nitrate (KNO_3) in an oven at 105°C for 24 h. Dissolve 0.7218 g in water and dilute to 1000 mL; $1.00\text{ mL} = 100\text{ }\mu\text{g NO}_3^- \text{-N}$. Preserve with 2 mL CHCl_3/L . This solution is stable for at least 6 months.

c. *Intermediate nitrate solution*: Dilute 100 mL stock nitrate solution to 1000 mL with water; $1.00\text{ mL} = 10.0\text{ }\mu\text{g NO}_3^- \text{-N}$. Preserve with 2 mL CHCl_3/L . This solution is stable for 6 months.

d. *Hydrochloric acid solution*, HCl , 1N.

4. Procedure

a. *Treatment of sample*: To 50 mL clear sample, filtered if necessary, add 1 mL HCl solution and mix thoroughly.

b. *Preparation of standard curve*: Prepare NO_3^- calibration standards in the range 0 to 7 mg $\text{NO}_3^- \text{-N/L}$ by diluting to 50 mL the following volumes of intermediate nitrate solution: 0, 1.00, 2.00, 4.00, 7.00 . . . 35.0 mL. Treat NO_3^- standards in same manner as samples.

c. *Spectrophotometric measurement*: Read absorbance or transmittance against redistilled water set at zero absorbance or 100% transmittance. Use a wavelength of 220 nm to obtain NO_3^- reading and a wavelength of 275 nm to determine interference due to dissolved organic matter.

5. Calculation

For samples and standards, subtract two times the absorbance reading at 275 nm from the reading at 220 nm to obtain absorbance due to NO_3^- . Construct a standard curve by plotting absorbance due to NO_3^- against $\text{NO}_3^- \text{-N}$ concentration of standard. Using corrected sample absorbances, obtain sample concentrations directly from standard curve. NOTE: If correction value is more than 10% of the reading at 220 nm, do not use this method.

6. Bibliography

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4500-NO₃⁻ C. (Reserved)

4500-NO₃⁻ D. Nitrate Electrode Method

1. General Discussion

a. *Principle:* The NO₃⁻ ion electrode is a selective sensor that develops a potential across a thin, porous, inert membrane that holds in place a water-immiscible liquid ion exchanger. The electrode responds to NO₃⁻ ion activity between about 10⁻⁵ and 10⁻¹ M (0.14 to 1400 mg NO₃⁻-N/L). The lower limit of detection is determined by the small but finite solubility of the liquid ion exchanger.

b. *Interferences:* Chloride and bicarbonate ions interfere when their weight ratios to NO₃⁻-N are >10 or >5, respectively. Ions that are potential interferences but do not normally occur at significant levels in potable waters are NO₂⁻, CN⁻, S²⁻, Br⁻, I⁻, ClO₃⁻, and ClO₄⁻. Although the electrodes function satisfactorily in buffers over the range pH 3 to 9, erratic responses have been noted where pH is not held constant. Because the electrode responds to NO₃⁻ activity rather than concentration, ionic strength must be constant in all samples and standards. Minimize these problems by using a buffer solution containing Ag₂SO₄ to remove Cl⁻, Br⁻, I⁻, S²⁻, and CN⁻, sulfamic acid to remove NO₂⁻, a buffer at pH 3 to eliminate HCO₃⁻ and to maintain a constant pH and ionic strength, and Al₂(SO₄)₃ to complex organic acids.

2. Apparatus

- pH meter, expanded-scale or digital*, capable of 0.1 mV resolution.
- Double-junction reference electrode*.*(61) Fill outer chamber with (NH₄)₂SO₄ solution.
- Nitrate ion electrode*:†(62) Carefully follow manufacturer's instructions regarding care and storage.
- Magnetic stirrer*: TFE-coated stirring bar.

3. Reagents

- Nitrate-free water*: Prepare as described in ¶ B.3a. Use for all solutions and dilutions.
- Stock nitrate solution*: Prepare as described in ¶ B.3b.
- Standard nitrate solutions*: Dilute 1.0, 10, and 50 mL stock nitrate solution to 100 mL with water to obtain standard solutions of 1.0, 10, and 50 mg NO₃⁻-N/L, respectively.

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d. *Buffer solution:* Dissolve 17.32 g $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$, 3.43 g Ag_2SO_4 , 1.28 g H_3BO_3 , and 2.52 g sulfamic acid ($\text{H}_2\text{NSO}_3\text{H}$), in about 800 mL water. Adjust to pH 3.0 by slowly adding 0.10N NaOH. Dilute to 1000 mL and store in a dark glass bottle.

e. *Sodium hydroxide, NaOH, 0.1N.*

f. *Reference electrode filling solution:* Dissolve 0.53 g $(\text{NH}_4)_2\text{SO}_4$ in water and dilute to 100 mL.

4. Procedure

a. *Preparation of calibration curve:* Transfer 10 mL of 1 mg NO_3^- -N/L standard to a 50-mL beaker, add 10 mL buffer, and stir with a magnetic stirrer. Immerse tips of electrodes and record millivolt reading when stable (after about 1 min). Remove electrodes, rinse, and blot dry. Repeat for 10-mg NO_3^- -N/L and 50-mg NO_3^- -N/L standards. Plot potential measurements against NO_3^- -N concentration on semilogarithmic graph paper, with NO_3^- -N concentration on the logarithmic axis (abscissa) and potential (in millivolts) on the linear axis (ordinate). A straight line with a slope of $+57 \pm 3$ mV/decade at 25°C should result. Recalibrate electrodes several times daily by checking potential reading of the 10 mg NO_3^- -N standard and adjusting the calibration control until the reading plotted on the calibration curve is displayed again.

b. *Measurement of sample:* Transfer 10 mL sample to a 50-mL beaker, add 10 mL buffer solution, and stir (for about 1 min) with a magnetic stirrer. Measure standards and samples at about the same temperature. Immerse electrode tips in sample and record potential reading when stable (after about 1 min). Read concentration from calibration curve.

5. Precision

Over the range of the method, precision of ± 0.4 mV, corresponding to 2.5% in concentration, is expected.

6. Bibliography

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4500-NO₃⁻ E. Cadmium Reduction Method

1. General Discussion

a. Principle: NO₃⁻ is reduced almost quantitatively to nitrite (NO₂⁻) in the presence of cadmium (Cd). This method uses commercially available Cd granules treated with copper sulfate (CuSO₄) and packed in a glass column.

The NO₂⁻ produced thus is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye that is measured colorimetrically. A correction may be made for any NO₂⁻ present in the sample by analyzing without the reduction step. The applicable range of this method is 0.01 to 1.0 mg NO₃⁻-N/L. The method is recommended especially for NO₃⁻ levels below 0.1 mg N/L where other methods lack adequate sensitivity.

b. Interferences: Suspended matter in the column will restrict sample flow. For turbid samples, see ¶ A.1. Concentrations of iron, copper, or other metals above several milligrams per liter lower reduction efficiency. Add EDTA to samples to eliminate this interference. Oil and grease will coat the Cd surface. Remove by pre-extraction with an organic solvent (see Section 5520). Residual chlorine can interfere by oxidizing the Cd column, reducing its efficiency. Check samples for residual chlorine (see DPD methods in Section 4500-Cl). Remove residual chlorine by adding sodium thiosulfate (Na₂S₂O₃) solution (Section 4500-NH₃.B.3d). Sample color that absorbs at about 540 nm interferes.

2. Apparatus

a. Reduction column: Purchase or construct the column*#(63) (Figure 4500-NO₃⁻:1) from a 100-mL volumetric pipet by removing the top portion. The column also can be constructed from two pieces of tubing joined end to end: join a 10-cm length of 3-cm-ID tubing to a 25-cm length of 3.5-mm-ID tubing. Add a TFE stopcock with metering valve¹ to control flow rate.

b. Colorimetric equipment: One of the following is required:

- 1) *Spectrophotometer*, for use at 543 nm, providing a light path of 1 cm or longer.
- 2) *Filter photometer*, with light path of 1 cm or longer and equipped with a filter having maximum transmittance near 540 nm.

3. Reagents

a. Nitrate-free water: See ¶ B.3a. The absorbance of a reagent blank prepared with this water should not exceed 0.01. Use for all solutions and dilutions.

b. Copper-cadmium granules: Wash 25 g new or used 20- to 100-mesh Cd granules†#(64)

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with 6N HCl and rinse with water. Swirl Cd with 100 mL 2% CuSO₄ solution for 5 min or until blue color partially fades. Decant and repeat with fresh CuSO₄ until a brown colloidal precipitate begins to develop. Gently flush with water to remove all precipitated Cu.

c. *Color reagent*: Prepare as directed in Section 4500-NO₂⁻.B.3b.

d. *Ammonium chloride-EDTA solution*: Dissolve 13 g NH₄Cl and 1.7 g disodium ethylenediamine tetraacetate in 900 mL water. Adjust to pH 8.5 with conc NH₄OH and dilute to 1 L.

e. *Dilute ammonium chloride-EDTA solution*: Dilute 300 mL NH₄Cl-EDTA solution to 500 mL with water.

f. *Hydrochloric acid*, HCl, 6N.

g. *Copper sulfate solution*, 2%: Dissolve 20 g CuSO₄·5H₂O in 500 mL water and dilute to 1 L.

h. *Stock nitrate solution*: Prepare as directed in ¶ B.3b.

i. *Intermediate nitrate solution*: Prepare as directed in ¶ B.3c.

j. *Stock nitrite solution*: See Section 4500-NO₂⁻.B.3e.

k. *Intermediate nitrite solution*: See Section 4500-NO₂⁻.B.3 f.

l. *Working nitrite solution*: Dilute 50.0 mL intermediate nitrite solution to 500 mL with nitrite-free water; 1.00 mL = 5 µg NO₂⁻-N.

4. Procedure

a. *Preparation of reduction column*: Insert a glass wool plug into bottom of reduction column and fill with water. Add sufficient Cu-Cd granules to produce a column 18.5 cm long. Maintain water level above Cu-Cd granules to prevent entrapment of air. Wash column with 200 mL dilute NH₄Cl-EDTA solution. Activate column by passing through it, at 7 to 10 mL/min, at least 100 mL of a solution composed of 25% 1.0 mg NO₃⁻-N/L standard and 75% NH₄Cl-EDTA solution.

b. *Treatment of sample*:

- 1) Turbidity removal—For turbid samples, see ¶ A.1.
- 2) pH adjustment—Adjust pH to between 7 and 9, as necessary, using a pH meter and dilute HCl or NaOH. This insures a pH of 8.5 after adding NH₄Cl- EDTA solution.
- 3) Sample reduction—To 25.0 mL sample or a portion diluted to 25.0 mL, add 75 mL NH₄Cl-EDTA solution and mix. Pour mixed sample into column and collect at a rate of 7 to 10 mL/min. Discard first 25 mL. Collect the rest in original sample flask. There is no need to wash columns between samples, but if columns are not to be reused for several hours or longer, pour

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50 mL dilute NH_4Cl -EDTA solution on to the top and let it pass through the system. Store Cu-Cd column in this solution and never let it dry.

4) Color development and measurement—As soon as possible, and not more than 15 min after reduction, add 2.0 mL color reagent to 50 mL sample and mix. Between 10 min and 2 h afterward, measure absorbance at 543 nm against a distilled water-reagent blank. NOTE: If NO_3^- concentration exceeds the standard curve range (about 1 mg N/L), use remainder of reduced sample to make an appropriate dilution and analyze again.

c. *Standards:* Using the intermediate NO_3^- -N solution, prepare standards in the range 0.05 to 1.0 mg NO_3^- -N/L by diluting the following volumes to 100 mL in volumetric flasks: 0.5, 1.0, 2.0, 5.0, and 10.0 mL. Carry out reduction of standards exactly as described for samples. Compare at least one NO_2^- standard to a reduced NO_3^- standard at the same concentration to verify reduction column efficiency. Reactivate Cu-Cd granules as described in ¶ 3b above when efficiency of reduction falls below about 75%.

5. Calculation

Obtain a standard curve by plotting absorbance of standards against NO_3^- -N concentration. Compute sample concentrations directly from standard curve. Report as milligrams oxidized N per liter (the sum of NO_3^- -N plus NO_2^- -N) unless the concentration of NO_2^- -N is separately determined and subtracted.

6. Precision and Bias

In a single laboratory using wastewater samples at concentrations of 0.04, 0.24, 0.55, and 1.04 mg $\text{NO}_3^- + \text{NO}_2^-$ -N/L, the standard deviations were ± 0.005 , ± 0.004 , ± 0.005 , and ± 0.01 , respectively. In a single laboratory using wastewater with additions of 0.24, 0.55, and 1.05 mg $\text{NO}_3^- + \text{NO}_2^-$ -N/L, the recoveries were 100%, 102%, and 100%, respectively.²

7. References

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4500-NO₃⁻ F. Automated Cadmium Reduction Method

1. General Discussion

- a. *Principle*: See ¶ E.1a.
- b. *Interferences*: Sample turbidity may interfere. Remove by filtration before analysis. Sample color that absorbs in the photometric range used for analysis also will interfere.
- c. *Application*: Nitrate and nitrite, singly or together in potable, surface, and saline waters and domestic and industrial wastewaters, can be determined over a range of 0.5 to 10 mg N/L.

2. Apparatus

Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the components shown in Figure 4500-NO₃⁻:2.

3. Reagents

- a. *Deionized distilled water*: See ¶ B.3a.
- b. *Copper sulfate solution*: Dissolve 20 g CuSO₄·5H₂O in 500 mL water and dilute to 1 L.
- c. *Wash solution*: Use water for unpreserved samples. For samples preserved with H₂SO₄, add 2 mL conc H₂SO₄/L wash water.
- d. *Copper-cadmium granules*: See ¶ E.3b.
- e. *Hydrochloric acid*, HCl, conc.
- f. *Ammonium hydroxide*, NH₄OH, conc.
- g. *Color reagent*: To approximately 800 mL water, add, while stirring, 100 mL conc H₃PO₄, 40 g sulfanilamide, and 2 g N-(1-naphthyl)-ethylenediamine dihydrochloride. Stir until dissolved and dilute to 1 L. Store in brown bottle and keep in the dark when not in use. This solution is stable for several months.
- h. *Ammonium chloride solution*: Dissolve 85 g NH₄Cl in water and dilute to 1 L. Add 0.5 mL polyoxyethylene 23 lauryl ether.*#(65)
- i. *Stock nitrate solution*: See ¶ B.3b.
- j. *Intermediate nitrate solution*: See ¶ B.3c.
- k. *Standard nitrate solutions*: Using intermediate NO₃⁻-N solution and water, prepare standards for calibration curve in appropriate nitrate range. Compare at least one NO₂⁻ standard

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to a NO_3^- standard at the same concentration to verify column reduction efficiency. To examine saline waters prepare standard solutions with the substitute ocean water described in Section 4500-NH₃.G.3g.

l. *Standard nitrite solution:* See Section 4500-NO₂⁻.B.3g.

4. Procedure

Set up manifold as shown in Figure 4500-NO₃⁻:2 and follow general procedure described by the manufacturer.

If sample pH is below 5 or above 9, adjust to between 5 and 9 with either conc HCl or conc NH₄OH.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against NO₃⁻-N concentration in standards. Compute sample NO₃⁻-N concentration by comparing sample response with standard curve.

6. Precision and Bias

Data obtained in three laboratories with an automated system based on identical chemical principles but having slightly different configurations are given in the table below. Analyses were conducted on four natural water samples containing exact increments of inorganic nitrate:

Increment as NO ₃ ⁻ -N µg/L	Standard Deviation µg N/L	Bias %	Bias µg N/L
290	12	+5.75	+17
350	92	+18.10	+63
2310	318	+4.47	+103
2480	176	-2.69	-67

In a single laboratory using surface water samples at concentrations of 100, 200, 800, and 2100 µg N/L, the standard deviations were 0, ±40, ±50, and ±50 µg/L, respectively, and at concentrations of 200 and 2200 µg N/L, recoveries were 100 and 96%, respectively.

Precision and bias for the system described herein are believed to be comparable.

7. Bibliography

FIORE, J. & J.E. O'BRIEN. 1962. Automation in sanitary chemistry—Parts 1 and 2. Determination of nitrates and nitrites. *Wastes Eng.* 33:128 & 238.

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4500-NO₃⁻ G. (Reserved)

4500-NO₃⁻ H. Automated Hydrazine Reduction Method

1. General Discussion

a. *Principle:* NO₃⁻ is reduced to NO₂⁻ with hydrazine sulfate. The NO₂⁻ (originally present) plus reduced NO₃⁻ is determined by diazotization with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye that is measured colorimetrically.

b. *Interferences:* Sample color that absorbs in the photometric range used will interfere. Concentrations of sulfide ion of less than 10 mg/L cause variations of NO₃⁻ and NO₂⁻ concentrations of ±10%.

c. *Application:* NO₃⁻ + NO₂⁻ in potable and surface water and in domestic and industrial wastes can be determined over a range of 0.01 to 10 mg N/L.

2. Apparatus

Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the components shown in Figure 4500-NO₃⁻:3.

3. Reagents

a. *Color developing reagent:* To approximately 500 mL water add 200 mL conc phosphoric acid and 10 g sulfanilamide. After sulfanilamide is dissolved completely, add 0.8 g N-(1-naphthyl)-ethylenediamine dihydrochloride. Dilute to 1 L with water, store in a dark bottle, and refrigerate. Solution is stable for approximately 1 month.

b. *Copper sulfate stock solution:* Dissolve 2.5 g CuSO₄·5H₂O in water and dilute to 1 L.

c. *Copper sulfate dilute solution:* Dilute 20 mL stock solution to 2 L.

d. *Sodium hydroxide stock solution, 10N:* Dissolve 400 g NaOH in 750 mL water, cool, and

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dilute to 1 L.

e. *Sodium hydroxide, 1.0N*: Dilute 100 mL stock NaOH solution to 1 L.

f. *Hydrazine sulfate stock solution*: Dissolve 27.5 g $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$ in 900 mL water and dilute to 1 L. This solution is stable for approximately 6 months. CAUTION: *Toxic if ingested. Mark container with appropriate warning.*

g. *Hydrazine sulfate dilute solution*: Dilute 22 mL stock solution to 1 L.

h. *Stock nitrate solution*: See ¶ B.3b.

i. *Intermediate nitrate solution*: See ¶ B.3c.

j. *Standard nitrate solutions*: Prepare NO_3^- calibration standards in the range 0 to 10 mg/L by diluting to 100 mL the following volumes of stock nitrate solution: 0, 0.5, 1.0, 2.0 . . . 10.0 mL. For standards in the range of 0.01 mg/L use intermediate nitrate solution. Compare at least one nitrite standard to a nitrate standard at the same concentration to verify the efficiency of the reduction.

k. *Standard nitrite solution*: See Section 4500- NO_2^- .B.3e, Section 4500- NO_2^- .B.3 f, and Section 4500- NO_2^- .B.3g.

4. Procedure

Set up manifold as shown in Figure 4500- NO_3^- :3 and follow general procedure described by manufacturer. Run a 2.0-mg NO_3^- -N/L and a 2.0-mg NO_2^- -N/L standard through the system to check for 100% reduction of nitrate to nitrite. The two peaks should be of equal height; if not, adjust concentration of the hydrazine sulfate solution: If the NO_3^- peak is lower than the NO_2^- peak, increase concentration of hydrazine sulfate until they are equal; if the NO_3^- peak is higher than the NO_2^- reduce concentration of hydrazine sulfate. When correct concentration has been determined, no further adjustment should be necessary.

5. Calculation

Prepare a standard curve by plotting response of processed standards against known concentrations. Compute concentrations of samples by comparing response with standard curve.

6. Precision and Bias

In a single laboratory using drinking water, surface water, and industrial waste at concentrations of 0.39, 1.15, 1.76, and 4.75 mg NO_3^- -N/L, the standard deviations were ± 0.02 , ± 0.01 , ± 0.02 , and ± 0.03 , respectively. In a single laboratory using drinking water at concentrations of 0.75 and 2.97 mg NO_3^- -N/L, the recoveries were 99% and 101%.¹

7. Reference

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1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes. U.S. Environmental Protection Agency, Washington, D.C.

8. Bibliography

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4500-NO₃⁻ I. Cadmium Reduction Flow Injection Method (PROPOSED)

1. General Discussion

a. Principle: The nitrate in the sample is reduced quantitatively to nitrite by passage of the sample through a copperized cadmium column. The resulting nitrite plus any nitrite originally in the sample is determined as a sum by diazotizing the nitrite with sulfanilamide followed by coupling with *N*-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water-soluble dye has a magenta color; absorbance of the color at 540 nm is proportional to the nitrate + nitrite in the sample. This sum also is known as total oxidized nitrogen (TON).

Nitrite alone can be determined by removing the cadmium column, recalibrating the method, and repeating the sample analyses. A TON and a nitrite FIA method also can be run in parallel for a set of samples. In this arrangement, the concentrations determined in the nitrite method can be subtracted from the corresponding concentrations determined in the TON method to give the resulting nitrate concentrations of the samples.

Also see Section 4500-NO₂⁻ and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against nitrate and nitrite contamination from reagents, water, glassware, and the sample preservation process.

Residual chlorine can interfere by oxidizing the cadmium reduction column. Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. Eliminate this interference by pre-extracting sample with an organic solvent.

Low results would be obtained for samples that contain high concentrations of iron, copper, or other metals. In this method, EDTA is added to the buffer to reduce this interference.

Also see Section 4500-NO₂⁻.B.1b and c and Section 4500-NO₃⁻.A.2 and Section 4500-NO₃⁻.B.1b.

2. Apparatus

Flow injection analysis equipment consisting of:

- a. FIA injection valve* with sample loop or equivalent.
- b. Multichannel proportioning pump.*

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c. *FIA manifold* (Figure 4500-NO₃⁻:4) with flow cell. Relative flow rates only are shown in Figure 4500-NO₃⁻:4. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

d. *Absorbance detector*, 540 nm, 10-nm bandpass.

e. *Injection valve control and data acquisition system*.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. *Ammonium chloride buffer*: CAUTION: *Fumes. Use a hood.* To a tared 1-L container add 800.0 g water, 126 g conc hydrochloric acid, HCl, 55.6 g ammonium hydroxide, NH₄OH, and 1.0 g disodium EDTA. Shake until dissolved. The pH of this buffer should be 8.5.

b. *Sulfanilamide color reagent*: To a tared, dark 1-L container add 876 g water, 170 g 85% phosphoric acid, H₃PO₄, 40.0 g sulfanilamide, and 1.0 g *N*-(1-naphthyl)ethylenediamine dihydrochloride (NED). Shake until wetted and stir with stir bar for 30 min until dissolved. This solution is stable for 1 month.

c. *Hydrochloric acid*, HCl, 1M: To a 100-mL container, add 92 g water, then add 9.6 g conc HCl. Stir or shake to mix.

d. *Copper sulfate solution*, 2%: To a 1-L container, add 20 g copper sulfate pentahydrate, CuSO₄·5H₂O, to 991 g water. Stir or shake to dissolve.

e. *Copperized cadmium granules*: Place 10 to 20 g coarse cadmium granules (0.3- to 1.5-mm-diam) in a 250-mL beaker. Wash with 50 mL acetone, then water, then two 50-mL portions 1M HCl (¶ 3c). Rinse several times with water. CAUTION: *Cadmium is toxic and carcinogenic. Collect and store all waste cadmium. When handling cadmium, wear gloves and follow the precautions described on the cadmium's Material Safety Data Sheet.*

Add 100 mL 2% copper sulfate solution (¶ 3d) to cadmium prepared above. Swirl for about 5 min, then decant the liquid and repeat with a fresh 100-mL portion of the 2% copper sulfate solution. Continue this process until the blue aqueous copper color persists. Decant and wash with at least five portions of ammonium chloride buffer (¶ 3a) to remove colloidal copper. The cadmium should be black or dark gray. The copperized cadmium granules may be stored in a bottle under ammonium chloride buffer.

f. *Stock nitrate standard*, 200 mg N/L: In a 1-L volumetric flask dissolve 1.444 g potassium nitrate, KNO₃, in about 600 mL water. Add 2 mL chloroform. Dilute to mark and invert to mix. This solution is stable for 6 months.

g. *Stock nitrite standard*, 200.0 mg N/L: In a 1-L volumetric flask dissolve 0.986 g sodium

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nitrite, NaNO_2 , or 1.214 g potassium nitrite, KNO_2 , in approximately 800 mL water. Add 2 mL chloroform. Dilute to mark and invert to mix. Refrigerate.

h. Standard solution: Prepare nitrate or nitrite standards in the desired concentration range, using the stock standards (¶ 3 *f* or *g*), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500- NO_3^- :4 and pack column with copperized cadmium granules. Follow methods supplied by column and instrument manufacturer or laboratory's standard operating procedure for this method. Follow quality control procedures outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus TON or nitrite concentration. The calibration curve is linear.

If TON includes measurable nitrite concentrations, it is important that the cadmium column be 100% efficient. If the efficiency is less, the nitrite in the sample will give a positive percent error equal to the difference from 100%, causing an error in TON and nitrate determinations. To measure efficiency of the cadmium column, prepare two calibration curves, one using nitrate standards and one using equimolar nitrite standards. The column efficiency is:

$$\text{Column efficiency} = 100\% \times (\text{slope of nitrate curve} / \text{slope of nitrite curve})$$

Determine column efficiency at least weekly.

6. Precision and Bias

In the studies described below, nitrate was measured. There was no significant concentration of nitrite in the samples.

a. Recovery and relative standard deviation: Table 4500- NO_3^- :I gives results of single-laboratory studies.

b. MDL: A 800- μL sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 2.00- μg N/L standard. These gave a mean of 1.82 μg N/L, a standard deviation of 0.098 μg N/L, and MDL of 0.25 μg N/L. A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagent flow rate.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

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4500-N_{org} NITROGEN (ORGANIC)*#(66)

4500-N_{org} A. Introduction

1. Selection of Method

The kjeldahl methods (B and C) determine nitrogen in the trinegative state. They fail to account for nitrogen in the form of azide, azine, azo, hydrazone, nitrate, nitrite, nitrile, nitro, nitroso, oxime, and semi-carbazone. “Kjeldahl nitrogen” is the sum of organic nitrogen and ammonia nitrogen.

The major factor that influences the selection of a macro- or semi-micro-kjeldahl method to determine organic nitrogen is its concentration. The macro-kjeldahl method is applicable for samples containing either low or high concentrations of organic nitrogen but requires a relatively large sample volume for low concentrations. In the semi-micro-kjeldahl method, which is applicable to samples containing high concentrations of organic nitrogen, the sample volume should be chosen to contain organic plus ammonia nitrogen in the range of 0.2 to 2 mg.

The block digestion method (D) is a micro method with an automated analysis step capable of measuring organic nitrogen as low as 0.1 mg/L when blanks are carefully controlled.

2. Storage of Samples

The most reliable results are obtained on fresh samples. If an immediate analysis is not possible, preserve samples for kjeldahl digestion by acidifying to pH 1.5 to 2.0 with concentrated H₂SO₄ and storing at 4°C. Do not use HgCl₂ because it will interfere with ammonia removal.

3. Interferences

a. Nitrate: During kjeldahl digestion, nitrate in excess of 10 mg/L can oxidize a portion of the ammonia released from the digested organic nitrogen, producing N₂O and resulting in a negative interference. When sufficient organic matter in a low state of oxidation is present, nitrate can be reduced to ammonia, resulting in a positive interference. The conditions under which significant interferences occur are not well defined and there is no proven way to eliminate the interference in conjunction with the kjeldahl methods described herein.

b. Inorganic salts and solids: The acid and salt content of the kjeldahl digestion reagent is intended to produce a digestion temperature of about 380°C. If the sample contains a very large quantity of salt or inorganic solids that dissolve during digestion, the temperature may rise above 400°C, at which point pyrolytic loss of nitrogen begins to occur. To prevent an excessive digestion temperature, add more H₂SO₄ to maintain the acid-salt balance. Not all salts cause precisely the same temperature rise, but adding 1 mL H₂SO₄/g salt in the sample gives reasonable results. Add the extra acid and the digestion reagent to both sample and reagent

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blank. Too much acid will lower the digestion temperature below 380°C and result in incomplete digestion and recovery. If necessary, add sodium hydroxide-sodium thiosulfate before the final distillation step to neutralize the excess acid.

Large amounts of salt or solids also may cause bumping during distillation. If this occurs, add more dilution water after digestion.

c. Organic matter: During kjeldahl digestion, H_2SO_4 oxidizes organic matter to CO_2 and H_2O . If a large amount of organic matter is present, a large amount of acid will be consumed, the ratio of salt to acid will increase, and the digestion temperature will increase. If enough organic matter is present, the temperature will rise above 400°C, resulting in pyrolytic loss of nitrogen. To prevent this, add to the digestion flask 10 mL conc H_2SO_4 /3 g COD. Alternately, add 50 mL more digestion reagent/g COD. Additional sodium hydroxide-sodium thiosulfate reagent may be necessary to keep the distillation pH high. Because reagents may contain traces of ammonia, treat the reagent blank identically with the samples.

4. Use of a Catalyst

Mercury has been the catalyst of choice for kjeldahl digestion. Because of its toxicity and problems associated with legal disposal of mercury residues, a less toxic catalyst is recommended. Digestion of some samples may be complete or nearly complete without the use of a catalyst. Effective digestion results from the use of a reagent having a salt/acid ratio of 1 g/mL with copper as catalyst (§ B.3a), and specified temperature (§ B.2a) and time (§ B.4c)). If a change is made in the reagent formula, report the change and indicate percentage recovery relative to the results for similar samples analyzed using the previous formula.

Before results are considered acceptable, determine nitrogen recovery from samples with known additions of nicotinic acid, to test completeness of digestion; and with ammonium chloride to test for loss of nitrogen.

4500-N_{org} B. Macro-Kjeldahl Method

1. General Discussion

a. Principle: In the presence of H_2SO_4 , potassium sulfate (K_2SO_4), and cupric sulfate (CuSO_4) catalyst, amino nitrogen of many organic materials is converted to ammonium. Free ammonia also is converted to ammonium. After addition of base, the ammonia is distilled from an alkaline medium and absorbed in boric or sulfuric acid. The ammonia may be determined colorimetrically, by ammonia-selective electrode, or by titration with a standard mineral acid.

b. Selection of ammonia measurement method: The sensitivity of colorimetric methods makes them particularly useful for determining organic nitrogen levels below 5 mg/L. The titrimetric and selective electrode methods of measuring ammonia in the distillate are suitable for determining a wide range of organic nitrogen concentrations. Selective electrode methods and

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automated colorimetric methods may be used for measurement of ammonia in digestate without distillation. Follow equipment manufacturer's instructions.

2. Apparatus

a. Digestion apparatus: Kjeldahl flasks with a total capacity of 800 mL yield the best results. Digest over a heating device adjusted so that 250 mL water at an initial temperature of 25°C can be heated to a rolling boil in approximately 5 min. For testing, preheat heaters for 10 min if gas-operated or 30 min if electric. A heating device meeting this specification should provide the temperature range of 375 to 385°C for effective digestion.

b. Distillation apparatus: See Section 4500-NH₃.B.2a.

c. Apparatus for ammonia determination: See Section 4500-NH₃.C.2, 4500-NH₃.D.2, Section 4500-NH₃.F.2, or Section 4500-NH₃.G.2.

3. Reagents

Prepare all reagents and dilutions in ammonia-free water.

All of the reagents listed for the determination of Nitrogen (Ammonia), Section 4500-NH₃.C.3, Section 4500-NH₃.D.3, Section 4500-NH₃.F.3, or Section 4500-NH₃.G.3, are required, plus the following:

a. Digestion reagent: Dissolve 134 g K₂SO₄ and 7.3 g CuSO₄ in about 800 mL water. Carefully add 134 mL conc H₂SO₄. When it has cooled to room temperature, dilute the solution to 1 L with water. Mix well. Keep at a temperature close to 20°C to prevent crystallization.

b. Sodium hydroxide-sodium thiosulfate reagent: Dissolve 500 g NaOH and 25 g Na₂S₂O₃·5H₂O in water and dilute to 1 L.

c. Borate buffer solution: See Section 4500-NH₃.B.3b.

d. Sodium hydroxide, NaOH, 6N.

4. Procedure

a. Selection of sample volume and sample preparation: Place a measured volume of sample in an 800-mL kjeldahl flask. Select sample size from the following tabulation:

Organic Nitrogen in Sample mg/L	Sample Size mL
0–1	500
1–10	250
10–20	100
20–50	50.0
50–100	25.0

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If necessary, dilute sample to 300 mL, neutralize to pH 7, and dechlorinate as described in Section 4500-NH₃.B.4b.

b. Ammonia removal: Add 25 mL borate buffer and then 6N NaOH until pH 9.5 is reached. Add a few glass beads or boiling chips such as Hengar Granules #12 and boil off 300 mL. If desired, distill this fraction and determine ammonia nitrogen. Alternately, if ammonia has been determined by the distillation method, use residue in distilling flask for organic nitrogen determination.

For sludge and sediment samples, weigh wet sample in a crucible or weighing bottle, transfer contents to a kjeldahl flask, and determine kjeldahl nitrogen. Follow a similar procedure for ammonia nitrogen and organic nitrogen determined by difference. Determinations of organic and kjeldahl nitrogen on dried sludge and sediment samples are not accurate because drying results in loss of ammonium salts. Measure dry weight of sample on a separate portion.

c. Digestion: Cool and add carefully 50 mL digestion reagent (or substitute 6.7 mL conc H₂SO₄, 6.7 g K₂SO₄, and 0.365 g CuSO₄) to distillation flask. Add a few glass beads and, after mixing, heat under a hood or with suitable ejection equipment to remove acid fumes. Boil briskly until the volume is greatly reduced (to about 25 to 50 mL) and copious white fumes are observed (fumes may be dark for samples high in organic matter). Then continue to digest for an additional 30 min. As digestion continues, colored or turbid samples will become transparent and pale green. After digestion, let cool, dilute to 300 mL with water, and mix. Tilt flask away from personnel and carefully add 50 mL sodium hydroxide-thiosulfate reagent to form an alkaline layer at flask bottom. Connect flask to a steamed-out distillation apparatus and swirl flask to insure complete mixing. The pH of the solution should exceed 11.0.

d. Distillation: Distill and collect 200 mL distillate. Use 50 mL indicating boric acid as absorbent solution when ammonia is to be determined by titration. Use 50 mL 0.04N H₂SO₄ solution as absorbent for manual phenate or electrode methods. Extend tip of condenser well below level of absorbent solution and do not let temperature in condenser rise above 29°C. Lower collected distillate free of contact with condenser tip and continue distillation during last 1 or 2 min to cleanse condenser.

e. Final ammonia measurement: Use the titration, ammonia-selective electrode, manual phenate, or automated phenate method, Section 4500-NH₃.C, Section 4500-NH₃.D, Section 4500-NH₃.F, and Section 4500-NH₃.G, respectively.

f. Standards: Carry a reagent blank and standards through all steps of the procedure.

5. Calculation

See Section 4500-NH₃.C.5, Section 4500-NH₃.D.5, Section 4500-NH₃.F.5, or Section 4500-NH₃.G.5.

6. Precision and Bias

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Two analysts in one laboratory prepared reagent water solutions of nicotinic acid and digested them by the macro-kjeldahl method. Ammonia in the distillate was determined by titration. Results are summarized in Table 4500-N_{org}:I.

7. Bibliography

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4500-N_{org} C. Semi-Micro-Kjeldahl Method

1. General Discussion

See Section 4500-N_{org}:B.1.

2. Apparatus

a. Digestion apparatus: Use kjeldahl flasks with a capacity of 100 mL in a semi-micro-kjeldahl digestion apparatus†#(67) equipped with heating elements to accommodate kjeldahl flasks and a suction outlet to vent fumes. The heating elements should provide the temperature range of 375 to 385°C for effective digestion.

b. Distillation apparatus: Use an all-glass unit equipped with a steam-generating vessel containing an immersion heater†#(68) (Figure 4500-N_{org}:1).

c. pH meter.

d. Apparatus for ammonia determination: See Section 4500-NH₃:C.2, Section 4500-NH₃:D.2, Section 4500-NH₃:F.2, or Section 4500-NH₃:G.2.

3. Reagents

All of the reagents listed for the determination of Nitrogen (Ammonia) (Section 4500-NH₃:B.3) and Nitrogen (Organic) macro-kjeldahl (Section 4500-N_{org}:B.3) are required.

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Prepare all reagents and dilutions with ammonia-free water.

4. Procedure

a. Selection of sample volume: Determine the sample size from the following tabulation:

Organic Nitrogen in Sample mg/L	Sample Size mL
4–40	50
8–80	25
20–200	10
40–400	5

For sludge and sediment samples weigh a portion of wet sample containing between 0.2 and 2 mg organic nitrogen in a crucible or weighing bottle. Transfer sample quantitatively to a 100-mL beaker by diluting it and rinsing the weighing dish several times with small quantities of water. Make the transfer using as small a quantity of water as possible and do not exceed a total volume of 50 mL. Measure dry weight of sample on a separate portion.

b. Ammonia removal: Pipet 50 mL sample or an appropriate volume diluted to 50 mL with water into a 100-mL beaker. Add 3 mL borate buffer and adjust to pH 9.5 with 6N NaOH, using a pH meter. Quantitatively transfer sample to a 100-mL kjeldahl flask and boil off 30 mL. Alternatively, if ammonia removal is not required, digest samples directly as described in ¶ c below. Distillation following this direct digestion yields kjeldahl nitrogen concentration rather than organic nitrogen.

c. Digestion: Carefully add 10 mL digestion reagent to kjeldahl flask containing sample. Add five or six glass beads (3- to 4-mm size) to prevent bumping during digestion. Set each heating unit on the micro-kjeldahl digestion apparatus to its medium setting and heat flasks under a hood or with suitable ejection equipment to remove fumes of SO₃. Continue to boil briskly until solution becomes transparent and pale green and copious fumes are observed. Then turn each heating unit up to its maximum setting and digest for an additional 30 min. Cool. Quantitatively transfer digested sample by diluting and rinsing several times into micro-kjeldahl distillation apparatus so that total volume in distillation apparatus does not exceed 30 mL. Add 10 mL sodium hydroxide-thiosulfate reagent and turn on steam.

d. Distillation: Control rate of steam generation to boil contents in distillation unit so that neither escape of steam from tip of condenser nor bubbling of contents in receiving flask occurs. Distill and collect 30 to 40 mL distillate below surface of 10 mL absorbent solution contained in a 125-mL erlenmeyer flask. Use indicating boric acid for a titrimetric finish. Use 10 mL 0.04N H₂SO₄ solution for collecting distillate for the phenate or electrode methods. Extend tip of condenser well below level of absorbent solution and do not let temperature in condenser rise

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above 29°C. Lower collected distillate free of contact with condenser tip and continue distillation during last 1 or 2 min to cleanse condenser.

e. Standards: Carry a reagent blank and standards through all steps of procedure and apply necessary correction to results.

f. Final ammonia measurement: Use the titration, ammonia-selective electrode, manual phenate, or automated phenate method, Section 4500-NH₃.C, Section 4500-NH₃.D, Section 4500-NH₃.F, and Section 4500-NH₃.G, respectively.

5. Calculation

See Section 4500-NH₃.C.5, Section 4500-NH₃.D.5, Section 4500-NH₃.F.5, or Section 4500-NH₃.G.5.

6. Precision and Bias

No data on the precision and bias of the semi-micro-kjeldahl method are available.

7. Bibliography

See Section 4500-N_{org}.B.7.

4500-N_{org} D. Block Digestion and Flow Injection Analysis (PROPOSED)

1. General Discussion

a. Principle: Samples of drinking, ground, and surface waters and of domestic and industrial wastes are digested in a block digester with sulfuric acid and copper sulfate as a catalyst. The digestion recovers nitrogen components of biological origin, such as amino acids, proteins, and peptides, as ammonia, but may not recover the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones, and some refractory tertiary amines. Nitrate is not recovered. See Section 4500-N for a discussion of the various forms of nitrogen found in waters and wastewaters, Section 4500-N_{org}.A and Section 4500-N_{org}.B for a discussion of kjeldahl nitrogen methods, and Section 4130, Flow Injection Analysis (FIA).

The digested sample is injected onto the FIA manifold where its pH is controlled by raising it to a known, basic pH by neutralization with a concentrated buffer. This in-line neutralization converts the ammonium cation to ammonia, and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction that follows. The ammonia thus produced is heated with salicylate and hypochlorite to produce a blue color that is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of EDTA in the buffer prevents precipitation of calcium and magnesium. The resulting peak's absorbance is measured at 660 nm. The peak area is proportional to the concentration of total

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kjeldahl nitrogen in the original sample.

b. Interferences: Remove large or fibrous particulates by filtering the sample through glass wool.

The main source of interference is ammonia. Ammonia is an airborne contaminant that is removed rapidly from ambient air by the digestion solution. Guard against ammonia contamination in reagents, water, glassware, and the sample preservation process. It is particularly important to prevent ammonia contamination in the sulfuric acid used for the digestion. Open sulfuric acid bottles away from laboratories in which ammonia or ammonium chloride have been used as reagents and store sulfuric acid away from such reagents. Ensure that the open ends of the block digester's tubes can be covered to prevent ammonia from being scrubbed from the fume hood make-up air during the digestion.

If a sample consumes more than 10% of the sulfuric acid during digestion, the pH-dependent color reaction will show a matrix effect. The color reaction buffer will accommodate a range of $5.4\% \pm 0.4\%$ H_2SO_4 (v/v) in the diluted digested sample. Sample matrices with a high concentration of carbohydrates or other organic material may consume more than 10% of the acid during digestion. If this effect is suspected, titrate digested sample with standardized sodium hydroxide to determine whether more than 10% of the sulfuric acid has been consumed during digestion. The block digester also should have a means to prevent loss of sulfuric acid from the digestion tubes during the digestion period.

Also see Section 4500-N_{org}.A and Section 4500-N_{org}.B.

2. Apparatus

Digestion and flow injection analysis equipment consisting of:

- a. Block digester* capable of maintaining a temperature of 380°C for 2 h.
- b. Digestion tubes* capable of being heated to 380°C for 2 h and having a cover to prevent ammonia contamination and loss of sulfuric acid.
- c. FIA injection valve* with sample loop or equivalent.
- d. Multichannel proportioning pump.*
- e. FIA manifold* (Figure 4500-N_{org}:2) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-N_{org}:2. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.
- f. Absorbance detector*, 660 nm, 10-nm bandpass.
- g. Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by

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weight/weight, use weight/volume.

a. *Digestion solution*: In a 1-L volumetric flask, dissolve 134.0 g potassium sulfate, K_2SO_4 , and 7.3 g copper sulfate, $CuSO_4$, in 800 mL water. Then add slowly while swirling 134 mL conc sulfuric acid, H_2SO_4 . Let cool, dilute to mark, and invert to mix.

b. *Carrier and diluent*: To a tared 1-L container, add 496 g digestion solution (§ 3a) and 600 g water. Shake until dissolved.

c. *Sodium hydroxide*, NaOH, 0.8M: To a tared 1-L plastic container, add 32.0 g NaOH and 985.0 g water. Stir or shake until dissolved.

d. *Buffer*: To a tared 1-L container add 941 g water. Add and completely dissolve 35.0 g sodium phosphate dibasic heptahydrate, $Na_2HPO_4 \cdot 7H_2O$. Add 20.0 g disodium EDTA (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add 50 g NaOH. Stir or shake until dissolved.

e. *Salicylate/nitroprusside*: To a tared 1-L dark container, add 150.0 g sodium salicylate (salicylic acid sodium salt), $C_6H_4(OH)(COO)Na$, 1.00 g sodium nitroprusside (sodium nitroferricyanide dihydrate), $Na_2Fe(CN)_5NO \cdot 2H_2O$, and 908 g water. Stir or shake until dissolved. Prepare fresh monthly.

f. *Hypochlorite*: To a tared 250-mL container, add 16 g commercial 5.25% sodium hypochlorite bleach solution*#(69) and 234 g deionized water. Shake to mix.

g. *Stock standard*, 250 mg N/L: In a 1-L volumetric flask dissolve 0.9540 g ammonium chloride, NH_4Cl (dried for 2 h at $110^\circ C$), in about 800 mL water. Dilute to mark and invert to mix.

h. *Standard ammonia solutions*: Prepare ammonia standards in desired concentration range, using the stock standard (§ 3g) and diluting with water.

i. *Simulated digested standards*: To prepare calibration standards without having to digest the standards prepared in § 3h, proceed as follows:

Stock standard, 5.00 mg N/L: To a tared 250-mL container add about 5.0 g stock standard (250 mg N/L). Divide actual weight of solution added by 0.02 and make up to this resulting total weight with diluent (§ 3b). Shake to mix. Prepare working standards from this stock standard, diluting with the diluent (§ 3b), not water.

4. Procedure

a. *Digestion procedure*: Carry both standards and samples through this procedure.

To a 75-mL block digester tube add 25.0 mL sample or standard and then add 10 mL digestion solution (§ 3a) and mix. Add four alundum granules to each tube for smooth boiling. Place tubes in preheated block digester for 1 h at $200^\circ C$. After 1 h, increase block temperature to $380^\circ C$ and continue to digest for 1 h at $380^\circ C$. Remove tubes from block and let cool for about 10 min. Dilute each to 25.0 mL with water and mix with vortex mixer. Cover tubes to prevent

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ammonia contamination.

b. FIA analysis: Set up a manifold equivalent to that in Figure 4500-N_{org}:2 and analyze digested standards and samples by method supplied by manufacturer or laboratory standard operating procedure. Follow quality control protocols described in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus ammonia concentration. The calibration curve is linear.

6. Precision and Bias

a. Recovery and relative standard deviation: Table 4500-N_{org}:II gives results of single-laboratory studies.

b. MDL: A 130- μ L sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 0.1-mg N/L standard. These gave a mean of 0.103 mg N/L, a standard deviation of 0.014 mg N/L, and MDL of 0.034 mg N/L. A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagents flow rate.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-O OXYGEN (DISSOLVED)*#(70)

4500-O A. Introduction

1. Significance

Dissolved oxygen (DO) levels in natural and wastewaters depend on the physical, chemical, and biochemical activities in the water body. The analysis for DO is a key test in water pollution and waste treatment process control.

2. Selection of Method

Two methods for DO analysis are described: the Winkler or iodometric method and its modifications and the electrometric method using membrane electrodes. The iodometric method¹ is a titrimetric procedure based on the oxidizing property of DO while the membrane electrode procedure is based on the rate of diffusion of molecular oxygen across a membrane.² The choice

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of procedure depends on the interferences present, the accuracy desired, and, in some cases, convenience or expedience.

3. References

1. WINKLER, L.W. 1888. The determination of dissolved oxygen in water. *Berlin. Deut. Chem. Ges.* 21:2843.
2. MANCY, K.H. & T. JAFFE. 1966. Analysis of Dissolved Oxygen in Natural and Waste Waters. Publ. No. 999-WP-37, U.S. Public Health Serv., Washington, D.C.

4500-O B. Iodometric Methods

1. Principle

The iodometric test is the most precise and reliable titrimetric procedure for DO analysis. It is based on the addition of divalent manganese solution, followed by strong alkali, to the sample in a glass-stoppered bottle. DO rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxides of higher valency states. In the presence of iodide ions in an acidic solution, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent to the original DO content. The iodine is then titrated with a standard solution of thiosulfate.

The titration end point can be detected visually, with a starch indicator, or electrometrically, with potentiometric or dead-stop techniques.¹ Experienced analysts can maintain a precision of $\pm 50 \mu\text{g/L}$ with visual end-point detection and a precision of $\pm 5 \mu\text{g/L}$ with electrometric end-point detection.^{1,2}

The liberated iodine also can be determined directly by simple absorption spectrophotometers.³ This method can be used on a routine basis to provide very accurate estimates for DO in the microgram-per-liter range provided that interfering particulate matter, color, and chemical interferences are absent.

2. Selection of Method

Before selecting a method consider the effect of interferences, particularly oxidizing or reducing materials that may be present in the sample. Certain oxidizing agents liberate iodine from iodides (positive interference) and some reducing agents reduce iodine to iodide (negative interference). Most organic matter is oxidized partially when the oxidized manganese precipitate is acidified, thus causing negative errors.

Several modifications of the iodometric method are given to minimize the effect of interfering materials.² Among the more commonly used procedures are the azide modification,⁴ the permanganate modification,⁵ the alum flocculation modification,⁶ and the copper sulfate-sulfamic acid flocculation modification.^{7,8} The azide modification (C) effectively

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removes interference caused by nitrite, which is the most common interference in biologically treated effluents and incubated BOD samples. Use the permanganate modification (D) in the presence of ferrous iron. When the sample contains 5 or more mg ferric iron salts/L, add potassium fluoride (KF) as the first reagent in the azide modification or after the permanganate treatment for ferrous iron. Alternately, eliminate Fe(III) interference by using 85 to 87% phosphoric acid (H_3PO_4) instead of sulfuric acid (H_2SO_4) for acidification. This procedure has not been tested for Fe(III) concentrations above 20 mg/L.

Use the alum flocculation modification (E) in the presence of suspended solids that cause interference and the copper sulfate-sulfamic acid flocculation modification (F) on activated-sludge mixed liquor.

3. Collection of Samples

Collect samples very carefully. Methods of sampling are highly dependent on source to be sampled and, to a certain extent, on method of analysis. Do not let sample remain in contact with air or be agitated, because either condition causes a change in its gaseous content. Samples from any depth in streams, lakes, or reservoirs, and samples of boiler water, need special precautions to eliminate changes in pressure and temperature. Procedures and equipment have been developed for sampling waters under pressure and unconfined waters (e.g., streams, rivers, and reservoirs). Sampling procedures and equipment needed are described in American Society for Testing and Materials Special Technical Publication No. 148-1 and in U.S. Geological Survey Water Supply Paper No. 1454.

Collect surface water samples in narrow-mouth glass-stoppered BOD bottles of 300-mL capacity with tapered and pointed ground-glass stoppers and flared mouths. Avoid entraining or dissolving atmospheric oxygen. In sampling from a line under pressure, attach a glass or rubber tube to the tap and extend to bottom of bottle. Let bottle overflow two or three times its volume and replace stopper so that no air bubbles are entrained.

Suitable samplers for streams, ponds, or tanks of moderate depth are of the APHA type shown in Figure 4500-O:1. Use a Kemmerer-type sampler for samples collected from depths greater than 2 m. Bleed sample from bottom of sampler through a tube extending to bottom of a 250- to 300-mL BOD bottle. Fill bottle to overflowing (overflow for approximately 10 s), and prevent turbulence and formation of bubbles while filling. Record sample temperature to nearest degree Celsius or more precisely.

4. Preservation of Samples

Determine DO immediately on all samples containing an appreciable oxygen or iodine demand. Samples with no iodine demand may be stored for a few hours without change after adding manganous sulfate (MnSO_4) solution, alkali-iodide solution, and H_2SO_4 , followed by shaking in the usual way. Protect stored samples from strong sunlight and titrate as soon as possible.

For samples with an iodine demand, preserve for 4 to 8 h by adding 0.7 mL conc H_2SO_4 and

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1 mL sodium azide solution (2 g NaN_3 /100 mL distilled water) to the BOD bottle. This will arrest biological activity and maintain DO if the bottle is stored at the temperature of collection or water-sealed and kept at 10 to 20°C. As soon as possible, complete the procedure, using 2 mL MnSO_4 solution, 3 mL alkali-iodide solution, and 2 mL conc H_2SO_4 .

5. References

1. POTTER, E.C. & G.E. EVERITT. 1957. Advances in dissolved oxygen microanalysis. *J. Appl. Chem.* 9:642.
2. MANCY, K.H. & T. JAFFE. 1966. Analysis of Dissolved Oxygen in Natural and Waste Waters. Publ. No. 99-WP-37, U.S. Public Health Serv., Washington, D.C.
3. OULMAN, C.S. & E.R. BAUMANN. 1956. A colorimetric method for determining dissolved oxygen. *Sewage Ind. Wastes* 28:1461.
4. ALSTERBERG, G. 1925. Methods for the determination of elementary oxygen dissolved in water in the presence of nitrite. *Biochem. Z.* 159: 36.
5. RIDEAL, S. & G.G. STEWART. 1901. The determination of dissolved oxygen in waters in the presence of nitrites and of organic matter. *Analyst* 26:141.
6. RUCHHOFT, C.C. & W.A. MOORE. 1940. The determination of biochemical oxygen demand and dissolved oxygen of river mud suspensions. *Ind. Eng. Chem., Anal. Ed.* 12:711.
7. PLACAK, O.R. & C.C. RUCHHOFT. 1941. Comparative study of the azide and Rideal-Stewart modifications of the Winkler method in the determination of biochemical oxygen demand. *Ind. Eng. Chem., Anal. Ed.* 13:12.
8. RUCHHOFT, C.C. & O.R. PLACAK. 1942. Determination of dissolved oxygen in activated-sludge sewage mixtures. *Sewage Works J.* 14:638.

4500-O C. Azide Modification

1. General Discussion

Use the azide modification for most wastewater, effluent, and stream samples, especially if samples contain more than 50 $\mu\text{g NO}_2^-$ -N/L and not more than 1 mg ferrous iron/L. Other reducing or oxidizing materials should be absent. If 1 mL KF solution is added before the sample is acidified and there is no delay in titration, the method is applicable in the presence of 100 to 200 mg ferric iron/L.

2. Reagents

a. Manganous sulfate solution: Dissolve 480 g $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 400 g $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$, or 364 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ in distilled water, filter, and dilute to 1 L. The MnSO_4 solution should not give a

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color with starch when added to an acidified potassium iodide (KI) solution.

b. Alkali-iodide-azide reagent:

1) For saturated or less-than-saturated samples—Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in distilled water and dilute to 1 L. Add 10 g NaN_3 dissolved in 40 mL distilled water. Potassium and sodium salts may be used interchangeably. This reagent should not give a color with starch solution when diluted and acidified.

2) For supersaturated samples—Dissolve 10 g NaN_3 in 500 mL distilled water. Add 480 g sodium hydroxide (NaOH) and 750 g sodium iodide (NaI), and stir until dissolved. There will be a white turbidity due to sodium carbonate (Na_2CO_3), but this will do no harm. CAUTION—*Do not acidify this solution because toxic hydrazoic acid fumes may be produced.*

c. Sulfuric acid, H_2SO_4 , conc: One milliliter is equivalent to about 3 mL alkali-iodide-azide reagent.

d. Starch: Use either an aqueous solution or soluble starch powder mixtures.

To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid, as a preservative, in 100 mL hot distilled water.

e. Standard sodium thiosulfate titrant: Dissolve 6.205 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in distilled water. Add 1.5 mL 6N NaOH or 0.4 g solid NaOH and dilute to 1000 mL. Standardize with bi-iodate solution.

f. Standard potassium bi-iodate solution, 0.0021M: Dissolve 812.4 mg $\text{KH}(\text{IO}_3)_2$ in distilled water and dilute to 1000 mL.

Standardization—Dissolve approximately 2 g KI, free from iodate, in an erlenmeyer flask with 100 to 150 mL distilled water. Add 1 mL 6N H_2SO_4 or a few drops of conc H_2SO_4 and 20.00 mL standard bi-iodate solution. Dilute to 200 mL and titrate liberated iodine with thiosulfate titrant, adding starch toward end of titration, when a pale straw color is reached. When the solutions are of equal strength, 20.00 mL 0.025M $\text{Na}_2\text{S}_2\text{O}_3$ should be required. If not, adjust the $\text{Na}_2\text{S}_2\text{O}_3$ solution to 0.025M.

3. Procedure

a. To the sample collected in a 250- to 300-mL bottle, add 1 mL MnSO_4 solution, followed by 1 mL alkali-iodide-azide reagent. If pipets are dipped into sample, rinse them before returning them to reagent bottles. Alternatively, hold pipet tips just above liquid surface when adding reagents. Stopper carefully to exclude air bubbles and mix by inverting bottle a few times. When precipitate has settled sufficiently (to approximately half the bottle volume) to leave clear supernate above the manganese hydroxide floc, add 1.0 mL conc H_2SO_4 . Restopper and mix by inverting several times until dissolution is complete. Titrate a volume corresponding to 200 mL original sample after correction for sample loss by displacement with reagents. Thus, for a total of 2 mL (1 mL each) of MnSO_4 and alkali-iodide-azide reagents in a 300-mL bottle, titrate $200 \times$

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$300/(300 - 2) = 201 \text{ mL}$.

b. Titrate with 0.025M $\text{Na}_2\text{S}_2\text{O}_3$ solution to a pale straw color. Add a few drops of starch solution and continue titration to first disappearance of blue color. If end point is overrun, back-titrate with 0.0021M bi-iodate solution added dropwise, or by adding a measured volume of treated sample. Correct for amount of bi-iodate solution or sample. Disregard subsequent recolorations due to the catalytic effect of nitrite or to traces of ferric salts that have not been complexed with fluoride.

4. Calculation

a. For titration of 200 mL sample, $1 \text{ mL } 0.025\text{M } \text{Na}_2\text{S}_2\text{O}_3 = 1 \text{ mg DO/L}$.

b. To express results as percent saturation at 101.3 kPa, use the solubility data in Table 4500-O:I. Equations for correcting solubilities to barometric pressures other than mean sea level and for various chlorinities are given below the table.

5. Precision and Bias

DO can be determined with a precision, expressed as a standard deviation, of about 20 $\mu\text{g/L}$ in distilled water and about 60 $\mu\text{g/L}$ in wastewater and secondary effluents. In the presence of appreciable interference, even with proper modifications, the standard deviation may be as high as 100 $\mu\text{g/L}$. Still greater errors may occur in testing waters having organic suspended solids or heavy pollution. Avoid errors due to carelessness in collecting samples, prolonging the completion of test, or selecting an unsuitable modification.

6. References

1. BENSON, B.B. & D. KRAUSE, JR. 1984. The concentration and isotopic fractionation of oxygen dissolved in freshwater and seawater in equilibrium with the atmosphere. *Limnol. Oceanogr.* 29:620.
2. BENSON, B.B. & D. KRAUSE, JR. 1980. The concentration and isotopic fractionation of gases dissolved in fresh water in equilibrium with the atmosphere: I. Oxygen. *Limnol. Oceanogr.* 25:662.
3. MORTIMER, C.H. 1981. The oxygen content of air-saturated fresh waters over ranges of temperature and atmospheric pressure of limnological interest. *Int. Assoc. Theoret. Appl. Limnol.*, Communication No. 22, Stuttgart, West Germany.
4. SULZER, F. & W.M. WESTGARTH. 1962. Continuous D. O. recording in activated sludge. *Water Sewage Works* 109: 376.
5. UNITED NATIONS EDUCATIONAL, SCIENTIFIC & CULTURAL ORGANIZATION. 1981. Background Papers and Supporting Data on the Practical Salinity Scale 1978. Tech. Paper Mar. Sci. No. 37.

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4500-O D. Permanganate Modification

1. General Discussion

Use the permanganate modification only on samples containing ferrous iron. Interference from high concentrations of ferric iron (up to several hundred milligrams per liter), as in acid mine water, may be overcome by the addition of 1 mL potassium fluoride (KF) and azide, provided that the final titration is made immediately after acidification.

This procedure is ineffective for oxidation of sulfite, thiosulfate, polythionate, or the organic matter in wastewater. The error with samples containing 0.25% by volume of digester waste from the manufacture of sulfite pulp may amount to 7 to 8 mg DO/L. With such samples, use the alkali-hypochlorite modification.¹ At best, however, the latter procedure gives low results, the deviation amounting to 1 mg/L for samples containing 0.25% digester wastes.

2. Reagents

All the reagents required for Method C, and in addition:

- a. *Potassium permanganate solution*: Dissolve 6.3 g KMnO_4 in distilled water and dilute to 1 L.
- b. *Potassium oxalate solution*: Dissolve 2 g $\text{K}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$ in 100 mL distilled water; 1 mL will reduce about 1.1 mL permanganate solution.
- c. *Potassium fluoride solution*: Dissolve 40 g $\text{KF} \cdot 2\text{H}_2\text{O}$ in distilled water and dilute to 100 mL.

3. Procedure

a. To a sample collected in a 250- to 300-mL bottle add, below the surface, 0.70 mL conc H_2SO_4 , 1 mL KMnO_4 solution, and 1 mL KF solution. Stopper and mix by inverting. Never add more than 0.7 mL conc H_2SO_4 as the first step of pretreatment. Add acid with a 1-mL pipet graduated to 0.1 mL. Add sufficient KMnO_4 solution to obtain a violet tinge that persists for 5 min. If the permanganate color is destroyed in a shorter time, add additional KMnO_4 solution, but avoid large excesses.

b. Remove permanganate color completely by adding 0.5 to 1.0 mL $\text{K}_2\text{C}_2\text{O}_4$ solution. Mix well and let stand in the dark to facilitate the reaction. Excess oxalate causes low results; add only enough $\text{K}_2\text{C}_2\text{O}_4$ to decolorize the KMnO_4 completely without an excess of more than 0.5 mL. Complete decolorization in 2 to 10 min. If it is impossible to decolorize the sample without adding a large excess of oxalate, the DO result will be inaccurate.

c. From this point the procedure closely parallels that in Section 4500-O.C.3. Add 1 mL MnSO_4 solution and 3 mL alkali-iodide-azide reagent. Stopper, mix, and let precipitate settle a

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short time; acidify with 2 mL conc H_2SO_4 . When 0.7 mL acid, 1 mL KF solution, 1 mL KMnO_4 solution, 1 mL $\text{K}_2\text{C}_2\text{O}_4$ solution, 1 mL MnSO_4 solution, and 3 mL alkali-iodide-azide (or a total of 7.7 mL reagents) are used in a 300-mL bottle, take $200 \times 300/(300 - 7.7) = 205$ mL for titration.

This correction is slightly in error because the KMnO_4 solution is nearly saturated with DO and 1 mL would add about 0.008 mg oxygen to the DO bottle. However, because precision of the method (standard deviation, 0.06 mL thiosulfate titration, or 0.012 mg DO) is 50% greater than this error, a correction is unnecessary. When substantially more KMnO_4 solution is used routinely, use a solution several times more concentrated so that 1 mL will satisfy the permanganate demand.

4. Reference

1. THERIAULT, E.J. & P.D. MCNAMEE. 1932. Dissolved oxygen in the presence of organic matter, hypochlorites, and sulfite wastes. *Ind. Eng. Chem., Anal. Ed.* 4:59.

4500-O E. Alum Flocculation Modification

1. General Discussion

Samples high in suspended solids may consume appreciable quantities of iodine in acid solution. The interference due to solids may be removed by alum flocculation.

2. Reagents

All the reagents required for the azide modification (Section 4500-O.C.2) and in addition:

a. *Alum solution*: Dissolve 10 g aluminum potassium sulfate, $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, in distilled water and dilute to 100 mL.

b. *Ammonium hydroxide*, NH_4OH , conc.

3. Procedure

Collect sample in a glass-stoppered bottle of 500 to 1000 mL capacity, using the same precautions as for regular DO samples. Add 10 mL alum solution and 1 to 2 mL conc NH_4OH . Stopper and invert gently for about 1 min. Let sample settle for about 10 min and siphon clear supernate into a 250- to 300-mL DO bottle until it overflows. Avoid sample aeration and keep siphon submerged at all times. Continue sample treatment as in Section 4500-O.C.3 or an appropriate modification.

4500-O F. Copper Sulfate-Sulfamic Acid Flocculation Modification

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1. General Discussion

This modification is used for biological flocs such as activated sludge mixtures, which have high oxygen utilization rates.

2. Reagents

All the reagents required for the azide modification (Section 4500-O.C.2) and, in addition:

Copper sulfate-sulfamic acid inhibitor solution: Dissolve 32 g technical-grade $\text{NH}_2\text{SO}_2\text{OH}$ without heat in 475 mL distilled water. Dissolve 50 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 500 mL distilled water. Mix the two solutions and add 25 mL conc acetic acid.

3. Procedure

Add 10 mL $\text{CuSO}_4\text{-NH}_2\text{SO}_2\text{OH}$ inhibitor to a 1-L glass-stoppered bottle. Insert bottle in a special sampler designed so that bottle fills from a tube near bottom and overflows only 25 to 50% of bottle capacity. Collect sample, stopper, and mix by inverting. Let suspended solids settle and siphon relatively clear supernatant liquor into a 250- to 300-mL DO bottle. Continue sample treatment as rapidly as possible by the azide (Section 4500-O.C.3) or other appropriate modification.

4500-O G. Membrane Electrode Method

1. General Discussion

Various modifications of the iodometric method have been developed to eliminate or minimize effects of interferences; nevertheless, the method still is inapplicable to a variety of industrial and domestic wastewaters.¹ Moreover, the iodometric method is not suited for field testing and cannot be adapted easily for continuous monitoring or for DO determinations in situ.

Polarographic methods using the dropping mercury electrode or the rotating platinum electrode have not been reliable always for the DO analysis in domestic and industrial wastewaters because impurities in the test solution can cause electrode poisoning or other interferences.^{2,3} With membrane-covered electrode systems these problems are minimized, because the sensing element is protected by an oxygen-permeable plastic membrane that serves as a diffusion barrier against impurities.⁴⁻⁶ Under steady-state conditions the current is directly proportional to the DO concentration.*#(71)

Membrane electrodes of the polarographic⁴ as well as the galvanic⁵ type have been used for DO measurements in lakes and reservoirs,⁸ for stream survey and control of industrial effluents,^{9,10} for continuous monitoring of DO in activated sludge units,¹¹ and for estuarine and oceanographic studies.¹² Being completely submersible, membrane electrodes are suited for analysis in situ. Their portability and ease of operation and maintenance make them particularly convenient for field applications. In laboratory investigations, membrane electrodes have been

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used for continuous DO analysis in bacterial cultures, including the BOD test.^{5,13}

Membrane electrodes provide an excellent method for DO analysis in polluted waters, highly colored waters, and strong waste effluents. They are recommended for use especially under conditions that are unfavorable for use of the iodometric method, or when that test and its modifications are subject to serious errors caused by interferences.

a. Principle: Oxygen-sensitive membrane electrodes of the polarographic or galvanic type are composed of two solid metal electrodes in contact with supporting electrolyte separated from the test solution by a selective membrane. The basic difference between the galvanic and the polarographic systems is that in the former the electrode reaction is spontaneous (similar to that in a fuel cell), while in the latter an external source of applied voltage is needed to polarize the indicator electrode. Polyethylene and fluorocarbon membranes are used commonly because they are permeable to molecular oxygen and are relatively rugged.

Membrane electrodes are commercially available in some variety. In all these instruments the “diffusion current” is linearly proportional to the concentration of molecular oxygen. The current can be converted easily to concentration units (e.g., milligrams per liter) by a number of calibration procedures.

Membrane electrodes exhibit a relatively high temperature coefficient largely due to changes in the membrane permeability.⁶ The effect of temperature on the electrode sensitivity, ϕ (microamperes per milligram per liter), can be expressed by the following simplified relationship:⁶

$$\log \phi = 0.43 mt + b$$

where:

- t = temperature, °C,
- m = constant that depends on the membrane material, and
- b = constant that largely depends on membrane thickness.

If values of ϕ and m are determined for one temperature (ϕ_0 and t_0), it is possible to calculate the sensitivity at any desired temperature (ϕ and t) as follows:

$$\log \phi = \log \phi_0 + 0.43 m (t - t_0)$$

Nomographic charts for temperature correction can be constructed easily⁷ and are available from some manufacturers. An example is shown in Figure 4500-O:2, in which, for simplicity, sensitivity is plotted versus temperature on semilogarithmic coordinates. Check one or two points frequently to confirm original calibration. If calibration changes, the new calibration should be parallel to the original, provided that the same membrane material is used.

Temperature compensation also can be made automatically by using thermistors in the electrode circuit.⁴ However, thermistors may not compensate fully over a wide temperature

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range. For certain applications where high accuracy is required, use calibrated nomographic charts to correct for temperature effect.

To use the DO membrane electrode in estuarine waters or in wastewaters with varying ionic strength, correct for effect of salting-out on electrode sensitivity.^{6,7} This effect is particularly significant for large changes in salt content. Electrode sensitivity varies with salt concentration according to the following relationship:

$$\log \phi_S = 0.43 m_S C_S + \log \phi_0$$

where:

ϕ_S, ϕ_0 = sensitivities in salt solution and distilled water, respectively,

C_S = salt concentration (preferably ionic strength), and

m_S = constant (salting-out coefficient).

If ϕ_0 and m_S are determined, it is possible to calculate sensitivity for any value of C_S . Conductivity measurements can be used to approximate salt concentration (C_S). This is particularly applicable to estuarine waters. Figure 4500-O:3 shows calibration curves for sensitivity of varying salt solutions at different temperatures.

b. Interference: Plastic films used with membrane electrode systems are permeable to a variety of gases besides oxygen, although none is depolarized easily at the indicator electrode. Prolonged use of membrane electrodes in waters containing such gases as hydrogen sulfide (H_2S) tends to lower cell sensitivity. Eliminate this interference by frequently changing and calibrating the membrane electrode.

c. Sampling: Because membrane electrodes offer the advantage of analysis in situ they eliminate errors caused by sample handling and storage. If sampling is required, use the same precautions suggested for the iodometric method.

2. Apparatus

Oxygen-sensitive membrane electrode, polarographic or galvanic, with appropriate meter.

3. Procedure

a. Calibration: Follow manufacturer's calibration procedure exactly to obtain guaranteed precision and accuracy. Generally, calibrate membrane electrodes by reading against air or a sample of known DO concentration (determined by iodometric method) as well as in a sample with zero DO. (Add excess sodium sulfite, Na_2SO_3 , and a trace of cobalt chloride, $CoCl_2$, to bring DO to zero.) Preferably calibrate with samples of water under test. Avoid an iodometric calibration where interfering substances are suspected. The following illustrate the recommended procedures:

1) Fresh water—For unpolluted samples where interfering substances are absent, calibrate in

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the test solution or distilled water, whichever is more convenient.

2) Salt water—Calibrate directly with samples of seawater or waters having a constant salt concentration in excess of 1000 mg/L.

3) Fresh water containing pollutants or interfering substances— Calibrate with distilled water because erroneous results occur with the sample.

4) Salt water containing pollutants or interfering substances— Calibrate with a sample of clean water containing the same salt content as the sample. Add a concentrated potassium chloride (KCl) solution (see Conductivity, Section 2510 and Table 2510:I) to distilled water to produce the same specific conductance as that in the sample. For polluted ocean waters, calibrate with a sample of unpolluted seawater.

5) Estuary water containing varying quantities of salt—Calibrate with a sample of uncontaminated seawater or distilled or tap water. Determine sample chloride or salt concentration and revise calibration to account for change of oxygen solubility in the estuary water.⁷

b. Sample measurement: Follow all precautions recommended by manufacturer to insure acceptable results. Take care in changing membrane to avoid contamination of sensing element and also trapping of minute air bubbles under the membrane, which can lead to lowered response and high residual current. Provide sufficient sample flow across membrane surface to overcome erratic response (see Figure 4500-O:4 for a typical example of the effect of stirring).

c. Validation of temperature effect: Check frequently one or two points to verify temperature correction data.

4. Precision and Bias

With most commercially available membrane electrode systems an accuracy of ± 0.1 mg DO/L and a precision of ± 0.05 mg DO/ L can be obtained.

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4500-O₃ OZONE (RESIDUAL)*#(72)

4500-O₃ A. Introduction

1. Sources

Ozone, a potent germicide, is used also as an oxidizing agent for the oxidation of organic compounds that produce taste and odor in drinking water, for the destruction of organic coloring matter, and for the oxidation of reduced iron or manganese salts to insoluble oxides.

2. Selection of Method

Ozone residual in water is determined by the indigo method. Residual ozone decays rapidly. Depending on water quality, the ozone residual half-life may be several seconds to a few minutes. Methods also are available for determining ozone in process gases.^{1,2}

3. References

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2. RAKNESS, K.L., L.D. DEMERS, B.D. BLANK & D.J. HENRY. 1996. Gas phase ozone concentration comparisons from a commercial UV meter and KI wet-chemistry tests. *Ozone: Sci. Eng.* 18:231.

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4500-O₃ B. Indigo Colorimetric Method

1. General Discussion

The indigo colorimetric method is quantitative, selective, and simple; it replaces methods based on the measurement of total oxidant. The method is applicable to lake water, river infiltrate, manganese-containing groundwaters, extremely hard groundwaters, and even biologically treated domestic wastewaters.

a. Principle: In acidic solution, ozone rapidly decolorizes indigo. The decrease in absorbance is linear with increasing concentration. The proportionality constant at 600 nm is $0.42 \pm 0.01/\text{cm}/\text{mg/L}$ ($\Delta = 20\,000/M\cdot\text{cm}$) compared to the ultraviolet absorption of pure ozone of $= 2950/M\cdot\text{cm}$ at 258 nm).¹

b. Interferences: Hydrogen peroxide (H₂O₂) and organic peroxides decolorize the indigo reagent very slowly. H₂O₂ does not interfere if ozone is measured in less than 6 h after adding reagents. Organic peroxides may react more rapidly. Fe(III) does not interfere. Mn(II) does not interfere but it is oxidized by ozone to forms that decolorize the reagent. Correct for this decolorization by making the measurement relative to a blank in which the ozone has been destroyed selectively. Without the corrective procedure, 0.1 mg/L ozonated manganese gives a response of about 0.08 mg/L apparent ozone. Chlorine also interferes. Low concentrations of chlorine (<0.1 mg/L) can be masked by malonic acid. Bromine, which can be formed by oxidation of Br⁻, interferes (1 mole HOBr corresponds to 0.4 mole ozone). In the presence of HOBr or chlorine in excess of 0.1 mg/L, an accurate measurement of ozone cannot be made with this method.

c. Minimum detectable concentration: For the spectrophotometric procedure using thermostated cells and a high-quality photometer, the low-range procedure will measure down to 2 µg O₃/L. The practical lower limit for residual measurement is 10 to 20 µg/L.

d. Sampling: React sample with indigo as quickly as possible, because the residual may decay rapidly. Avoid loss of ozone residual due to off-gassing during sample collection. Do not run sample down side of flask. Add sample so that completely decolorized zones are eliminated quickly by swirling or stirring.

2. Apparatus

Photometer: Spectrophotometer or filter colorimeter for use at 600 ± 10 nm.

3. Reagents

a. Indigo stock solution: Add about 500 mL distilled water and 1 mL conc phosphoric acid to a 1-L volumetric flask. With stirring, add 770 mg potassium indigo trisulfonate, C₁₆H₇N₂O₁₁S₃K₃ (use only high-grade reagent, commercially available at about 80 to 85%

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purity). Fill to mark with distilled water. A 1:100 dilution exhibits an absorbance of 0.20 ± 0.010 cm at 600 nm. The stock solution is stable for about 4 months when stored in the dark. Discard when absorbance of a 1:100 dilution falls below 0.16/ cm. Do not change concentration of dye for higher ranges of ozone residual. Volume of dye used may be adjusted.

b. *Indigo reagent I*: To a 1-L volumetric flask add 20 mL indigo stock solution, 10 g sodium dihydrogen phosphate (NaH_2PO_4), and 7 mL conc phosphoric acid. Dilute to mark. Prepare solution fresh when its absorbance decreases to less than 80% of its initial value, typically within a week.

c. *Indigo reagent II*: Proceed as with indigo reagent I, but add 100 mL indigo stock solution instead of 20 mL.

d. *Malonic acid reagent*: Dissolve 5 g malonic acid in water and dilute to 100 mL.

e. *Glycine reagent*: Dissolve 7 g glycine in water and dilute to 100 mL.

4. Procedure

a. *Spectrophotometric, volumetric procedure*:

1) Concentration range 0.01 to 0.1 mg O_3/L —Add 10.0 mL indigo reagent I to each of two 100-mL volumetric flasks. Fill one flask (blank) to mark with distilled water. Fill other flask to mark with sample. Measure absorbance of both solutions at ± 10 nm as soon as possible but at least within 4 h. Preferably use 10-cm cells. Calculate the ozone concentration from the difference between the absorbances found in sample and blank (§ 5a below). (NOTE: A maximum delay of 4 h before spectrophotometric reading can be tolerated only for drinking water samples. For other sample types that cannot be read immediately, determine the relationship between time and absorbance.)

2) Range 0.05 to 0.5 mg O_3/L —Proceed as above using 10.0 mL indigo reagent II instead of reagent I. Preferably measure absorbance in 4- or 5-cm cells.

3) Concentrations greater than 0.3 mg O_3/L —Proceed using indigo reagent II, but for these higher ozone concentrations use a correspondingly smaller sample volume. Dilute resulting mixture to 100 mL with distilled water.

4) Control of interferences—In presence of low chlorine concentration (<0.1 mg/L), place 1 mL malonic acid reagent in both flasks before adding sample and/or filling to mark. Measure absorbance as soon as possible, within 60 min (Br^- , Br_2 , and HOBr are only partially masked by malonic acid).

In presence of manganese prepare a blank solution using sample, in which ozone is selectively destroyed by addition of glycine. Place 0.1 mL glycine reagent in 100-mL volumetric flask (blank) and 10.0 mL indigo reagent II in second flask (sample). Pipet exactly the same volume of sample into each flask. Adjust dose so that decolorization in second flask is easily visible but complete bleaching does not result (maximum 80 mL).

Insure that pH of glycine/sample mixture in blank flask (before adding indigo) is not below 6

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because reaction between ozone and glycine becomes very slow at low pH. Stopper flasks and mix by carefully inverting. Add 10.0 mL indigo reagent II to blank flask only 30 to 60 s after sample addition. Fill both flasks to the mark with ozone-free water and mix thoroughly. Measure absorbance of both solutions at comparable contact times of approximately 30 to 60 min (after this time, residual manganese oxides further discolor indigo only slowly and the drift of absorbance in blank and sample become comparable). Reduced absorbance in blank flask results from manganese oxides while that in sample flask is due to ozone plus manganese oxide.

5) Calibration—Because ozone is unstable, base measurements on known and constant loss of absorbance of the indigo reagent ($f = 0.42 + 0.01/\text{cm}/\text{mg O}_3/\text{L}$). For maximum accuracy analyze the lot of potassium indigo trisulfonate (no commercial lot has been found to deviate from $f = 0.42$) using the iodometric procedure.

When using a filter photometer, readjust the conversion factor, f , by comparing photometer sensitivity with absorbance at 600 nm by an accurate spectrophotometer.

b. Spectrophotometric, gravimetric procedure:

1) Add 10.0 mL indigo reagent II to 100-mL volumetric flask and fill flask (blank) to mark with distilled water. Obtain tare weight of a second flask (volumetric or erlenmeyer). Add 10.0 mL indigo reagent II to second flask. Fill directly with sample (do not run water down side), and swirl second flask until blue solution has turned to a light blue color. Weigh flask containing indigo and sample.

2) Preferably using 10-cm cells, measure absorbance of both solutions at 600 ± 10 nm as soon as possible, but at least within 4 h. NOTE: A maximum delay of 4 h before spectrophotometric reading is suitable only for drinking water samples. For other sample types, test the time drift.

5. Calculations

a. Spectrophotometric, volumetric method:

$$\text{mg O}_3/\text{L} = \frac{100 \times \Delta A}{f \times b \times V}$$

where:

ΔA = difference in absorbance between sample and blank,

b = path length of cell, cm,

V = volume of sample, mL (normally 90 mL), and

$f = 0.42$.

The factor f is based on a sensitivity factor of 20 000/cm for the change of absorbance (600

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nm) per mole of added ozone per liter. It was calibrated by iodometric titration. The UV absorbance of ozone in pure water may serve as a secondary standard: the factor $f = 0.42$ corresponds to an absorption coefficient for aqueous ozone, $= 2950/M \cdot \text{cm}$ at 258 nm.

b. *Spectrophotometric, gravimetric method:*

$$\text{mg O}_3/\text{L} = \frac{(A_B \times 100) - (A_S \times V_T)}{f \times V_S \times b}$$

where:

A_B, A_S = absorbance of blank and sample, respectively,

V_S = volume of sample, mL = [(final weight – tare weight) g $\times$ 1.0 mL/g] – 10 mL,

V_T = total volume of sample plus indigo, mL = (final weight – tare weight) g $\times$ 1.0 mL/g,

b = path length of cell, cm, and

$f = 0.42$ (see ¶ a above).

6. Precision and Bias

For the spectrophotometric volumetric procedure in the absence of interferences, the relative error is less than 5% without special sampling setups. In laboratory testing this may be reduced to 1%. No data are available for the spectrophotometric gravimetric procedure.

Because this method is based on the differences in absorbance between the sample and blank (ΔA) the method is not applicable in the presence of chlorine. If the manganese content exceeds the ozone, precision is reduced. If the ratio of manganese to ozone is less than 10:1, ozone concentrations above 0.02 mg/L may be determined with a relative error of less than 20%.

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4500-P PHOSPHORUS*#(73)

4500-P A. Introduction

1. Occurrence

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates (pyro-, meta-, and other polyphosphates), and organically bound phosphates. They occur in solution, in particles or detritus, or in the bodies of aquatic organisms.

These forms of phosphate arise from a variety of sources. Small amounts of orthophosphate or certain condensed phosphates are added to some water supplies during treatment. Larger quantities of the same compounds may be added when the water is used for laundering or other cleaning, because these materials are major constituents of many commercial cleaning preparations. Phosphates are used extensively in the treatment of boiler waters. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm runoff and to a lesser extent with melting snow. Organic phosphates are formed primarily by biological processes. They are contributed to sewage by body wastes and food residues, and also may be formed from orthophosphates in biological treatment processes or by receiving water biota.

Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water. In instances where phosphate is a growth-limiting nutrient, the discharge of raw or treated wastewater, agricultural drainage, or certain industrial wastes to that water may stimulate the growth of photosynthetic aquatic micro- and macroorganisms in nuisance quantities.

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Phosphates also occur in bottom sediments and in biological sludges, both as precipitated inorganic forms and incorporated into organic compounds.

2. Definition of Terms

Phosphorus analyses embody two general procedural steps: (a) conversion of the phosphorus form of interest to dissolved orthophosphate, and (b) colorimetric determination of dissolved orthophosphate. The separation of phosphorus into its various forms is defined analytically but the analytical differentiations have been selected so that they may be used for interpretive purposes.

Filtration through a 0.45- μm -pore-diam membrane filter separates dissolved from suspended forms of phosphorus. No claim is made that filtration through 0.45- μm filters is a true separation of suspended and dissolved forms of phosphorus; it is merely a convenient and replicable analytical technique designed to make a gross separation.

Membrane filtration is selected over depth filtration because of the greater likelihood of obtaining a consistent separation of particle sizes. Prefiltration through a glass fiber filter may be used to increase the filtration rate.

Phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the sample are termed “reactive phosphorus.” While reactive phosphorus is largely a measure of orthophosphate, a small fraction of any condensed phosphate present usually is hydrolyzed unavoidably in the procedure. Reactive phosphorus occurs in both dissolved and suspended forms.

Acid hydrolysis at boiling-water temperature converts dissolved and particulate condensed phosphates to dissolved orthophosphate. The hydrolysis unavoidably releases some phosphate from organic compounds, but this may be reduced to a minimum by judicious selection of acid strength and hydrolysis time and temperature. The term “acid-hydrolyzable phosphorus” is preferred over “condensed phosphate” for this fraction.

The phosphate fractions that are converted to orthophosphate only by oxidation destruction of the organic matter present are considered “organic” or “organically bound” phosphorus. The severity of the oxidation required for this conversion depends on the form—and to some extent on the amount—of the organic phosphorus present. Like reactive phosphorus and acid-hydrolyzable phosphorus, organic phosphorus occurs both in the dissolved and suspended fractions.

The total phosphorus as well as the dissolved and suspended phosphorus fractions each may be divided analytically into the three chemical types that have been described: reactive, acid-hydrolyzable, and organic phosphorus. Figure 4500-P:1 shows the steps for analysis of individual phosphorus fractions. As indicated, determinations usually are conducted only on the unfiltered and filtered samples. Suspended fractions generally are determined by difference; however, they may be determined directly by digestion of the material retained on a glass-fiber filter.

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3. Selection of Method

a. Digestion methods: Because phosphorus may occur in combination with organic matter, a digestion method to determine total phosphorus must be able to oxidize organic matter effectively to release phosphorus as orthophosphate. Three digestion methods are given in Section 4500-P.B.3, Section 4500-P.B.4, and Section 4500-P.B.5. The perchloric acid method, the most drastic and time-consuming method, is recommended only for particularly difficult samples such as sediments. The nitric acid-sulfuric acid method is recommended for most samples. By far the simplest method is the persulfate oxidation technique. Persulfate oxidation is coupled with ultraviolet light for a more efficient digestion in an automated in-line digestion/determination by flow injection analysis (4500-P.I). It is recommended that persulfate oxidation methods be checked against one or more of the more drastic digestion techniques and be adopted if identical recoveries are obtained.

After digestion, determine liberated orthophosphate by Method C, D, E, F, G, or H. The colorimetric method used, rather than the digestion procedure, governs in matters of interference and minimum detectable concentration.

b. Colorimetric method: Three methods of orthophosphate determination are described. Selection depends largely on the concentration range of orthophosphate. The vanadomolybdophosphoric acid method (C) is most useful for routine analysis in the range of 1 to 20 mg P/L. The stannous chloride method (D) or the ascorbic acid method (E) is more suited for the range of 0.01 to 6 mg P/L. An extraction step is recommended for the lower levels of this range and when interferences must be overcome. Automated versions of the ascorbic acid method (F, G, and H) also are presented. Careful attention to procedure may allow application of these methods to very low levels of phosphorus, such as those found in unimpaired fresh water.

Ion chromatography (Section 4110) and capillary ion electrophoresis (Section 4140) are useful for determination of orthophosphate in undigested samples.

4. Precision and Bias

To aid in method selection, Table 4500-P:I presents the results of various combinations of digestions, hydrolysis, and colorimetric techniques for three synthetic samples of the following compositions:

Sample 1: 100 µg orthophosphate phosphorus (PO_4^{3-} -P/L), 80 µg condensed phosphate phosphorus/L (sodium hexametaphosphate), 30 µg organic phosphorus/L (adenylic acid), 1.5 mg NH_3 -N/L, 0.5 mg NO_3 -N/L, and 400 mg Cl^- /L.

Sample 2: 600 µg PO_4^{3-} -P/L, 300 µg condensed phosphate phosphorus/L (sodium hexametaphosphate), 90 µg organic phosphorus/L (adenylic acid), 0.8 mg NH_3 -N/L, 5.0 mg NO_3^- -N/L, and 400 mg Cl^- /L.

Sample 3: 7.00 mg PO_4^{3-} -P/L, 3.00 mg condensed phosphate phosphorus/L (sodium

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hexametaphosphate), 0.230 mg organic phosphorus/L (adenylic acid), 0.20 mg $\text{NH}_3\text{-N/L}$, 0.05 mg $\text{NO}_3^- \text{- N/L}$, and 400 mg Cl^-/L .

5. Sampling and Storage

If dissolved phosphorus forms are to be differentiated, filter sample immediately after collection. Preserve by freezing at or below -10°C . In some cases 40 mg HgCl_2/L may be added to the samples, especially when they are to be stored for long periods before analysis. CAUTION: HgCl_2 is a hazardous substance; take appropriate precautions in disposal; use of HgCl_2 is not encouraged. Do not add either acid or CHCl_3 as a preservative when phosphorus forms are to be determined. If total phosphorus alone is to be determined, add H_2SO_4 or HCl to $\text{pH}<2$ and cool to 4°C , or freeze without any additions.

Do not store samples containing low concentrations of phosphorus in plastic bottles unless kept in a frozen state because phosphates may be adsorbed onto the walls of plastic bottles.

Rinse all glass containers with hot dilute HCl , then rinse several times in reagent water. Never use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis.

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4500-P B. Sample Preparation

For information on selection of digestion method (§s 3 through 5 below), see 4500-P.A.3a.

1. Preliminary Filtration

Filter samples for determination of dissolved reactive phosphorus, dissolved acid-hydrolyzable phosphorus, and total dissolved phosphorus through 0.45- μm membrane filters. A glass fiber filter may be used to prefilter hard-to-filter samples.

Wash membrane filters by soaking in distilled water before use because they may contribute significant amounts of phosphorus to samples containing low concentrations of phosphate. Use

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one of two washing techniques: (a) soak 50 filters in 2 L distilled water for 24 h; (b) soak 50 filters in 2 L distilled water for 1 h, change distilled water, and soak filters an additional 3 h. Membrane filters also may be washed by running several 100-mL portions of distilled water through them. This procedure requires more frequent determination of blank values to ensure consistency in washing and to evaluate different lots of filters.

2. Preliminary Acid Hydrolysis

The acid-hydrolyzable phosphorus content of the sample is defined operationally as the difference between reactive phosphorus as measured in the untreated sample and phosphate found after mild acid hydrolysis. Generally, it includes condensed phosphates such as pyro-, tripoly-, and higher-molecular-weight species such as hexametaphosphate. In addition, some natural waters contain organic phosphate compounds that are hydrolyzed to orthophosphate under the test conditions. Polyphosphates generally do not respond to reactive phosphorus tests but can be hydrolyzed to orthophosphate by boiling with acid.

After hydrolysis, determine reactive phosphorus by a colorimetric method (C, D, or E). Interferences, precision, bias, and sensitivity will depend on the colorimetric method used.

a. Apparatus:

Autoclave or pressure cooker, capable of operating at 98 to 137 kPa.

b. Reagents:

1) *Phenolphthalein indicator aqueous solution*.

2) *Strong acid solution*: Slowly add 300 mL conc H_2SO_4 to about 600 mL distilled water.

When cool, add 4.0 mL conc HNO_3 and dilute to 1 L.

3) Sodium hydroxide, NaOH, 6N.

c. *Procedure*: To 100-mL sample or a portion diluted to 100 mL, add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add strong acid solution dropwise, to just discharge the color. Then add 1 mL more.

Boil gently for at least 90 min, adding distilled water to keep the volume between 25 and 50 mL. Alternatively, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa. Cool, neutralize to a faint pink color with NaOH solution, and restore to the original 100-mL volume with distilled water.

Prepare a calibration curve by carrying a series of standards containing orthophosphate (see colorimetric method C, D, or E) through the hydrolysis step. Do not use orthophosphate standards without hydrolysis, because the salts added in hydrolysis cause an increase in the color intensity in some methods.

Determine reactive phosphorus content of treated portions, using Method C, D, or E. This gives the sum of polyphosphate and orthophosphate in the sample. To calculate its content of acid-hydrolyzable phosphorus, determine reactive phosphorus in a sample portion that has not been hydrolyzed, using the same colorimetric method as for treated sample, and subtract.

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3. Perchloric Acid Digestion

a. Apparatus:

- 1) *Hot plate:* A 30- × 50-cm heating surface is adequate.
- 2) *Safety shield.*
- 3) *Safety goggles.*
- 4) *Erlenmeyer flasks, 125-mL, acid-washed and rinsed with distilled water.*

b. Reagents:

- 1) *Nitric acid, HNO_3 , conc.*
- 2) *Perchloric acid, $\text{HClO}_4 \cdot 2\text{H}_2\text{O}$, purchased as 70 to 72% HClO_4 , reagent-grade.*
- 3) *Sodium hydroxide, NaOH , 6N.*
- 4) *Methyl orange indicator solution.*
- 5) *Phenolphthalein indicator aqueous solution.*

c. *Procedure:* CAUTION—Heated mixtures of HClO_4 and organic matter may explode violently. Avoid this hazard by taking the following precautions: (a) Do not add HClO_4 to a hot solution that may contain organic matter. (b) Always initiate digestion of samples containing organic matter with HNO_3 . Complete digestion using the mixture of HNO_3 and HClO_4 . (c) Do not fume with HClO_4 in ordinary hoods. Use hoods especially constructed for HClO_4 fuming or a glass fume eradicator*⁽⁷⁴⁾ connected to a water pump. (d) Never let samples being digested with HClO_4 evaporate to dryness.

Measure sample containing the desired amount of phosphorus (this will be determined by whether Method C, D, or E is to be used) into a 125-mL erlenmeyer flask. Acidify to methyl orange with conc HNO_3 , add another 5 mL conc HNO_3 , and evaporate on a steam bath or hot plate to 15 to 20 mL.

Add 10 mL each of conc HNO_3 and HClO_4 to the 125-mL conical flask, cooling the flask between additions. Add a few boiling chips, heat on a hot plate, and evaporate gently until dense white fumes of HClO_4 just appear. If solution is not clear, cover neck of flask with a watch glass and keep solution barely boiling until it clears. If necessary, add 10 mL more HNO_3 to aid oxidation.

Cool digested solution and add 1 drop aqueous phenolphthalein solution. Add 6N NaOH solution until the solution just turns pink. If necessary, filter neutralized solution and wash filter liberally with distilled water. Make up to 100 mL with distilled water.

Determine the PO_4^{3-} -P content of the treated sample by Method C, D, or E.

Prepare a calibration curve by carrying a series of standards containing orthophosphate (see Method C, D, or E) through digestion step. Do not use orthophosphate standards without

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treatment.

4. Sulfuric Acid-Nitric Acid Digestion

a. Apparatus:

1) *Digestion rack*: An electrically or gas-heated digestion rack with provision for withdrawal of fumes is recommended. Digestion racks typical of those used for micro-kjeldahl digestions are suitable.

2) *Micro-kjeldahl flasks*.

b. Reagents:

1) *Sulfuric acid*, H_2SO_4 , conc.

2) *Nitric acid*, HNO_3 , conc.

3) *Phenolphthalein indicator aqueous solution*.

4) *Sodium hydroxide*, NaOH , 1N.

c. *Procedure*: Into a micro-kjeldahl flask, measure a sample containing the desired amount of phosphorus (this is determined by the colorimetric method used). Add 1 mL conc H_2SO_4 and 5 mL conc HNO_3 .

Digest to a volume of 1 mL and then continue until solution becomes colorless to remove HNO_3 .

Cool and add approximately 20 mL distilled water, 0.05 mL (1 drop) phenolphthalein indicator, and as much 1N NaOH solution as required to produce a faint pink tinge. Transfer neutralized solution, filtering if necessary to remove particulate material or turbidity, into a 100-mL volumetric flask. Add filter washings to flask and adjust sample volume to 100 mL with distilled water.

Determine phosphorus by Method C, D, or E, for which a separate calibration curve has been constructed by carrying standards through the acid digestion procedure.

5. Persulfate Digestion Method

a. Apparatus:

1) *Hot plate*: A 30- × 50-cm heating surface is adequate.

2) *Autoclave*: An autoclave or pressure cooker capable of developing 98 to 137 kPa may be used in place of a hot plate.

3) *Glass scoop*, to hold required amounts of persulfate crystals.

b. Reagents:

1) *Phenolphthalein indicator aqueous solution*.

2) *Sulfuric acid solution*: Carefully add 300 mL conc H_2SO_4 to approximately 600 mL distilled water and dilute to 1 L with distilled water.

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3) *Ammonium persulfate*, $(\text{NH}_4)_2\text{S}_2\text{O}_8$, solid, or potassium persulfate, $\text{K}_2\text{S}_2\text{O}_8$, solid.

4) *Sodium hydroxide*, NaOH , 1N.

c. *Procedure*: Use 50 mL or a suitable portion of thoroughly mixed sample. Add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add H_2SO_4 solution dropwise to just discharge the color. Then add 1 mL H_2SO_4 solution and either 0.4 g solid $(\text{NH}_4)_2\text{S}_2\text{O}_8$ or 0.5 g solid $\text{K}_2\text{S}_2\text{O}_8$.

Boil gently on a preheated hot plate for 30 to 40 min or until a final volume of 10 mL is reached. Organophosphorus compounds such as AMP may require as much as 1.5 to 2 h for complete digestion. Cool, dilute to 30 mL with distilled water, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH . Alternatively, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa. Cool, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH . Make up to 100 mL with distilled water. In some samples a precipitate may form at this stage, but do not filter. For any subsequent subdividing of the sample, shake well. The precipitate (which is possibly a calcium phosphate) redissolves under the acid conditions of the colorimetric reactive phosphorus test. Determine phosphorus by Method C, D, or E, for which a separate calibration curve has been constructed by carrying standards through the persulfate digestion procedure.

6. Bibliography

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4500-P C. Vanadomolybdophosphoric Acid Colorimetric Method

1. General Discussion

a. *Principle*: In a dilute orthophosphate solution, ammonium molybdate reacts under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow color is proportional to phosphate concentration.

b. *Interference*: Positive interference is caused by silica and arsenate only if the sample is heated. Negative interferences are caused by arsenate, fluoride, thorium, bismuth, sulfide, thiosulfate, thiocyanate, or excess molybdate. Blue color is caused by ferrous iron but this does not affect results if ferrous iron concentration is less than 100 mg/L. Sulfide interference may be removed by oxidation with bromine water. Ions that do not interfere in concentrations up to 1000

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mg/L are Al^{3+} , Fe^{3+} , Mg^{2+} , Ca^{2+} , Ba^{2+} , Sr^{2+} , Li^{+} , Na^{+} , K^{+} , NH_4^{+} , Cd^{2+} , Mn^{2+} , Pb^{2+} , Hg^{+} , Hg^{2+} , Sn^{2+} , Cu^{2+} , Ni^{2+} , Ag^{+} , U^{4+} , Zr^{4+} , AsO_3^{-} , Br^{-} , CO_3^{2-} , ClO_4^{-} , CN^{-} , IO_3^{-} , SiO_4^{4-} , NO_3^{-} , NO_2^{-} , SO_4^{2-} , SO_3^{2-} , pyrophosphate, molybdate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate, and salicylate. If HNO_3 is used in the test, Cl^{-} interferes at 75 mg/L.

c. *Minimum detectable concentration:* The minimum detectable concentration is 200 $\mu\text{g P/L}$ in 1-cm spectrophotometer cells.

2. Apparatus

a. *Colorimetric equipment:* One of the following is required:

- 1) *Spectrophotometer*, for use at 400 to 490 nm.
- 2) *Filter photometer*, provided with a blue or violet filter exhibiting maximum transmittance between 400 and 470 nm.

The wavelength at which color intensity is measured depends on sensitivity desired, because sensitivity varies tenfold with wavelengths 400 to 490 nm. Ferric iron causes interference at low wavelengths, particularly at 400 nm. A wavelength of 470 nm usually is used. Concentration ranges for different wavelengths are:

P Range mg/L	Wavelength nm
1.0– 5.0	400
2.0–10	420
4.0–18	470

b. *Acid-washed glassware:* Use acid-washed glassware for determining low concentrations of phosphorus. Phosphate contamination is common because of its absorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with hot dilute HCl and rinse well with distilled water. Preferably, reserve the glassware only for phosphate determination, and after use, wash and keep filled with water until needed. If this is done, acid treatment is required only occasionally.

c. *Filtration apparatus and filter paper.**(75)

3. Reagents

a. *Phenolphthalein indicator aqueous solution.*

b. *Hydrochloric acid*, HCl, 1 + 1. H_2SO_4 , HClO_4 , or HNO_3 may be substituted for HCl. The acid concentration in the determination is not critical but a final sample concentration of 0.5N is recommended.

c. *Activated carbon.*†(76) Remove fine particles by rinsing with distilled water.

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d. Vanadate-molybdate reagent:

1) *Solution A:* Dissolve 25 g ammonium molybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$, in 300 mL distilled water.

2) *Solution B:* Dissolve 1.25 g ammonium metavanadate, NH_4VO_3 , by heating to boiling in 300 mL distilled water. Cool and add 330 mL conc HCl. Cool Solution B to room temperature, pour Solution A into Solution B, mix, and dilute to 1 L.

e. Standard phosphate solution: Dissolve in distilled water 219.5 mg anhydrous KH_2PO_4 and dilute to 1000 mL; 1.00 mL = 50.0 $\mu\text{g PO}_4^{3-}\text{-P}$.

4. Procedure

a. Sample pH adjustment: If sample pH is greater than 10, add 0.05 mL (1 drop) phenolphthalein indicator to 50.0 mL sample and discharge the red color with 1 + 1 HCl before diluting to 100 mL.

b. Color removal from sample: Remove excessive color in sample by shaking about 50 mL with 200 mg activated carbon in an erlenmeyer flask for 5 min and filter to remove carbon. Check each batch of carbon for phosphate because some batches produce high reagent blanks.

c. Color development in sample: Place 35 mL or less of sample, containing 0.05 to 1.0 mg P, in a 50-mL volumetric flask. Add 10 mL vanadate-molybdate reagent and dilute to the mark with distilled water. Prepare a blank in which 35 mL distilled water is substituted for the sample. After 10 min or more, measure absorbance of sample versus a blank at a wavelength of 400 to 490 nm, depending on sensitivity desired (see ¶ 2a above). The color is stable for days and its intensity is unaffected by variation in room temperature.

d. Preparation of calibration curve: Prepare a calibration curve by using suitable volumes of standard phosphate solution and proceeding as in ¶ 4c. When ferric ion is low enough not to interfere, plot a family of calibration curves of one series of standard solutions for various wavelengths. This permits a wide latitude of concentrations in one series of determinations. Analyze at least one standard with each set of samples.

5. Calculation

$$\text{mg P/L} = \frac{\text{mg P (in 50 mL final volume)} \times 1000}{\text{mL sample}}$$

6. Precision and Bias

See Table 4500-P:I.

7. Bibliography

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4500-P D. Stannous Chloride Method

1. General Discussion

a. Principle: Molybdophosphoric acid is formed and reduced by stannous chloride to intensely colored molybdenum blue. This method is more sensitive than Method C and makes feasible measurements down to 7 µg P/L by use of increased light path length. Below 100 µg P/L an extraction step may increase reliability and lessen interference.

b. Interference: See Section 4500-P.C.1b.

c. Minimum detectable concentration: The minimum detectable concentration is about 3 µg P/L. The sensitivity at 0.3010 absorbance is about 10 µg P/L for an absorbance change of 0.009.

2. Apparatus

The same apparatus is required as for Method C, except that a pipetting bulb is required for the extraction step. Set spectrophotometer at 625 nm in the measurement of benzene-isobutanol extracts and at 690 nm for aqueous solutions. If the instrument is not equipped to read at 690 nm, use a wavelength of 650 nm for aqueous solutions, with somewhat reduced sensitivity and precision.

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3. Reagents

a. *Phenolphthalein indicator aqueous solution.*

b. *Strong-acid solution:* Prepare as directed in Section 4500-P.B.2b2).

c. *Ammonium molybdate reagent I:* Dissolve 25 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 175 mL distilled water. Cautiously add 280 mL conc H_2SO_4 to 400 mL distilled water. Cool, add molybdate solution, and dilute to 1 L.

d. *Stannous chloride reagent I:* Dissolve 2.5 g fresh $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 100 mL glycerol. Heat in a water bath and stir with a glass rod to hasten dissolution. This reagent is stable and requires neither preservatives nor special storage.

e. *Standard phosphate solution:* Prepare as directed in Section 4500-P.C.3e.

f. *Reagents for extraction:*

1) *Benzene-isobutanol solvent:* Mix equal volumes of benzene and isobutyl alcohol. (CAUTION—This solvent is highly flammable.)

2) *Ammonium molybdate reagent II:* Dissolve 40.1 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in approximately 500 mL distilled water. Slowly add 396 mL ammonium molybdate reagent I. Cool and dilute to 1 L.

3) *Alcoholic sulfuric acid solution:* Cautiously add 20 mL conc H_2SO_4 to 980 mL methyl alcohol with continuous mixing.

4) *Dilute stannous chloride reagent II:* Mix 8 mL stannous chloride reagent I with 50 mL glycerol. This reagent is stable for at least 6 months.

4. Procedure

a. *Preliminary sample treatment:* To 100 mL sample containing not more than 200 μg P and free from color and turbidity, add 0.05 mL (1 drop) phenolphthalein indicator. If sample turns pink, add strong acid solution dropwise to discharge the color. If more than 0.25 mL (5 drops) is required, take a smaller sample and dilute to 100 mL with distilled water after first discharging the pink color with acid.

b. *Color development:* Add, with thorough mixing after each addition, 4.0 mL molybdate reagent I and 0.5 mL (10 drops) stannous chloride reagent I. Rate of color development and intensity of color depend on temperature of the final solution, each 1°C increase producing about 1% increase in color. Hence, hold samples, standards, and reagents within 2°C of one another and in the temperature range between 20 and 30°C .

c. *Color measurement:* After 10 min, but before 12 min, using the same specific interval for all determinations, measure color photometrically at 690 nm and compare with a calibration curve, using a distilled water blank. Light path lengths suitable for various concentration ranges are as follows:

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Approximate P Range mg/L	Light Path cm
0.3–2	0.5
0.1–1	2
0.007–0.2	10

Always run a blank on reagents and distilled water. Because the color at first develops progressively and later fades, maintain equal timing conditions for samples and standards. Prepare at least one standard with each set of samples or once each day that tests are made. The calibration curve may deviate from a straight line at the upper concentrations of the 0.3 to 2.0-mg/L range.

d. Extraction: When increased sensitivity is desired or interferences must be overcome, extract phosphate as follows: Pipet a 40-mL sample, or one diluted to that volume, into a 125-mL separatory funnel. Add 50.0 mL benzene-isobutanol solvent and 15.0 mL molybdate reagent II. Close funnel at once and shake vigorously for exactly 15 s. If condensed phosphate is present, any delay will increase its conversion to orthophosphate. Remove stopper and withdraw 25.0 mL of separated organic layer, using a pipet with safety bulb. Transfer to a 50-mL volumetric flask, add 15 to 16 mL alcoholic H_2SO_4 solution, swirl, add 0.50 mL (10 drops) dilute stannous chloride reagent II, swirl, and dilute to the mark with alcoholic H_2SO_4 . Mix thoroughly. After 10 min, but before 30 min, read against the blank at 625 nm. Prepare blank by carrying 40 mL distilled water through the same procedure used for the sample. Read phosphate concentration from a calibration curve prepared by taking known phosphate standards through the same procedure used for samples.

5. Calculation

Calculate as follows:

a. Direct procedure:

$$\text{mg P/L} = \frac{\text{mg P (in approximately 104.5 mL final volume)} \times 1000}{\text{mL sample}}$$

b. Extraction procedure:

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$$\text{mg P/L} = \frac{\text{mg P (in 50 mL final volume)} \times 1000}{\text{mL sample}}$$

6. Precision and Bias

See Table 4500-P:I.

4500-P E. Ascorbic Acid Method

1. General Discussion

a. Principle: Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid—phosphomolybdic acid—that is reduced to intensely colored molybdenum blue by ascorbic acid.

b. Interference: Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.1 mg As/L interfere with the phosphate determination. Hexavalent chromium and NO_2^- interfere to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L. Sulfide (Na_2S) and silicate do not interfere at concentrations of 1.0 and 10 mg/L.

c. Minimum detectable concentration: Approximately 10 $\mu\text{g P/L}$. P ranges are as follows:

Approximate P Range <i>mg/L</i>	Light Path <i>cm</i>
0.30–2.0	0.5
0.15–1.30	1.0
0.01–0.25	5.0

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer*, with infrared phototube for use at 880 nm, providing a light path of 2.5 cm or longer.

2) *Filter photometer*, equipped with a red color filter and a light path of 0.5 cm or longer.

b. Acid-washed glassware: See Section 4500-P.C.2b.

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3. Reagents

- a. *Sulfuric acid*, H_2SO_4 , 5N: Dilute 70 mL conc H_2SO_4 to 500 mL with distilled water.
- b. *Potassium antimonyl tartrate solution*: Dissolve 1.3715 g $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O}$ in 400 mL distilled water in a 500-mL volumetric flask and dilute to volume. Store in a glass-stoppered bottle.
- c. *Ammonium molybdate solution*: Dissolve 20 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 500 mL distilled water. Store in a glass-stoppered bottle.
- d. *Ascorbic acid*, 0.1M: Dissolve 1.76 g ascorbic acid in 100 mL distilled water. The solution is stable for about 1 week at 4°C.
- e. *Combined reagent*: Mix the above reagents in the following proportions for 100 mL of the combined reagent: 50 mL 5N H_2SO_4 , 5 mL potassium antimonyl tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. *Mix after addition of each reagent*. Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 h.
- f. *Stock phosphate solution*: See Section 4500-P.C.3e.
- g. *Standard phosphate solution*: Dilute 50.0 mL stock phosphate solution to 1000 mL with distilled water; 1.00 mL = 2.50 μg P.

4. Procedure

- a. *Treatment of sample*: Pipet 50.0 mL sample into a clean, dry test tube or 125-mL erlenmeyer flask. Add 0.05 mL (1 drop) phenolphthalein indicator. If a red color develops add 5N H_2SO_4 solution dropwise to just discharge the color. Add 8.0 mL combined reagent and mix thoroughly. After at least 10 min but no more than 30 min, measure absorbance of each sample at 880 nm, using reagent blank as the reference solution.
- b. *Correction for turbidity or interfering color*: Natural color of water generally does not interfere at the high wavelength used. For highly colored or turbid waters, prepare a blank by adding all reagents except ascorbic acid and potassium antimonyl tartrate to the sample. Subtract blank absorbance from absorbance of each sample.
- c. *Preparation of calibration curve*: Prepare individual calibration curves from a series of six standards within the phosphate ranges indicated in ¶ 1c above. Use a distilled water blank with the combined reagent to make photometric readings for the calibration curve. Plot absorbance vs. phosphate concentration to give a straight line passing through the origin. Test at least one phosphate standard with each set of samples.

5. Calculation

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$$\text{mg P/L} = \frac{\text{mg P (in approximately 58 mL final volume)} \times 1000}{\text{mL sample}}$$

6. Precision and Bias

The precision and bias values given in Table 4500-P:I are for a single-solution procedure given in the 13th edition. The present procedure differs in reagent-to-sample ratios, no addition of solvent, and acidity conditions. It is superior in precision and bias to the previous technique in the analysis of both distilled water and river water at the 228- $\mu\text{g P/L}$ level (Table 4500-P:II).

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4500-P F. Automated Ascorbic Acid Reduction Method

1. General Discussion

a. Principle: Ammonium molybdate and potassium antimonyl tartrate react with orthophosphate in an acid medium to form an antimony-phosphomolybdate complex, which, on reduction with ascorbic acid, yields an intense blue color suitable for photometric measurement.

b. Interferences: As much as 50 mg Fe^{3+}/L , 10 mg Cu/L , and 10 mg SiO_2/L can be tolerated. High silica concentrations cause positive interference.

In terms of phosphorus, the results are high by 0.005, 0.015, and 0.025 mg/L for silica concentrations of 20, 50, and 100 mg/L, respectively. Salt concentrations up to 20% (w/v) cause an error of less than 1%. Arsenate (AsO_4^{3-}) is a positive interference.

Eliminate interference from NO_2^- and S^{2-} by adding an excess of bromine water or a saturated potassium permanganate (KMnO_4) solution. Remove interfering turbidity by filtration

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before analysis. Filter samples for total or total hydrolyzable phosphorus only after digestion. Sample color that absorbs in the photometric range used for analysis also will interfere. See also Section 4500-P.E.1b.

c. Application: Orthophosphate can be determined in potable, surface, and saline waters as well as domestic and industrial wastewaters over a range of 0.001 to 10.0 mg P/L when photometric measurements are made at 650 to 660 or 880 nm in a 15-mm or 50-mm tubular flow cell. Determine higher concentrations by diluting sample. Although the automated test is designed for orthophosphate only, other phosphorus compounds can be converted to this reactive form by various sample pretreatments described in Section 4500-P.B.1, Section 4500-P.B.2, and Section 4500-P.B.5.

2. Apparatus

a. Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-P:2. A flow cell of 15 or 50 mm and a filter of 650 to 660 or 880 nm may be used.

b. Hot plate or autoclave.

c. Acid-washed glassware: See Section 4500-P.C.2b.

3. Reagents

a. Potassium antimonyl tartrate solution: Dissolve 0.3 g $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O}$ in approximately 50 mL distilled water and dilute to 100 mL. Store at 4°C in a dark, glass-stoppered bottle.

b. Ammonium molybdate solution: Dissolve 4 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 100 mL distilled water. Store in a plastic bottle at 4°C.

c. Ascorbic acid solution: See Section 4500-P.E.3d.

d. Combined reagent: See Section 4500-P.E.3e.

e. Dilute sulfuric acid solution: Slowly add 140 mL conc H_2SO_4 to 600 mL distilled water. When cool, dilute to 1 L.

f. Ammonium persulfate, $(\text{NH}_4)_2\text{S}_2\text{O}_8$, crystalline.

g. Phenolphthalein indicator aqueous solution.

h. Stock phosphate solution: Dissolve 439.3 mg anhydrous KH_2PO_4 , dried for 1 h at 105°C, in distilled water and dilute to 1000 mL; 1.00 mL = 100 µg P.

i. Intermediate phosphate solution: Dilute 100.0 mL stock phosphate solution to 1000 mL with distilled water; 1.00 mL = 10.0 µg P.

j. Standard phosphate solutions: Prepare a suitable series of standards by diluting appropriate volumes of intermediate phosphate solution.

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4. Procedure

Set up manifold as shown in Figure 4500-P:2 and follow the general procedure described by the manufacturer.

Add 0.05 mL (1 drop) phenolphthalein indicator solution to approximately 50 mL sample. If a red color develops, add H_2SO_4 (¶ 3e) dropwise to just discharge the color.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against P concentration in standards. Compute sample P concentration by comparing sample response with standard curve.

6. Precision and Bias

Six samples were analyzed in a single laboratory in septuplicate. At an average PO_4^{3-} concentration of 0.340 mg/L, the average deviation was 0.015 mg/L. The coefficient of variation was 6.2%. In two samples with added PO_4^{3-} , recoveries were 89 and 96%.

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4500-P G. Flow Injection Analysis for Orthophosphate (PROPOSED)

1. General Discussion

a. *Principle:* The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex that absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

Also see Section 4500-P.A, Section 4500-P.B, and Section 4500-P.F, and Section 4130, Flow Injection Analysis (FIA).

b. *Interferences:* Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation

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process.

Silica forms a pale blue complex that also absorbs at 880 nm. This interference is generally insignificant because a silica concentration of approximately 30 mg/L would be required to produce a 0.005 mg P/L positive error in orthophosphate.

Concentrations of ferric iron greater than 50 mg/L cause a negative error due to competition with the complex for the reducing agent ascorbic acid. Treat samples high in iron with sodium bisulfite to eliminate this interference, as well as the interference due to arsenates.

Glassware contamination is a problem in low-level phosphorus determinations. Wash glassware with hot dilute HCl and rinse with reagent water. Commercial detergents are rarely needed but, if they are used, use special phosphate-free preparations.

Also see Section 4500-P.F.

2. Apparatus

Flow injection analysis equipment consisting of:

- a. *FIA injection valve* with sample loop or equivalent.
- b. *Multichannel proportioning pump*.
- c. *FIA manifold* (Figure 4500-P:3) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-P:3. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.
- d. *Absorbance detector*, 880 nm, 10-nm bandpass.
- e. *Injection valve control and data acquisition system*.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. *Stock ammonium molybdate solution*: To a tared 1-L container add 40.0 g ammonium molybdate tetrahydrate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$ and 983 g water. Mix with a magnetic stirrer for at least 4 h. Store in plastic and refrigerate.

b. *Stock antimony potassium tartrate solution*: To a 1-L dark, tared container add 3.0 g antimony potassium tartrate (potassium antimonyl tartrate hemihydrate), $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot 1/2\text{H}_2\text{O}$, and 995 g water. Mix with a magnetic stirrer until dissolved. Store in a dark bottle and refrigerate.

c. *Working molybdate color reagent*: To a tared 1-L container add 680 g water, then add 64.4 g conc sulfuric acid. (CAUTION: *This solution becomes very hot!*) Swirl to mix. When mixture can be handled comfortably, add 213 g stock ammonium molybdate solution (§ 3a) and 72.0 g stock antimony potassium tartrate solution (§ 3b). Shake and degas with helium.

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d. *Ascorbic acid solution:* To a tared 1-L container, add 60.0 g granular ascorbic acid and 975 g water. Stir or shake until dissolved. Degas this reagent with helium, then add 1.0 g dodecyl sulfate, $\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$, stirring gently to mix. Prepare fresh weekly.

e. *Stock orthophosphate standard, 25.00 mg P/L:* In a 1-L volumetric flask dissolve 0.1099 g primary standard grade anhydrous potassium phosphate monobasic (KH_2PO_4) that has been dried for 1 h at 105°C in about 800 mL water. Dilute to mark with water and invert to mix.

f. *Standard orthophosphate solutions:* Prepare orthophosphate standards in desired concentration range, using stock standard (¶ 3e) and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-P:3 and follow method supplied by manufacturer or laboratory standard operating procedure. Use quality control protocols outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus orthophosphate concentration. The calibration curve is linear.

6. Precision and Bias

a. *Recovery and relative standard deviation:* Table 4500-P:III gives results of single-laboratory studies.

b. *MDL:* A 700- μL sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 5.0- μg P/L standard. These gave a mean of 5.26 μg P/L, a standard deviation of 0.264 μg P/L, and MDL of 0.67 μg P/L.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-P H. Manual Digestion and Flow Injection Analysis for Total Phosphorus (PROPOSED)

1. General Discussion

a. *Principle:* Polyphosphates are converted to the orthophosphate form by a sulfuric acid digestion and organic phosphorus is converted to orthophosphate by a persulfate digestion. When the resulting solution is injected onto the manifold, the orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a

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complex. This complex is reduced with ascorbic acid to form a blue complex that absorbs light at 880 nm. The absorbance is proportional to the concentration of total phosphorus in the sample.

See Section 4500-P.A for a discussion of the various forms of phosphorus found in waters and wastewaters, Section 4500-P.B for a discussion of sample preparation and digestion, and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: See Section 4500-P.G.1b.

2. Apparatus

Digestion and flow injection analysis equipment consisting of:

a. Hotplate or autoclave.

b. FIA injection valve with sample loop or equivalent.

c. Multichannel proportioning pump.

d. FIA manifold (Figure 4500-P:4) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-P:4. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

Prepare reagents listed in Section 4500-P.G.3a, b, d, e, and f, and in addition:

a. Sulfuric acid carrier, H₂SO₄, 0.13M: To a tared 1-L container add 993 g water, then add 13.3 g conc H₂SO₄. Shake carefully to mix. Degas daily. Prepare fresh weekly.

b. Molybdate color reagent: To a tared 1-L container add 694 g water, then add 38.4 g conc H₂SO₄. (CAUTION: *The solution becomes very hot!*) Swirl to mix. When mixture can be handled comfortably, add 72.0 g stock antimony potassium tartrate (§ G.3b) and 213 g stock ammonium molybdate (§ G.3a). Shake to mix, and degas.

4. Procedure

See Section 4500-P.B.4 or 5 for digestion procedures. Carry both standards and samples through the digestion. The resulting solutions should be about 0.13M in sulfuric acid to match the concentration of the carrier. If the solutions differ more than 10% from this concentration, adjust concentration of carrier's sulfuric acid to match that of digested samples.

Set up a manifold equivalent to that in Figure 4500-P:4 and analyze digested samples and standards by following method supplied by manufacturer or laboratory's standard operating procedure. Use quality control protocols outlined in Section 4020.

5. Calculations

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Prepare standard curves by plotting absorbance of standards processed through the manifold versus phosphorus concentration. The calibration curve is linear.

6. Precision and Bias

a. *MDL*: A 780- μ L sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 3.5- μ g P/L standard. These gave a mean of 3.53 μ g P/L, a standard deviation of 0.82 μ g P/L, and MDL of 2.0 μ g P/L. The MDL is limited mainly by the precision of the digestion.

b. *Precision study*: Ten injections of a 100.0- μ g P/L standard gave a percent relative standard deviation of 0.3%.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-P I. In-line UV/Persulfate Digestion and Flow Injection Analysis for Total Phosphorus (PROPOSED)

1. General Discussion

a. *Principle*: Organic phosphorus is converted in-line to orthophosphate by heat, ultraviolet radiation, and persulfate digestion. At the same time, inorganic polyphosphates are converted to orthophosphate by in-line sulfuric acid digestion. The digestion processes occur before sample injection. A portion of the digested sample is then injected and its orthophosphate concentration determined by the flow injection method described in Section 4500-P.H.1.

See Section 4500-P.A for a discussion of the various forms of phosphorus found in waters and wastewaters, Section 4500-P.B for a discussion of sample preparation and digestion, and Section 4130, Flow Injection Analysis (FIA).

b. *Interferences*: See Section 4500-P.G.1b.

2. Apparatus

Flow injection analysis equipment consisting of:

- a. *FIA injection valve* with sample loop or equivalent.
- b. *Multichannel proportioning pump*.
- c. *FIA manifold* (Figure 4500-P:5) with tubing heater, in-line ultraviolet digestion fluidics including a debubbler consisting of a gas-permeable TFE membrane and its holder, and flow cell. Relative flow rates only are shown in Figure 4500-P:5. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material

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such as TFE. The block marked “UV” should consist of TFE tubing irradiated by a mercury discharge ultraviolet lamp emitting radiation at 254 nm.

- d. *Absorbance detector*, 880 nm, 10-nm bandpass.
- e. *Injection valve control and data acquisition system*.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and all reagents with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. *Digestion reagent 1*: To a tared 1-L container, add 893.5 g water, then slowly add 196.0 g sulfuric acid, H_2SO_4 . CAUTION: *This solution becomes very hot!* Prepare weekly. Degas before using.

b. *Digestion reagent 2*: To a tared 1-L container, add 1000 g water, then add 26 g potassium persulfate, $\text{K}_2\text{S}_4\text{O}_8$. Mix with a magnetic stirrer until dissolved. Prepare weekly. Degas before using.

c. *Sulfuric acid carrier*, 0.71M: To a tared 1-L container, slowly add 70 g H_2SO_4 to 962 g water. Add 5 g sodium chloride, NaCl. Let cool, then degas with helium. Add 1.0 g sodium dodecyl sulfate. Invert to mix. Prepare weekly.

d. *Stock ammonium molybdate*: To a tared 1-L container add 40.0 g ammonium molybdate tetrahydrate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$, and 983 g water. Mix with a magnetic stirrer for at least 4 h. The solution can be stored in plastic for up to 2 months if refrigerated.

e. *Stock antimony potassium tartrate*: To a 1-L dark, plastic, tared container add 3.0 g antimony potassium tartrate (potassium antimonyl tartrate trihydrate), $\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2\cdot 3\text{H}_2\text{O}$, and 995 g water. Mix with a magnetic stirrer until dissolved. The solution can be stored in a dark plastic container for up to 2 months if refrigerated.

f. *Molybdate color reagent*: To a tared 1-L container add 715 g water, then 213 g stock ammonium molybdate (¶ 3e) and 72.0 g stock antimony potassium tartrate (¶ 3 f). Add and dissolve 22.8 g sodium hydroxide, NaOH. Shake and degas with helium. Prepare weekly.

g. *Ascorbic acid*: To a tared 1-L container add 70.0 g ascorbic acid and 975 g water. Mix with a magnetic stirrer until dissolved. Degas with helium. Add 1.0 g sodium dodecyl sulfate. Mix with a magnetic stirrer. Prepare fresh every 2 d.

h. *Stock orthophosphate standard*, 1000 mg P/L: In a 1-L volumetric flask dissolve 4.396 g primary standard grade anhydrous potassium phosphate monobasic, KH_2PO_4 (dried for 1 h at 105°C), in about 800 mL water. Dilute to mark with water and invert to mix. Prepare monthly.

i. *Standard solutions*: Prepare orthophosphate standards in desired concentration range, using stock orthophosphate standards (¶ 3i), and diluting with water. If the samples are preserved

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with sulfuric acid, ensure that stock standard and diluted standards solutions are of the same concentration.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-P:5 and follow method supplied by manufacturer or laboratory's standard operating procedure. Use quality control procedures described in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through manifold versus phosphorus concentration. The calibration curve is linear.

Verify digestion efficiency by determining tripolyphosphate and trimethylphosphate standards at regular intervals. In the concentration range of the method, the recovery of either of these compounds should be >95%.

6. Precision and Bias

a. MDL: A 390- μ L sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 0.10-mg P/L orthophosphate standard. These gave a mean of 0.10 mg P/L, a standard deviation of 0.003 mg P/L, and MDL of 0.007 mg P/L.

b. Precision of recovery study: Ten injections of a 10.0-mg P/L trimethylphosphate standard gave a mean percent recovery of 98% and a percent relative standard deviation of 0.8%.

c. Recovery of total phosphorus: Two organic and two inorganic complex phosphorus compounds were determined in triplicate at three concentrations. The results are shown in Table 4500-P:IV.

d. Comparison of in-line digestion with manual digestion method: Samples from a wastewater treatment plant influent and effluent and total phosphorus samples at 2.0 mg P/L were determined in duplicate with both manual persulfate digestion followed by the method in Section 4500-PH and in-line digestion method. Table 4500-P:V gives the results of this comparison, and Figure 4500-P:6 shows the correlation between manual and in-line total phosphorus methods.

4500-KMnO₄ POTASSIUM PERMANGANATE*(77)

4500-KMnO₄ A. Introduction

1. Occurrence and Significance

Potassium permanganate, KMnO₄, has been widely used in both potable and nonpotable water sources. It has been applied to water supplies to remove taste, odor, color, iron,

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manganese, and sulfides and to control trihalomethanes (THMs) and zebra mussels. Municipal and industrial waste treatment facilities use potassium permanganate for odor control, toxic pollutant destruction, bio-augmentation, and grease removal.

Potassium permanganate is produced as a dark black-purple crystalline material. It has a solubility in water of 60 g/L at 20°C. The color of potassium permanganate solutions ranges from faint pink (dilute) to deep purple (concentrated). Under normal conditions the solid material is stable. However, as with all oxidizing agents, avoid contact with acids, peroxides, and all combustible organic or readily oxidizable materials.

2. Sampling and Storage

If kept dry, solid potassium permanganate may be stored indefinitely. Potassium permanganate solutions, made in oxidant-demand-free water, are stable for long periods of time if kept in an amber bottle out of direct sunlight.¹ For samples obtained from other water sources (those having an oxidant demand), analyze potassium permanganate on site, as soon as possible after sample collection.

3. Reference

1. DAY, R.A. & A.L. UNDERWOOD. 1986. Quantitative Analysis, 5th ed. Prentice-Hall, Englewood Cliffs, N.J.

4500-KMnO₄ B. Spectrophotometric Method

1. General Discussion

a. Principle: This method is a direct determination of aqueous potassium permanganate solutions. The concentration (pink to violet color) is directly proportional to the absorbance as measured at 525 nm. The concentrations are best determined from the light-absorbing characteristics of a filtered sample by means of a spectrophotometer.

b. Application: This method is applicable to ground and surface waters.

c. Interference: Turbidity and manganese dioxide interfere. Remove by the filtration methods described below. Other color-producing compounds also interfere. Compensate for color by using an untreated sample to zero the spectrophotometer.

d. Minimum detectable concentration: As a guide, use the following light paths for the indicated KMnO₄ concentrations measured at 525nm. Sample dilution may be required, depending on the initial concentration.

Cell Path Length cm	Range mg KMnO ₄ /L	Expected Absorbance for 1-mg/L KMnO ₄ Solution

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Cell Path Length cm	Range mg KMnO_4 /L	Expected Absorbance for 1-mg/L KMnO_4
		Solution
1	0.5–100	0.016
2.5	0.2–25	0.039
5	0.1–20	0.078

2. Apparatus

a. *Photometric equipment:* Use one of the following:

1) *Spectrophotometer*, for use at a wavelength of 525 nm and providing a light path of 1 cm or longer.

2) *Filter photometer*, equipped with a filter having a maximum transmittance at or near 525 nm and providing a light path of 1 cm or longer.

b. *Filtration apparatus.* *#(78)

c. *Filters:* Use 0.22- μm filters that do not react with KMnO_4 (or smallest glass fiber filters available).

3. Reagents

Use potassium-permanganate-demand-free water (¶ e below) for all reagent preparation and dilutions.

a. *Calcium chloride solution*, CaCl_2 , 1M: Dissolve 111 g CaCl_2 in water and dilute to 1 L.

b. *Sulfuric acid*, H_2SO_4 , 20%: Add 20 g conc H_2SO_4 slowly, with stirring, to 80 mL water. After cooling, adjust final volume to 100 mL.

c. *Sodium oxalate*, $\text{Na}_2\text{C}_2\text{O}_4$, primary standard.

d. *Sodium thiosulfate solution*, $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, 0.019M: Dissolve 0.471 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in water and dilute to 100 mL.

e. *Potassium-permanganate-demand-free water:* Add one small crystal KMnO_4 to 1 L distilled or deionized water; let stand. After 1 to 2 d, a residual pink color should be present; if not, discard and obtain better-quality water or increase permanganate added. Redistill in an all-borosilicate-glass apparatus and discard initial 50 mL distillate. Collect distillate fraction that is free of permanganate: a red color with DPD reagent (see Section 4500-Cl.F.2b) indicates presence of permanganate.

f. *Potassium permanganate standard*, KMnO_4 , 0.006M: Dissolve 1.000 g KMnO_4 in water and dilute to 1000 mL. Standardize as follows: Accurately weigh about 0.1 g primary standard grade sodium oxalate and dissolve it in 150 mL water in a 250-mL erlenmeyer flask. Add 20 mL

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20% H_2SO_4 and heat to 70 to 80°C. Titrate the warm oxalate solution with the potassium permanganate standard until a pink coloration persists (60 s). Calculate the potassium permanganate concentration:

$$\text{mg KMnO}_4/\text{L} = \frac{W \times 1000}{2.1197 \times V}$$

where:

W = weight of sodium oxalate, mg, and

V = mL KMnO_4 titrant.

4. Procedure

a. Calibration of photometric equipment: Prepare calibration curve by diluting standardized potassium permanganate solution. Make dilutions appropriate for the cell path length and range desired. Plot absorbance (y axis) versus KMnO_4 concentration (x axis). Calculate a best-fit line through the points. Preferably perform a calibration check with a known KMnO_4 standard before any analysis to ensure that equipment is in proper working order.

b. Potassium permanganate analysis: Check zero on spectrophotometer at 525 nm with deionized water. If the water is soft (i.e., <40 mg/L hardness as CaCO_3), add 1 mL CaCl_2 solution/L sample (111 mg/L as CaCl_2) to aid in removal of any colloidal manganese dioxide and suspended solids. Pass 50 mL sample through a 0.22- μm filter. Rinse spectrophotometer cell with two or three portions of filtrate. Fill cell and check that no air bubbles are present in the solution or on the sides of the cell. Measure absorbance at 525 nm (Reading A). For best results, minimize time between filtration and reading absorbance. To 100 mL sample, add 0.1 mL CaCl_2 solution. Add 0.1 mL sodium thiosulfate solution per 1 mg/L KMnO_4 (based on Reading A). Pass through a 0.22- μm filter and measure absorbance (Reading B).

5. Calculation

$$\text{Correct absorbance} = A - B$$

where:

A = absorbance of sample, and

B = absorbance of blank.

Compare corrected absorbance value obtained with the calibration curve and report the corresponding value as milligrams potassium permanganate per liter.

6. Precision and Bias

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Based on the results obtained by eight analysts in a single laboratory, the overall precision (pooled standard deviation) was determined to be 0.035 mg/L for a 1-cm cell. The mean recovery for 12 measurements at 4 initial concentrations was 98%. The method detection level (MDL), as determined using Method 1030, was 0.083 mg KMnO_4 /L.

4500-SiO₂ SILICA*#(79)

4500-SiO₂ A. Introduction

1. Occurrence and Significance

Silicon does not occur free in nature, but rather as free silica (SiO_2) in coarsely crystalline (quartz, rock crystal, amethyst, etc.) and microcrystalline (flint, chert, jasper, etc.) varieties of quartz, the major component of sand and sandstone. Silicon is found in combination with other elements in silicates, represented by feldspar, hornblende, mica, asbestos, and other clay minerals. Silicates also occur in rocks such as granite, basalt, and shale. Silicon therefore is usually reported as silica (SiO_2) when rocks, sediments, soils, and water are analyzed. The average abundance of silica in different rock types is 7 to 80%, in typical soils 50 to 80%, and in surface and groundwater 14 mg/L.

The common aqueous forms of silica are H_4SiO_4 and H_3SiO_4^- . In the presence of magnesium, it can form scale deposits in boilers and in steam turbines. It is considered a nonessential trace element for most plants, but essential for most animals. Chronic exposure to silica dust can be toxic. There is no U.S. EPA drinking water standard MCL for silica.

A more complete discussion of the occurrence and chemistry of silica in natural waters is available.¹

2. Selection of Method

Perform analyses by the electrothermal atomic absorption method (3113B) or one of the colorimetric methods (C, D, E, or F), depending on the fraction to be measured. The inductively coupled plasma mass spectrometric method (3125) or the inductively coupled plasma method (3120) also may be applied successfully in most cases (with lower detection limits), even though silica is not specifically listed as an analyte in the method.

Methods 3120 and 3125 determine total silica. Methods C, D, E, and F determine molybdate-reactive silica. As noted in Section 4500-SiO₂.C.4, it is possible to convert other forms of silica to the molybdate-reactive form for determination by these methods. Method 3111D determines more than one form of silica. It will determine all dissolved silica and some colloiddally dispersed silica. The determination of silica present in micrometer and submicrometer particles will depend on the size distribution, composition, and structure of the particles; thus

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Method 3111D cannot be said to determine total silica.

Method C is recommended for relatively pure waters containing from 0.4 to 25 mg SiO₂/L. As with most colorimetric methods, the range can be extended, if necessary, by diluting, by concentrating, or by varying the light path. Interferences due to tannin, color, and turbidity are more severe with this method than with Method D. Moreover, the yellow color produced by Method C has a limited stability and attention to timing is necessary. When applicable, however, it offers greater speed and simplicity than Method D because one reagent fewer is used; one timing step is eliminated; and many natural waters can be analyzed without dilution, which is not often the case with Method D. Method D is recommended for the low range, from 0.04 to 2 mg SiO₂/L. This range also can be extended if necessary. Such extension may be desirable if interference is expected from tannin, color, or turbidity. A combination of factors renders Methods D, E, and F less susceptible than Method C to those interferences; also, the blue color in Methods D, E, and F is more stable than the yellow color in Method C. However, many samples will require dilution because of the high sensitivity of the method. Permanent artificial color standards are not available for the blue color developed in Method D.

The yellow color produced by Method C and the blue color produced by Methods D, E, and F are affected by high concentrations of salts. With seawater the yellow color intensity is decreased by 20 to 35% and the blue color intensity is increased by 10 to 15%. When waters of high ionic strength are analyzed by these methods, use silica standards of approximately the same ionic strengths.²

Method E or F may be used where large numbers of samples are analyzed regularly. Method 3111D is recommended for broad-range use. Although Method 3111D is usable from 1 to 300 mg SiO₂/L, optimal results are obtained from about 20 to 300 mg/L. The range can be extended upward by dilution if necessary. This method is rapid and does not require any timing step.

The inductively coupled plasma method (3120) also may be used in analyses for silica.

3. Sampling and Storage

Collect samples in bottles of polyethylene, other plastic, or hard rubber, especially if there will be a delay between collection and analysis. Borosilicate glass is less desirable, particularly with waters of pH above 8 or with seawater, in which cases a significant amount of silica in the glass can dissolve. Freezing to preserve samples for analysis of other constituents can lower soluble silica values by as much as 20 to 40% in waters that have a pH below 6. Do not acidify samples for preservation because silica precipitates in acidic solutions.

4. References

1. HEM, J.D. 1985. Study and interpretation of the Chemical Characteristics of Natural Water, 3rd ed. U.S. Geol. Surv. Water Supply Pap. No. 2254.
2. FANNING, K.A. & M.E.Q. PILSON. 1973. On the spectrophotometric determination of dissolved silica in natural waters. *Anal. Chem.* 45:136.

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5. Bibliography

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- VAIL, J.G. 1952. The Soluble Silicates, Their Properties and Uses. Reinhold Publishing Corp., New York, N.Y. Vol. 1, pp. 95–97, 100–161.

4500-SiO₂ B. (Reserved)

4500-SiO₂ C. Molybdosilicate Method

1. General Discussion

a. Principle: Ammonium molybdate at pH approximately 1.2 reacts with silica and any phosphate present to produce heteropoly acids. Oxalic acid is added to destroy the molybdophosphoric acid but not the molybdosilicic acid. Even if phosphate is known to be absent, the addition of oxalic acid is highly desirable and is a mandatory step in both this method and Method D. The intensity of the yellow color is proportional to the concentration of “molybdate-reactive” silica. In at least one of its forms, silica does not react with molybdate even though it is capable of passing through filter paper and is not noticeably turbid. It is not known to what extent such “unreactive” silica occurs in waters. Terms such as “colloidal,” “crystalloidal,” and “ionic” have been used to distinguish among various forms of silica but such terminology cannot be substantiated. “Molybdate-unreactive” silica can be converted to the “molybdate-reactive” form by heating or fusing with alkali. Molybdate-reactive or unreactive does not imply reactivity, or lack of it, toward *other* reagents or processes.

b. Interference: Because both apparatus and reagents may contribute silica, avoid using glassware as much as possible and use reagents low in silica. Also, make a blank determination to correct for silica so introduced. In both this method and Method D, tannin, large amounts of iron, color, turbidity, sulfide, and phosphate interfere. Treatment with oxalic acid eliminates interference from phosphate and decreases interference from tannin. If necessary, use photometric compensation to cancel interference from color or turbidity.

c. Minimum detectable concentration: Approximately 1 mg SiO₂/L can be detected in 50-mL nessler tubes.

2. Apparatus

a. Platinum dishes, 100-mL.

b. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 410 nm, providing a light path of 1 cm or longer. See Table 4500-SiO₂:I for light path selection.

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2) *Filter photometer*, providing a light path of 1 cm or longer and equipped with a violet filter having maximum transmittance near 410 nm.

3) *Nessler tubes*, matched, 50-mL, tall form.

3. Reagents

For best results, set aside and use batches of chemicals low in silica. Use distilled reagent water in making reagents and dilutions. Store all reagents in plastic containers to guard against high blanks.

a. *Sodium bicarbonate*, NaHCO_3 , powder.

b. *Sulfuric acid*, H_2SO_4 , 1N.

c. *Hydrochloric acid*, HCl , 1 + 1.

d. *Ammonium molybdate reagent*: Dissolve 10 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ in water, with stirring and gentle warming, and dilute to 100 mL. Filter if necessary. Adjust to pH 7 to 8 with silica-free NH_4OH or NaOH and store in a polyethylene bottle to stabilize. (If the pH is not adjusted, a precipitate gradually forms. If the solution is stored in glass, silica may leach out and cause high blanks.) If necessary, prepare silica-free NH_4OH by passing gaseous NH_3 into distilled water contained in a plastic bottle.

e. *Oxalic acid solution*: Dissolve 7.5 g $\text{H}_2\text{C}_2\text{O}_4\cdot\text{H}_2\text{O}$ in water and dilute.

f. *Stock silica solution*: Dissolve 4.73 g sodium metasilicate nonahydrate, $\text{Na}_2\text{SiO}_3\cdot 9\text{H}_2\text{O}$, in water and dilute to 1000 mL. For work of highest accuracy, analyze 100.0-mL portions by the gravimetric method.¹ Store in a tightly stoppered plastic bottle.

g. *Standard silica solution*: Dilute 10.00 mL stock solution to 1000 mL with water; 1.00 mL = 10.0 μg SiO_2 . Calculate exact concentration from concentration of stock silica solution. Store in a tightly stoppered plastic bottle.

h. *Permanent color solutions*:

1) *Potassium chromate solution*: Dissolve 630 mg K_2CrO_4 in water and dilute to 1 L.

2) *Borax solution*: Dissolve 10 g sodium borate decahydrate, $\text{Na}_2\text{B}_4\text{O}_7\cdot 10\text{H}_2\text{O}$, in water and dilute to 1 L.

4. Procedure

a. *Color development*: To 50.0 mL sample add in rapid succession 1.0 mL 1 + 1 HCl and 2.0 mL ammonium molybdate reagent. Mix by inverting at least six times and let stand for 5 to 10 min. Add 2.0 mL oxalic acid solution and mix thoroughly. Read color after 2 min but before 15 min, measuring time from addition of oxalic acid. Because the yellow color obeys Beer's law, measure photometrically or visually.

b. To detect the presence of molybdate-unreactive silica, digest sample with NaHCO_3 before

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color development. This digestion is not necessarily sufficient to convert all molybdate-unreactive silica to the molybdate-reactive form. Complex silicates and higher silica polymers may require extended fusion with alkali at high temperatures or digestion under pressure for complete conversion. Omit digestion if all the silica is known to react with molybdate.

Prepare a clear sample by filtration if necessary. Place 50.0 mL, or a smaller portion diluted to 50 mL, in a 100-mL platinum dish. Add 200 mg silica-free NaHCO_3 and digest on a steam bath for 1 h. Cool and add slowly, with stirring, 2.4 mL 1N H_2SO_4 . Do not interrupt analysis but proceed *at once* with remaining steps. Transfer quantitatively to a 50-mL nessler tube and make up to mark with water. (Tall-form 50-mL nessler tubes are convenient for mixing even if the solution subsequently is transferred to an absorption cell for photometric measurement.)

c. Preparation of standards: If NaHCO_3 pretreatment is used, add to the standards (approximately 45 mL total volume) 200 mg NaHCO_3 and 2.4 mL 1N H_2SO_4 , to compensate both for the slight amount of silica introduced by the reagents and for the effect of the salt on color intensity. Dilute to 50.0 mL.

d. Correction for color or turbidity: Prepare a special blank for every sample that needs such correction. Carry two identical portions of each such sample through the procedure, including NaHCO_3 treatment if this is used. To one portion add all reagents as directed in ¶ 4a preceding. To the other portion add HCl and oxalic acid but no molybdate. Adjust photometer to zero absorbance with the blank containing no molybdate before reading absorbance of molybdate-treated sample.

e. Photometric measurement: Prepare a calibration curve from a series of approximately six standards to cover the optimum ranges cited in Table 4500-SiO₂:I. Follow direction of ¶ 4a above on suitable portions of standard silica solution diluted to 50.0 mL in nessler tubes. Set photometer at zero absorbance with water and read all standards, including a reagent blank, against water. Plot micrograms silica in the final (55 mL) developed solution against photometer readings. Run a reagent blank and at least one standard with each group of samples to confirm that the calibration curve previously established has not shifted.

f. Visual comparison: Make a set of permanent artificial color standards, using K_2CrO_4 and borax solutions. Mix liquid volumes specified in Table 4500-SiO₂:II and place them in well-stoppered, appropriately labeled 50-mL nessler tubes. Verify correctness of these permanent artificial standards by comparing them visually against standards prepared by analyzing portions of the standard silica solution. Use permanent artificial color standards only for visual comparison.

5. Calculation

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$$\text{mg SiO}_2/\text{L} = \frac{\mu\text{g SiO}_2 \text{ (in 55 mL final volume)}}{\text{mL sample}}$$

Report whether NaHCO_3 digestion was used.

6. Precision and Bias

A synthetic sample containing 5.0 mg SiO_2/L , 10 mg Cl^-/L , 0.20 mg $\text{NH}_3\text{-N}/\text{L}$, 1.0 mg $\text{NO}_3^- \text{-N}/\text{L}$, 1.5 mg organic N/L, and 10.0 mg $\text{PO}_4^{3-}/\text{L}$ in distilled water was analyzed in 19 laboratories by the molybdosilicate method with a relative standard deviation of 14.3% and a relative error of 7.8%.

Another synthetic sample containing 15.0 mg SiO_2/L , 200 mg Cl^-/L , 0.800 mg $\text{NH}_3\text{-N}/\text{L}$, 1.0 mg $\text{NO}_3^- \text{-N}/\text{L}$, 0.800 mg organic N/L, and 5.0 mg $\text{PO}_4^{3-}/\text{L}$ in distilled water was analyzed in 19 laboratories by the molybdosilicate method, with a relative standard deviation of 8.4% and a relative error of 4.2%.

A third synthetic sample containing 30.0 mg SiO_2/L , 400 mg Cl^-/L , 1.50 mg $\text{NH}_3\text{-N}/\text{L}$, 1.0 mg $\text{NO}_3^- \text{-N}/\text{L}$, 0.200 mg organic N/L, and 0.500 mg $\text{PO}_4^{3-}/\text{L}$, in distilled water was analyzed in 20 laboratories by the molybdosilicate method, with a relative standard deviation of 7.7% and a relative error of 9.8%.

All results were obtained after sample digestion with NaHCO_3 .

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4500-SiO₂ D. Heteropoly Blue Method

1. General Discussion

- a. *Principle:* The principles outlined under Method C, ¶ 1a, also apply to this method. The yellow molybdosilicic acid is reduced by means of aminonaphtholsulfonic acid to heteropoly blue. The blue color is more intense than the yellow color of Method C and provides increased sensitivity.
- b. *Interference:* See Section 4500-SiO₂.C.1b.
- c. *Minimum detectable concentration:* Approximately 20 µg SiO₂/L can be detected in 50-mL nessler tubes and 50 µg SiO₂/L spectrophotometrically with a 1-cm light path at 815 nm.

2. Apparatus

- a. *Platinum dishes*, 100-mL.
- b. *Colorimetric equipment:* One of the following is required:
 - 1) *Spectrophotometer*, for use at approximately 815 nm. The color system also obeys Beer's law at 650 nm, with appreciably reduced sensitivity. Use light path of 1 cm or longer. See Table 4500-SiO₂:I for light path selection.
 - 2) *Filter photometer*, provided with a red filter exhibiting maximum transmittance in the wavelength range of 600 to 815 nm. Sensitivity improves with increasing wavelength. Use light path of 1 cm or longer.
 - 3) *Nessler tubes*, matched, 50-mL, tall form.

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3. Reagents

For best results, set aside and use batches of chemicals low in silica. Store all reagents in plastic containers to guard against high blanks. Use distilled water that does not contain detectable silica after storage in glass.

All of the reagents listed in Section 4500-SiO₂.C.3 are required, and in addition:

Reducing agent: Dissolve 500 mg 1-amino-2-naphthol-4-sulfonic acid and 1 g Na₂SO₃ in 50 mL distilled water, with gentle warming if necessary; add this to a solution of 30 g NaHSO₃ in 150 mL distilled water. Filter into a plastic bottle. Discard when solution becomes dark. Prolong reagent life by storing in a refrigerator and away from light. Do not use aminonaphtholsulfonic acid that is incompletely soluble or that produces reagents that are dark even when freshly prepared.*#(80)

4. Procedure

a. *Color development:* Proceed as in 4500-SiO₂.C.4a up to and including the words, “Add 2.0 mL oxalic acid solution and mix thoroughly.” Measuring time from the moment of adding oxalic acid, wait at least 2 min but not more than 15 min, add 2.0 mL reducing agent, and mix thoroughly. After 5 min, measure blue color photometrically or visually. If NaHCO₃ pretreatment is used, follow 4500-SiO₂.C.4b.

b. *Photometric measurement:* Prepare a calibration curve from a series of approximately six standards to cover the optimum range indicated in Table 4500-SiO₂:I. Carry out the steps described above on suitable portions of standard silica solution diluted to 50.0 mL in nessler tubes; pretreat standards if NaHCO₃ digestion is used (see Section 4500-SiO₂.C.4b). Adjust photometer to zero absorbance with distilled water and read all standards, including a reagent blank, against distilled water. If necessary to correct for color or turbidity in a sample, see Section 4500-SiO₂.C.4d. To the special blank add HCl and oxalic acid, but no molybdate or reducing agent. Plot micrograms silica in the final 55 mL developed solution against absorbance. Run a reagent blank and at least one standard with each group of samples to check the calibration curve.

c. *Visual comparison:* Prepare a series of not less than 12 standards, covering the range 0 to 120 µg SiO₂, by placing the calculated volumes of standard silica solution in 50-mL nessler tubes, diluting to mark with distilled water, and developing color as described in ¶ a preceding.

5. Calculation

$$\text{mg SiO}_2/\text{L} = \frac{\mu\text{g SiO}_2 \text{ (in 55 mL final volume)}}{\text{mL sample}}$$

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Report whether NaHCO_3 digestion was used.

6. Precision and Bias

A synthetic sample containing 5.0 mg SiO_2/L , 10 mg Cl^-/L , 0.200 mg $\text{NH}_3\text{-N}/\text{L}$, 1.0 mg $\text{NO}_3^- \text{-N}/\text{L}$, 1.5 mg organic N/L, and 10.0 mg $\text{PO}_4^{3-}/\text{L}$ in distilled water was analyzed in 11 laboratories by the heteropoly blue method, with a relative standard deviation of 27.2% and a relative error of 3.0%.

A second synthetic sample containing 15 mg SiO_2/L , 200 mg Cl^-/L , 0.800 mg $\text{NH}_3\text{-N}/\text{L}$, 1.0 mg $\text{NO}_3^- \text{-N}/\text{L}$, 0.800 mg organic N/L, and 5.0 mg $\text{PO}_4^{3-}/\text{L}$ in distilled water was analyzed in 11 laboratories by the heteropoly blue method, with a relative standard deviation of 18.0% and a relative error of 2.9%.

A third synthetic sample containing 30.0 mg SiO_2/L , 400 mg Cl^-/L , 1.50 mg $\text{NH}_3\text{-N}/\text{L}$, 1.0 mg $\text{NO}_3^- \text{-N}/\text{L}$, 0.200 mg organic N/L, and 0.500 mg $\text{PO}_4^{3-}/\text{L}$ in distilled water was analyzed in 10 laboratories by the heteropoly blue method with a relative standard deviation of 4.9% and a relative error of 5.1%.

All results were obtained after sample digestion with NaHCO_3 .

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4500-SiO₂ E. Automated Method for Molybdate-Reactive Silica

1. General Discussion

a. *Principle:* This method is an adaptation of the heteropoly blue method (Method D) utilizing a continuous-flow analytical instrument.

b. *Interferences:* See Section 4500-SiO₂.C.1b. If particulate matter is present, filter sample or use a continuous filter as an integral part of the system.

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c. *Application:* This method is applicable to potable, surface, domestic, and other waters containing 0 to 20 mg SiO₂/L. The range of concentration can be broadened to 0 to 80 mg/L by substituting a 15-mm flow cell for the 50-mm flow cell shown in Figure 4500-SiO₂:1.

2. Apparatus

Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-SiO₂:1.

3. Reagents

a. *Sulfuric acid*, H₂SO₄, 0.05M (0.1N).

b. *Ammonium molybdate reagent:* Dissolve 10 g (NH₄)₆Mo₇O₂₄·4H₂O in 1 L 0.05M H₂SO₄. Filter and store in an amber plastic bottle.

c. *Oxalic acid solution:* Dissolve 50 g oxalic acid in 900 mL distilled water and dilute to 1 L.

d. *Reducing agent:* Dissolve 120 g NaHSO₃ and 4 g Na₂SO₃ in 800 mL warm distilled water. Add 2 g 1-amino-2-naphthol-4-sulfonic acid, mix well, and dilute to 1 L. Filter into amber plastic bottle for storage.

To prepare working reagent, dilute 100 mL to 1 L with distilled water. Make working reagent daily.

e. *Standard silica solution:* See 4500-SiO₂.C.3g.

4. Procedure

Set up manifold as shown in Figure 4500-SiO₂:1 and follow the general procedure described by the manufacturer. Determine absorbance at 660 nm. Use quality control procedures given in Section 4020.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against SiO₂ concentration in standards. Compute sample SiO₂ concentration by comparing sample response with standard curve.

6. Precision and Bias

For 0 to 20 mg SiO₂/L, when a 50-mm flow cell was used at 40 samples/h, the detection limit was 0.1 mg/L, sensitivity (concentration giving 0.398 absorbance) was 7.1 mg/L, and the coefficient of variation (95% confidence level at 7.1 mg/L) was 1.6%. For 0 to 80 mg SiO₂/L, when a 15-mm flow cell was used at 50 samples/h, detection limit was 0.5 mg/L, sensitivity was 31 mg/L, and coefficient of variation at 31 mg/L was 1.5%.

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4500-SiO₂ F. Flow Injection Analysis for Molybdate-Reactive Silicate (PROPOSED)

1. General Discussion

Silicate reacts with molybdate under acidic conditions to form yellow beta-molybdosilicic acid. This acid is subsequently reduced with stannous chloride to form a heteropoly blue complex that has an absorbance maximum at 820 nm. Oxalic acid is added to reduce the interference from phosphate.

Collect samples in polyethylene or other plastic bottles and refrigerate at 4°C. Chemical preservation for silica is not recommended. Adding acid may cause polymerization of reactive silicate species. Freezing decreases silicate concentrations, especially at concentrations greater than 100 µg SiO₂/L. If filtration is required, preferably use a 0.45-µm TFE filter. Samples may be held for 28 d.

Also see Section 4500-SiO₂.A, Section 4500-SiO₂.D, and Section 4500-SiO₂.E, and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through inert filter.

The interference due to phosphates is reduced by the addition of oxalic acid as a reagent on the flow injection manifold. By the following method, a solution of 1000 µg P/L was determined as 20 µg SiO₂/L. Verify extent of phosphate interference by determining a solution of phosphate at the highest concentration that is expected to occur.

Tannin and large amounts of iron or sulfides are interferences. Remove sulfides by boiling an acidified sample. Add disodium EDTA to eliminate interference due to iron. Treat with oxalic acid to decrease interference from tannin.

Sample color and turbidity can interfere. Determine presence of these interferences by analyzing samples without the presence of the molybdate.

Avoid silica contamination by storing samples, standards, and reagents in plastic. Do not use glass-distilled water for reagents or standards.

2. Apparatus

Flow injection analysis equipment consisting of:

- a. FIA injection valve* with sample loop or equivalent.
- b. Multichannel proportioning pump.*
- c. FIA manifold* (Figure 4500-SiO₂:2) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-SiO₂:2. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*#(81)
- d. Absorbance detector*, 820 nm, 10-nm bandpass.

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e. *Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. All reagents can also be prepared on a weight/volume basis if desired.

a. *Molybdate*: To a tared 500-mL container add 20.0 g ammonium molybdate tetrahydrate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$. Add 486 g warm water and 14.8 g conc sulfuric acid (H_2SO_4). Stir or shake until dissolved. Store in plastic and refrigerate. Prepare fresh monthly and discard if precipitate or blue color is observed.

b. *Oxalic acid*: To a tared 500-mL container add 50.0 g oxalic acid ($\text{HO}_2\text{CCO}_2\text{H}\cdot 2\text{H}_2\text{O}$) and 490 g water. Stir or shake until dissolved. Store in plastic.

c. *Stannous chloride*: To a tared 1-L container, add 978 g water. Add 40.0 g conc H_2SO_4 . Dissolve 2.0 g hydroxylamine hydrochloride in this solution. Then dissolve 0.30 g stannous chloride. Prepare fresh weekly.

d. *Stock silicate standard*, 100 mg SiO_2/L : In a 1-L volumetric flask dissolve 0.473 g sodium metasilicate nonahydrate ($\text{Na}_2\text{SiO}_3\cdot 9\text{H}_2\text{O}$) in approximately 800 mL water. Dilute to mark and invert three times. Alternatively, use a commercially prepared standard solution, especially if nonstoichiometry of the solid metasilicate is suspected; the original degree of polymerization of the sodium metasilicate, which depends on storage time, can affect free silica concentration of the resulting solution.

e. *Standard silicate solutions*: Prepare silicate standards in the desired concentration range, using the stock standard (§ 3d), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-SiO₂:2 and follow method supplied by manufacturer or laboratory standard operating procedure.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus silicate concentration. The calibration curve is linear.

6. Precision and Bias

Twenty-one replicates of a 5.0- $\mu\text{g SiO}_2/\text{L}$ standard were analyzed with a 780- μL sample loop by a published MDL method.¹ These gave a mean of 4.86 $\mu\text{g SiO}_2/\text{L}$, a standard deviation of 0.31 $\mu\text{g SiO}_2/\text{L}$, and an MDL of 0.78 $\mu\text{g SiO}_2/\text{L}$.

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7. Quality Control

Follow procedures outlined in Section 4020.

8. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and Procedure for the Determination of Method Detection Limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-S²⁻ SULFIDE*#(82)

4500-S²⁻ A. Introduction

1. Occurrence and Significance

Sulfide often is present in groundwater, especially in hot springs. Its common presence in wastewaters comes partly from the decomposition of organic matter, sometimes from industrial wastes, but mostly from the bacterial reduction of sulfate. Hydrogen sulfide escaping into the air from sulfide-containing wastewater causes odor nuisances. The threshold odor concentration of H₂S in clean water is between 0.025 and 0.25 µg/L. Gaseous H₂S is very toxic and has claimed the lives of numerous workers in sewers. At levels toxic to humans it interferes with the olfactory system, giving a false sense of the safe absence of H₂S. It attacks metals directly and indirectly has caused serious corrosion of concrete sewers because it is oxidized biologically to H₂SO₄ on the pipe wall. Dissolved H₂S is toxic to fish and other aquatic organisms.

2. Categories of Sulfides

From an analytical standpoint, three categories of sulfide in water and wastewater are distinguished.

a. Total sulfide includes dissolved H₂S and HS⁻, as well as acid-soluble metallic sulfides present in suspended matter. The S²⁻ is negligible, amounting to less than 0.5% of the dissolved sulfide at pH 12, less than 0.05% at pH 11, etc. Copper and silver sulfides are so insoluble that they do not respond in ordinary sulfide determinations; they can be ignored for practical purposes.

b. Dissolved sulfide is that remaining after suspended solids have been removed by flocculation and settling.

c. Un-ionized hydrogen sulfide may be calculated from the concentration of dissolved sulfide, the sample pH, and the practical ionization constant of H₂S.

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Figure 4500-S²⁻:1 shows analytical flow paths for sulfide determinations under various conditions and options.

3. Sampling and Storage

Take samples with minimum aeration. Either analyze samples immediately after collection or preserve for later analysis with zinc acetate solution. To preserve a sample for a total sulfide determination put zinc acetate and sodium hydroxide solutions into bottle before filling it with sample. Use 4 drops of 2N zinc acetate solution per 100 mL sample. Increase volume of zinc acetate solution if the sulfide concentration is expected to be greater than 64 mg/L. The final pH should be at least 9. Add more NaOH if necessary. Fill bottle completely and stopper.

4. Qualitative Tests

A qualitative test for sulfide often is useful. It is advisable in the examination of industrial wastes containing interfering substances that may give a false negative result in the methylene blue method (D).

a. Antimony test: To about 200 mL sample, add 0.5 mL saturated solution of potassium antimony tartrate and 0.5 mL 6N HCl in excess of phenolphthalein alkalinity.

Yellow antimony sulfide (Sb_2S_3) is discernible at a sulfide concentration of 0.5 mg/L. Comparisons with samples of known sulfide concentration make the technique roughly quantitative. The only known interferences are metallic ions such as lead, which hold the sulfide so firmly that it does not produce Sb_2S_3 , and dithionite, which decomposes in acid solution to produce sulfide.

b. Silver-silver sulfide electrode test: Dilute sample 1:1 with alkaline antioxidant reagent (see ¶ G.3a below). Measure electrode potential relative to a double-junction reference electrode and estimate the sulfide concentration from an old calibration curve or the example calibration curve in the electrode manual. This gives a reasonable estimate of sulfide concentration if the electrode is in good condition.

c. Lead acetate paper and silver foil tests: Confirm odors attributed to H_2S with lead acetate paper. On exposure to the vapor of a slightly acidified sample, the paper becomes blackened by formation of PbS. A strip of silver foil is more sensitive than lead acetate paper. Clean the silver by dipping in NaCN solution and rinse. CAUTION: *NaCN is toxic, handle with care.* Silver is suitable particularly for long-time exposure in the vicinity of possible H_2S sources because black Ag_2S is permanent whereas PbS slowly oxidizes.

5. Selection of Quantitative Methods

Iodine oxidizes sulfide in acid solution. A titration based on this reaction is an accurate method for determining sulfide at concentrations above 1 mg/L if interferences are absent and if loss of H_2S is avoided. The iodometric method (F) is useful for standardizing the methylene blue colorimetric methods (D, E, and I) and is suitable for analyzing samples freshly taken from wells

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or springs. The method can be used for wastewater and partly oxidized water from sulfur springs if interfering substances are removed first. The automated methylene blue method with distillation (I) is useful for a variety of samples containing more than 1 mg S⁻/L.

The methylene blue method (D) is based on the reaction of sulfide, ferric chloride, and dimethyl-*p*-phenylenediamine to produce methylene blue. Ammonium phosphate is added after color development to remove ferric chloride color. The procedure is applicable at sulfide concentrations between 0.1 and 20.0 mg/L. The automated methylene blue method (E) is similar to Method D. A gas dialysis technique separates the sulfide from the sample matrix. Gas dialysis eliminates most interferences, including turbidity and color. The addition of the antioxidant ascorbic acid improves sulfide recoveries. The method is applicable at sulfide concentrations between 0.002 and 0.100 mg/L.

Potentiometric methods utilizing a silver electrode (G) may be suitable. From the potential of the electrode relative to a reference electrode an estimate can be made of the sulfide concentration, but careful attention to details of procedures and frequent standardizations are needed to secure good results. The electrode is useful particularly as an end-point indicator for titration of dissolved sulfide with silver nitrate. The ion-selective electrode method is unaffected by sample color or turbidity and is applicable for concentrations greater than 0.03 mg/L.

6. Preparation of Sulfide Standards

Take care in preparing reliable stock solutions of sulfide for calibration and quality control. Prepare sulfide standards from sodium sulfide nonahydrate (Na₂S·9H₂O) crystals. These crystals usually have excess water present on the surface, in addition to a layer of contamination from oxidation products (polysulfides, polythionates, and sulfate) of sulfide reacting with atmospheric oxygen. Further, solutions of sulfide are prone to ready oxidation by dissolved and atmospheric oxygen. Use reagent water to prepare sulfide standards and sample dilutions. Boil and degas with either argon or nitrogen while cooling. Purchase the smallest amount of solid standards possible and keep no longer than 1 year. Preferably handle and store solid sulfide standards and stock solutions in an inert atmosphere glove bag or glove box to reduce contamination due to oxidation.

Preferably remove single crystals of Na₂S·9H₂O from reagent bottle with nonmetallic tweezers; quickly rinse in degassed reagent water to remove surface contamination. Blot crystal dry with a tissue, then rapidly transfer to a tared, stoppered weighing bottle containing 5 to 10 mL degassed reagent water. Repeat procedure until desired amount of sodium sulfide is in weighing bottle. Determine amount of Na₂S·9H₂O in weighing bottle by difference, then multiply the weight by 0.133 to determine the amount of S²⁻. Avoid excess agitation and mixing of the solution with atmospheric oxygen. Quantitatively transfer and dilute entire contents of weighing bottle to an appropriate size volumetric flask with degassed reagent water to prepare a known concentration sulfide stock solution (3.750 g Na₂S·9H₂O diluted to a final volume of 500 mL will give a stock solution of which 1.00 mL = 1.00 mg S²⁻). Alternatively, purchase

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precertified stock solutions of sulfide. Verify concentration of stock solution daily using the iodometric method (F). Store stock solution with minimum headspace for no more than 1 week.

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4500-S²⁻- B. Separation of Soluble and Insoluble Sulfides

Unless the sample is entirely free from suspended solids (dissolved sulfide equals total sulfide), to measure dissolved sulfide first remove insoluble matter. This can be done by producing an aluminum hydroxide floc that is settled, leaving a clear supernatant for analysis.

1. Apparatus

Glass bottles with stoppers: Use 100 mL if sulfide will be determined by the methylene blue method and 500 to 1000 mL if by the iodometric method.

2. Reagents

a. *Sodium hydroxide solution*, NaOH, 6N.

b. *Aluminum chloride solution:* Because of the hygroscopic and caking tendencies of this chemical, purchase 100-g bottles of AlCl₃·6H₂O. Dissolve contents of a previously unopened 100-g bottle in 144 mL distilled water.

3. Procedure

a. To a 100-mL glass bottle add 0.2 mL (nominally 4 drops) 6N NaOH. Fill bottle with sample and immediately add 0.2 mL (4 drops) AlCl₃ solution. Stopper bottle with no air under stopper. Rotate back and forth about a transverse axis vigorously for 1 min or longer to flocculate contents. Vary volumes of these added chemicals to get good clarification without using excessively large amounts and to produce a pH of 6 to 9. If a 500- or 1000-mL bottle is used, add proportionally larger amounts of reagents.

b. Let settle until reasonably clear supernatant can be drawn off. With proper flocculation, this may take 5 to 15 min. Do not wait longer than necessary.

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c. Either analyze the supernatant immediately or preserve with 2N zinc acetate (see Section 4500-S²⁻.C).

4500-S²⁻.C. Sample Pretreatment to Remove Interfering Substances or to Concentrate the Sulfide

The iodometric method suffers interference from reducing substances that react with iodine, including thiosulfate, sulfite, and various organic compounds, both solid and dissolved.

Strong reducing agents also interfere in the methylene blue method (D) by preventing formation of the blue color. Thiosulfate at concentrations about 10 mg/L may retard color formation or completely prevent it. Ferrocyanide produces a blue color. Sulfide itself prevents the reaction if its concentration is very high, in the range of several hundred milligrams per liter. To avoid the possibility of false negative results, use the antimony method to obtain a qualitative result in industrial wastes likely to contain sulfide but showing no color by the methylene blue method. Iodide, which is likely to be present in oil-field wastewaters, may diminish color formation if its concentration exceeds 2 mg/L. Many metals (e.g., Hg, Cd, Cu) form insoluble sulfides and give low recoveries.

Eliminate interferences due to sulfite, thiosulfate, iodide, and many other soluble substances, but not ferrocyanide, by first precipitating ZnS, removing the supernatant, and replacing it with distilled water. Use the same procedure, even when not needed for removal of interferences, to concentrate sulfide. The automated methylene blue method (E) is relatively free from interferences because gas dialysis separates the sulfide from the sample matrix.

1. Apparatus

Glass bottles with stoppers: See Section 4500-S²⁻.B.1.

2. Reagents

a. *Zinc acetate solution:* Dissolve 220 g $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$ in 870 mL water; this makes 1 L solution.

b. *Sodium hydroxide solution,* NaOH, 6N.

3. Procedure

a. Put 0.20 mL (4 drops) zinc acetate solution and 0.10 mL (2 drops) 6N NaOH into a 100-mL glass bottle, fill with sample, and add 0.10 mL (2 drops) 6N NaOH solution. Stopper with no air bubbles under stopper and mix by rotating back and forth vigorously about a transverse axis. For the iodometric procedure, use a 500-mL bottle or other convenient size, with proportionally larger volumes of reagents. Vary volume of reagents added according to sample so that the resulting precipitate is not excessively bulky and settles readily. Add enough NaOH to raise the pH above 9. Let precipitate settle for 30 min. The treated sample is relatively stable and can be held for several hours. However, if much iron is present, oxidation may be fairly

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rapid.

b. If the iodometric method is to be used, collect precipitate on a glass fiber filter and continue at once with titration according to the procedure of Method F. If the methylene blue method (D) is used, let precipitate settle for 30 min and decant as much supernatant as possible without loss of precipitate. Refill bottle with distilled water, shake to resuspend precipitate, and quickly withdraw a sample. If interfering substances are present in high concentration, settle, decant, and refill a second time. If sulfide concentration is known to be low, add only enough water to bring volume to one-half or one-fifth of original volume. Use this technique for analyzing samples of very low sulfide concentrations. After determining the sulfide concentration colorimetrically, multiply the result by the ratio of final to initial volume. No concentration or pretreatment steps to remove interferences are necessary for Method E.

4500-S²⁻- D. Methylene Blue Method

1. Apparatus

- a. *Matched test tubes*, approximately 125 mm long and 15 mm OD.
- b. *Droppers*, delivering 20 drops/mL methylene blue solution. To obtain uniform drops hold dropper in a vertical position and let drops form slowly.
- c. If photometric rather than visual color determination will be used, either:
 - 1) *Spectrophotometer*, for use at a wavelength of 664 nm with cells providing light paths of 1 cm and 1 mm, or other path lengths, or
 - 2) *Filter photometer*, with a filter providing maximum transmittance near 660 nm.

2. Reagents

- a. *Amine-sulfuric acid stock solution*: Dissolve 27 g *N,N*-dimethyl-*p*-phenylenediamine oxalate*#(83) in an iced mixture of 50 mL conc H₂SO₄ and 20 mL distilled water. Cool and dilute to 100 mL with distilled water. Use fresh oxalate because an old supply may be oxidized and discolored to a degree that results in interfering colors in the test. Store in a dark glass bottle. When this stock solution is diluted and used in the procedure with a sulfide-free sample, it first will be pink but then should become colorless within 3 min.
- b. *Amine-sulfuric acid reagent*: Dilute 25 mL amine-sulfuric acid stock solution with 975 mL 1 + 1 H₂SO₄. Store in a dark glass bottle.
- c. *Ferric chloride solution*: Dissolve 100 g FeCl₃·6H₂O in 40 mL water.
- d. *Sulfuric acid solution*, H₂SO₄, 1 + 1.
- e. *Diammonium hydrogen phosphate solution*: Dissolve 400 g (NH₄)₂HPO₄ in 800 mL distilled water.
- f. *Methylene blue solution I*: Use USP grade dye or one certified by the Biological Stain

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Commission. The dye content should be reported on the label and should be 84% or more. Dissolve 1.0 g in distilled water and make up to 1 L. This solution will be approximately the correct strength, but because of variation between different lots of dye, standardize against sulfide solutions of known strength and adjust its concentration so that 0.05 mL (1 drop) = 1.0 mg sulfide/L.

Standardization—Prepare five known-concentration sulfide standards ranging from 1 to 8 mg/L as described in 4500-S²⁻.A.6, or proceed as follows: Put several grams of clean, washed crystals of Na₂S·9H₂O into a small beaker. Add somewhat less than enough water to cover crystals. Stir occasionally for a few minutes, then pour solution into another vessel. This solution reacts slowly with oxygen but the change is insignificant if analysis is performed within a few hours. Prepare solution daily. To 1 L distilled water add 1 drop of Na₂S solution and mix.

Immediately determine sulfide concentration by the methylene blue procedure and by the iodometric procedure. Repeat, using more than 1 drop Na₂S solution or smaller volumes of water, until at least five tests have been made, with a range of sulfide concentrations between 1 and 8 mg/L. Calculate average percent error of the methylene blue result as compared to the iodometric result. If the average error is negative, that is, methylene blue results are lower than iodometric results, dilute methylene blue solution by the same percentage, so that a greater volume will be used in matching colors. If methylene blue results are high, increase solution strength by adding more dye.

g. Methylene blue solution II: Dilute 10.00 mL of adjusted methylene blue solution I to 100 mL with reagent water.

3. Procedure

a. Color development: Transfer 7.5 mL sample to each of two matched test tubes, using a special wide-tip pipet or filling to marks on test tubes. If sample has been preserved with zinc acetate, shake vigorously before taking subsample. Add to Tube A 0.5 mL amine-sulfuric acid reagent and 0.15 mL (3 drops) FeCl₃ solution. Mix immediately by inverting slowly, only once. (Excessive mixing causes low results by loss of H₂S as a gas before it has had time to react). To Tube B add 0.5 mL 1 + 1 H₂SO₄ and 0.15 mL (3 drops) FeCl₃ solution and mix. The presence of S²⁻ will be indicated by the appearance of blue color in Tube A. Color development usually is complete in about 1 min, but a longer time often is required for fading out of the initial pink color. Wait 3 to 5 min and add 1.6 mL (NH₄)₂HPO₄ solution to each tube. Wait 3 to 15 min and make color comparisons. If zinc acetate was used, wait at least 10 min before making a visual color comparison.

b. Color determination:

1) Visual color estimation—Add methylene blue solution I or II, depending on sulfide concentration and desired accuracy, dropwise, to the second tube, until color matches that developed in first tube. If the concentration exceeds 20 mg/L, repeat test with a portion of

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sample diluted tenfold.

With methylene blue solution I, adjusted so that 0.05 mL (1 drop) = 1.0 mg S²⁻/L when 7.5 mL of sample are used:

$$\text{mg S}^{2-}/\text{L} = \text{no. drops solution I} + 0.1 (\text{no. drops solution II})$$

2) Photometric color measurement—A cell with a light path of 1 cm is suitable for measuring sulfide concentrations from 0.1 to 2.0 mg/L. Use shorter or longer light paths for higher or lower concentrations. This method is suitable for sample concentrations up to 20 mg/L. Zero instrument with a portion of treated sample from Tube B. Prepare calibration curves on basis of colorimetric tests made on Na₂S solutions simultaneously analyzed by the iodometric method, plotting concentration vs. absorbance. A linear relationship between concentration and absorbance can be assumed from 0 to 1.0 mg/L.

Read sulfide concentration from calibration curve.

4. Precision and Bias

In a study by two chemists working in the same laboratory, the standard deviation estimated from 34 sets of duplicate sulfide measurements was 0.04 mg/L for concentrations between 0.2 and 1.5 mg/L. The average recoveries of known additions were 92% for 40 samples containing 0.5 to 1.5 mg/L and 89% for samples containing less than 0.1 mg/L.

5. Bibliography

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NUSBAUM, I. 1965. Determining sulfides in water and waste water. *Water Sewage Works* 112:113.

4500-S²⁻ E. Gas Dialysis, Automated Methylene Blue Method

1. Apparatus

a. *Automated analytical equipment:* An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-S²⁻:2.

The sampler is equipped with a mixer to stir samples before analysis and the gas dialysis membrane, which is maintained at room temperature, separates H₂S from the sample matrix.

2. Reagents

a. *N,N-dimethyl-p-phenylenediamine stock solution:* Dissolve 1 g N,N-dimethyl-p-phenylenediamine dihydrochloride in 500 mL 6N HCl. Prepare fresh monthly. Store in an amber bottle.

b. *N,N-dimethyl-p-phenylenediamine working solution:* Dilute 190 mL

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N,N-dimethyl-*p*-phenylenediamine stock solution to 1 L. Store in an amber bottle. Prepare weekly.

c. Ferric chloride stock solution: Dissolve 13.5 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 500 mL 5N HCl. Store in an amber bottle. Prepare fresh monthly.

d. Working ferric chloride solution: Dilute 190 mL ferric chloride stock solution to 1 L. Store in an amber bottle. Prepare fresh weekly.

e. Hydrochloric acid, HCl, 6N:

f. Sodium hydroxide stock solution, NaOH, 1N.

g. Sodium hydroxide, NaOH, 0.01N: Dilute 10 mL NaOH stock solution to 1 L.

h. Sulfide stock solution, 1.00 mg S^{2-} /1.00 mL: See Section 4500- S^{2-} .A.6.

i. Sulfide intermediate standard solution: Dilute 10 mL sulfide stock solution to 1 L with water. Prepare fresh daily. Standardize by iodometric titration method, Section 4500- S^{2-} .F. 1 mL $\approx 0.01 \text{ mg } \text{S}^{2-}$.

j. Sulfide tertiary standard solution: Dilute 50 mL sulfide intermediate solution to 500 mL with 0.01N NaOH. Prepare fresh daily. Use standardization value from ¶ 2i to determine exact concentration. 1.00 mL $\approx 0.001 \text{ mg } \text{S}^{2-}$.

k. Working sulfide standard solutions: Prepare a suitable series of standards by diluting appropriate volumes of sulfide tertiary standing solutions with 0.01N NaOH. Prepare fresh daily.

l. Zinc acetate preservative solution: Dissolve 220 g $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$ in 870 mL water (this makes 1 L solution).

3. Procedure

For unpreserved, freshly collected samples and sulfide working standards, add, in order, 4 drops 2N zinc acetate, 0.5 mL 6N NaOH, and 400 mg ascorbic acid/100 mL. For preserved samples, add 0.5 mL 6N NaOH and 400 mg ascorbic acid/100 mL. Shake well.

Let precipitate settle for at least 30 min. Pour a portion of well-mixed sample or working standard into a sample cup. Set up manifold as shown in Figure 4500- S^{2-} :2 and follow the general procedure described by the manufacturer. Determine absorbance at 660 nm.

4. Calculation

Prepare standard curves by plotting peak heights of standards processed through the manifold against S^{2-} concentration in the standards. Compute S^{2-} sample concentration by comparing sample response with standard curve.

5. Precision and Bias

In a single laboratory, samples with S^{2-} concentrations of 0.012, 0.015, 0.034, and 0.085 mg/L had standard deviations of 0.001, 0.001, 0.001, and 0.001 mg/L, respectively, with coefficients of variation of 8.3%, 6.3%, 2.9%, and 1.2%, respectively. In two environmental

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samples with added S^{2-} , recoveries were 104.2% and 97.6%.

6. Bibliography

FRANCOM, D., L.R. GOODWIN & F.P. DIEKEN. 1989. Determination of low level sulfides in environmental waters by automated gas dialysis/methylene blue colorimetry. *Anal. Lett.* 22:2587.

4500- S^{2-} F. Iodometric Method

1. Reagents

- a. *Hydrochloric acid*, HCl, 6N.
- b. *Standard iodine solution*, 0.0250N: Dissolve 20 to 25 g KI in a little water and add 3.2 g iodine. After iodine has dissolved, dilute to 1000 mL and standardize against 0.0250N $Na_2S_2O_3$, using starch solution as indicator.
- c. *Standard sodium thiosulfate solution*, 0.0250N: See Section 4500-O.C.2e.
- d. *Starch solution*: See Section 4500-O.C.2d.

2. Procedure

- a. Measure from a buret into a 500-mL flask an amount of iodine solution estimated to be an excess over the amount of sulfide present. Add distilled water, if necessary, to bring volume to about 20 mL. Add 2 mL 6N HCl. Pipet 200 mL sample into flask, discharging sample under solution surface. If iodine color disappears, add more iodine until color remains. Back-titrate with $Na_2S_2O_3$ solution, adding a few drops of starch solution as end point is approached, and continuing until blue color disappears.
- b. If sulfide was precipitated with zinc and ZnS filtered out, return filter with precipitate to original bottle and add about 100 mL water. Add iodine solution and HCl and titrate as in ¶ 2a above.

3. Calculation

One milliliter 0.0250N iodine solution reacts with 0.4 mg S^{2-} :

$$\text{mg } S^{2-}/L = \frac{[(A \times B) - (C \times D)] \times 16\,000}{\text{mL sample}}$$

where:

- A = mL iodine solution,
B = normality of iodine solution,

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C = mL $\text{Na}_2\text{S}_2\text{O}_3$ solution, and

D = normality of $\text{Na}_2\text{S}_2\text{O}_3$ solution.

4. Precision

The precision of the end point varies with the sample. In clean waters it should be determinable within 1 drop, which is equivalent to 0.1 mg/L in a 200-mL sample.

4500-S²⁻ G. Ion-Selective Electrode Method

1. General Discussion

a. Principle: The potential of a silver/sulfide ion-selective electrode (ISE) is related to the sulfide ion activity. An alkaline antioxidant reagent (AAR) is added to samples and standards to inhibit oxidation of sulfide by oxygen and to provide a constant ionic strength and pH. Use of the AAR allows calibration in terms of total dissolved sulfide concentration. All samples and standards must be at the same temperature. Sulfide concentrations between 0.032 mg/L ($1 \times 10^{-6}M$) and 100 mg/L can be measured without preconcentration. For lower concentrations, preconcentration is necessary.

b. Interferences: Humic substances may interfere with Ag/S-ISE measurements. For highly colored water (high concentration of humic substances), use the method of standard additions to check results. Sulfide is oxidized by dissolved oxygen. Sulfide oxidation may cause potential readings to drift in the direction of decreasing concentration, i.e., to more positive values. Flush surface of samples and standards with nitrogen to minimize contact with atmospheric oxygen for low-level measurements. Temperature changes may cause potentials to drift either upward or downward. Therefore, let standards and samples come to the same temperature. If samples cannot be analyzed immediately, preserve dissolved sulfide by precipitating with zinc acetate (Section 4500-S²⁻.C).

2. Apparatus

a. Silver/sulfide electrode:(84)*

b. Double-junction reference electrode.

c. Electrode polishing strips.†(85)

d. pH meter with millivolt scale, capable of 0.1-mV resolution. Meters that can be calibrated in concentration and that perform standard-additions calculations are available.

e. Electrochemical cell: Make suitable cell from a 150-mL beaker and a sheet of rigid plastic (PVC or acrylic) with holes drilled to allow insertion of the electrodes and a tube for flushing the headspace with nitrogen. Alternatively, purchase a polarographic cell with gas transfer tube.‡(86)

f. Gas dispersion tube: Use to deaerate water for preparing reagents and standards.

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g. Magnetic stirrer and stirring bar: Use a piece of styrofoam or cardboard to insulate the cell from the magnetic stirrer.

3. Reagents

a. Alkaline antioxidant reagent (AAR): To approximately 600 mL deaerated reagent water (DRW) in a 1-L volumetric flask, add 80 g NaOH, 35 g ascorbic acid, and 67 g Na₂H₂EDTA. Swirl to dissolve and dilute to 1 L. The color of freshly prepared AAR will range from colorless to yellow. Store in a tightly capped brown glass bottle. Discard when solution becomes brown.

b. Lead perchlorate, 0.1M: Dissolve 4.60 g Pb(ClO₄)₂·3H₂O in 100 mL reagent water. Standardize by titrating with Na₂H₂EDTA. Alternatively, use commercially available 0.1M Pb(ClO₄)₂ solutions.

c. Sulfide stock solution, 130 mg/L: See 4500-S²⁻.A.6, and dilute 13.0 mL of 1.00 mg S²⁻/mL stock to 100.0 mL with AAR. Alternatively, add 500 mL AAR and 10 g Na₂S·9H₂O to a 1-L volumetric flask; dissolve. Dilute to 1 L with DRW. Use deaerated artificial seawater (DASW), Table 8010:III, or 0.7M NaCl if sulfide concentrations are to be determined in seawater. Standardize stock solution by titrating with 0.1M Pb(ClO₄)₂. Pipet 50 mL sulfide stock solution into the electrochemical cell. (Use 10 mL with a small-volume polarographic cell.) Insert Ag/S electrode and reference electrode and read initial potential. Titrate with 0.1M Pb(ClO₄)₂. Let electrode potential stabilize and record potential after each addition. Locate equivalence point as in Section 4500-Cl⁻.D.4a. Alternatively, linearize the titration curve.¹ Calculate the function F_1 for points before the equivalence point.

$$F_1 = (V_o + V)10^{\frac{E}{m}}$$

where:

V_o = volume of stock solution, mL,

V = titrant volume, mL,

E = potential, mV, and

m = slope of calibration curve, mV/log unit.

Plot F_1 as a function of titrant volume. Extrapolate to find the intersection with the x-axis; that is, the equivalence point. Calculate sulfide concentration in the stock solution from:

$$C = \frac{V_{eq}[Pb]}{V_o}$$

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where:

- C = sulfide concentration, mg/L,
- V_{eq} = equivalence volume, mL,
- $[Pb]$ = concentration of Pb in titrant, mg/L, and
- V_o = volume of stock solution, mL.

Store stock solution in a tightly capped bottle for 1 week or less. The stock solution also can be standardized iodometrically (see Section 4500-S²⁻.E). CAUTION: *Store in a fume hood.*

d. Sulfide standards: Prepare sulfide standards daily by serial dilution of stock. Add AAR and Zn(C₂H₃O₂)₂ solutions to 100-mL volumetric flasks. Add sulfide solutions and dilute to volume with DRW (or DASW). Refer to Table 4500-S²⁻.I for volumes. Prepare at least one standard with a concentration less than the lowest sample concentration.

4. Procedure

Check electrode performance and calibrate daily. Check electrode potential in a sulfide standard every 2 h. The procedure depends on the sulfide concentration and the time between sample collection and sulfide determination. If the total sulfide concentration is greater than 0.03 mg/L ($1 \times 10^{-6}M$) and the time delay is only a few minutes, sulfide can be determined directly. Otherwise, precipitate ZnS and filter as described in Section 4500-S²⁻.C.

a. Check electrode performance: Pipet 50 mL AAR, 50 mL DWR, and 1 mL sulfide stock solution into the measurement cell. Place Ag/S and reference electrodes in the solution and read potential. Add 10 mL stock solution and read potential. The change in potential should be -28 ± 2 mV. If it is not, follow the troubleshooting procedure in the electrode manual.

b. Calibration: Place electrodes in the most dilute standard but use calibration standards that bracket the sulfide concentrations in the samples. Record potential when the rate of change is less than 0.3 mV/min. (This may take up to 30 min for very low sulfide concentrations, i.e., less than 0.03 mg/L.) Rinse electrodes, blot dry with a tissue, and read potential of the next highest standard. For a meter that can be calibrated directly in concentration, follow manufacturer's directions. For other meters, plot potential as a function of the logarithm (base 10) of the sulfide concentration. For potentials in the linear range, calculate the slope and intercept of the linear portion of the calibration plot.

c. Sulfide determination by comparison with calibration curve, no ZnS precipitation: Add 40 mL AAR, 0.15 mL (3 drops) zinc acetate, and 50 mL sample to a 100-mL volumetric flask. Dilute to 100 mL with AAR. Pour into the electrochemical cell and insert the electrodes. Record potential when the rate of change is less than 0.3 mV/min. Read sulfide concentration from the calibration curve. Alternatively, for potentials in the linear range, calculate the sulfide concentration from:

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$$S_{Tot} = 10^{\frac{E-b}{m}}$$

where:

E = electrode potential and

b and m are the intercept and slope of the calibration curve. For a meter that can be calibrated directly in concentration, follow the manufacturer's directions.

d. Sulfide determination by comparison with calibration curve, with ZnS precipitation: Place filter with ZnS precipitate in a 150-mL beaker containing a stir bar. Wash sample bottle with 50 mL AAR and 20 mL DRW and pour the washings into the beaker. Stir to dissolve precipitate. Remove filter with forceps while rinsing it into the beaker with a minimum amount of DRW. Quantitatively transfer to a 100-mL volumetric flask and dilute to mark with DRW. Pour into the electrochemical cell and place the electrodes in the solution. Measure potential as in ¶ 4c above. Calculate sulfide concentration (¶ 4c).

e. Sulfide determination by standard addition with or without ZnS precipitation: Measure the Ag/S-ISE electrode potential as in ¶ c or d above. Add sulfide stock solution and measure potential again. Calculate sulfide concentration as follows:

$$C_o = \frac{fC_s}{(1 + f)10^{\frac{E_i - E_o}{m}} - 1}$$

where:

C_o and C_s = sulfide concentrations in sample and known addition,

E_o and E_s = potentials measured for sample and known addition,

m = slope of calibration curve (approximately 28 mV/log S^{2-} , and

f = ratio of known-addition volume to sample volume.

f. Sulfide determination by titration: Use the same procedure as for standardizing the sulfide stock solution (¶ 3c). The minimum sulfide concentration for determination by titration is 0.3 mg/L ($10^{-5}M$).

5. Precision

For sulfide determination by comparison with the calibration curve, the relative standard deviation varies with the sulfide concentration. RSD values of 23% for 0.0091 mg/L and 5% for 0.182 mg/L have been reported.² (0.0091 µg/L was below the range for which the potential varied linearly with the logarithm of the sulfide concentration, i.e., the Nernstian range.) For

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sulfide determination by standard addition, the precision is greatest if the amount of sulfide added is as large as possible while staying within the linear range.³

6. References

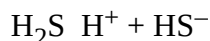
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4500-S²⁻-H. Calculation of Un-ionized Hydrogen Sulfide

Hydrogen sulfide (H₂S) and bisulfide ion (HS⁻), which together constitute dissolved sulfide, are in equilibrium with hydrogen ions:



The conditional ionization constant, which is valid for the temperature and ionic strength of the water of interest, relates the concentrations of H₂S and HS⁻:

$$K'_1 = \frac{[\text{H}^+][\text{HS}^-]}{[\text{H}_2\text{S}]}$$

The conditional constant is used to calculate the distribution of dissolved sulfide between the two species. The conditional ionization constant of H₂S is approximately 7.0. It differs from 7.0 by less than 0.2 log units for the ionic strengths and temperatures likely to be encountered in water-quality monitoring. The fraction of sulfide present as H₂S can be estimated with an error of less than 40% from Figure 4500-S²⁻-3. If more accuracy is needed, use the methods given below.

1. Calculation for Fresh Water and Brackish Water (*I* < 0.1*M*)

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Calculate the dissociation constant for zero ionic strength (pK_1) and the temperature of interest.¹ If the temperature is 25°C, then pK_1 is 6.98. Otherwise:

$$pK_1(T) = 32.55 + 1519.44/T - 15.672 \log_{10} T + 0.02722T$$

where T is temperature (°K, i.e., T °C + 273.15). Next, calculate the ionic strength I as in Table 2330:I, the Debye-Huckel A parameter, and the negative logarithm of the monovalent ion activity coefficient (pf_m):

$$A = 0.7083 - 2.277 \times 10^{-3}T + 5.399 \times 10^{-6}T^2$$

$$A = 0.7083 - 2.277 \times 10^{-3}T + 5.399 \times 10^{-6}T^2$$

$$pf_m = A \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3I \right)$$

Calculate the conditional ionization constant, K'_1 , and the hydrogen ion concentration, $[H^+]$:

$$K'_1 = 10^{-pK_1 + 2 pf_m}$$

$$[H^+] = 10^{-pH + pf_m}$$

Finally, calculate the un-ionized hydrogen sulfide concentration, $[H_2S]$, from the total sulfide concentration, S_T :

$$[H_2S] = \frac{S_T}{1 + \frac{K'_1}{[H^+]}}$$

Sample calculation: Total sulfide concentration 0.32 mg/L ($1.0 \times 10^{-5}M$), pH 6.75, ionic strength 0.02M, temperature 15.5°C.

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$$pK_1 = 32.55 \times \frac{1519.44}{288.65} - 15.672 \times \log_{10}(288.65) + 0.02722 \times 288.65 = 7.11$$

$$A = 0.7083 - 2.277 \times 10^{-3} \times 288.65 + 5.399 \times 10^{-6} \times (288.65)^2 = 0.501$$

$$pf_m = 0.501 \times \left(\frac{\sqrt{0.02}}{1 + \sqrt{0.02}} - 0.3 \times 0.02 \right) = 0.059$$

$$K'_1 = 10^{-7.11 + 2 \times 0.059} = 1.014 \times 10^{-7}$$

$$[H^+] = 10^{-6.75 + 0.059} = 2.037 \times 10^{-7}$$

$$[H_2S] = \frac{1 \times 10^{-5}}{1 + \frac{1.014 \times 10^{-7}}{2.037 \times 10^{-7}}} = 6.68 \times 10^{-6} M = 0.21 \text{ mg/L (as S)}$$

2. Calculation for Seawater and Estuarine Water

This procedure differs only in calculating the conditional ionization constant, which can be calculated accurately.¹ The (potentially) largest source of error in calculating un-ionized hydrogen sulfide in seawater is the hydrogen ion concentration. Calibrate the pH electrode in artificial seawater at the temperature of the water of interest.² Alternatively, if the pH electrode is calibrated using NIST buffers (as in Section 4500-H), measure pH of dilute acid (10^{-4} – $10^{-3}N$ HNO_3 , HCl , or $HClO_4$) in artificial seawater diluted to the salinity of the water of interest and at the temperature of interest and calculate a correction factor.³ (Prepare artificial seawater as in Table 8010:III, substituting $NaCl$ for NaF , $NaHCO_3$, and $Na_2SiO_3 \cdot 9H_2O$ on an equimolar basis.)

Calculate pK'_1 as outlined in Section 4500-S²⁻.H.1. Calculate the coefficients A and B^1 (A and B are not Debye-Huckel parameters):

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$$A = -0.2391 + \frac{35.685}{T}$$
$$B = 0.0109 - \frac{0.3776}{T}$$

Calculate pK'_1 :

$$pK'_1 = pK_1 + A\sqrt{S} + BS$$

where:

S = salinity, g/kg.

Calculate K'_1 :

$$K'_1 = 10^{-pK'_1}$$

Sample calculation: Total sulfide concentration 0.32 mg/L ($1 \times 10^{-5}M$), pH 6.75, salinity 35 g/kg ($I = 0.7M$), temperature 15.5°C.

$$A = -0.2391 + \frac{35.685}{288.65}$$
$$= -0.115$$
$$B = 0.0109 - \frac{0.3776}{288.65}$$
$$= 0.00959$$

From 4500-S²⁻.H.1, $pK_1 = 7.11$.

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$$\begin{aligned} pK'_1 &= 7.11 - 0.115\sqrt{35} + 0.00959 \times 35 \\ &= 6.77 \end{aligned}$$

$$\begin{aligned} K'_1 &= 10^{-6.77} \\ &= 1.70 \times 10^{-7} \end{aligned}$$

$$\begin{aligned} pf_m &= 0.501 \times \left(\frac{\sqrt{0.7}}{1 + \sqrt{0.7}} - 0.3 \times 0.7 \right) \\ &= 0.12 \end{aligned}$$

$$\begin{aligned} [H^+] &= 10^{-6.75+0.12} \\ &= 2.34 \times 10^{-7} \end{aligned}$$

$$\begin{aligned} [H_2S] &= \frac{1 \times 10^{-5}}{1 + \frac{1.7 \times 10^{-7}}{2.3 \times 10^{-7}}} \\ &= 5.8 \times 10^{-6} M \\ &= 0.19 \text{ mg/L (as S)} \end{aligned}$$

3. References

1. MILLERO, F.J. 1986. The thermodynamics and kinetics of the hydrogen sulfide system in natural waters. *Mar. Chem.* 18:121.
2. MILLERO, F.J. 1986. The pH of estuarine waters. *Limnol. Oceanogr.* 31 :839.
3. SIGEL, H., A.D. ZUBERBUHLER & O. YAMAUCHI. 1991. Comments on potentiometric pH titrations and the relationship between pH-meter reading and hydrogen ion concentration. *Anal. Chim. Acta.* 255:63.

4. Bibliography

ARCHER, D.G. & P. WANG. 1990. The dielectric constant of water and Debye-Huckel Limiting Law Slopes. *J. Phys. Chem. Ref. Data* 12: 817.

4500-S²⁻ I. Distillation, Methylene Blue Flow Injection Analysis (PROPOSED)

1. General Discussion

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a. Principle: Water and wastewater samples are distilled into a sodium hydroxide trapping solution and the distillate is analyzed. Hydrogen sulfide (H_2S) reacts in acid media and in the presence of ferric chloride with two molecules of *N, N*-dimethyl-*p*-phenylenediamine to form methylene blue. The resulting color is read at 660 nm.

b. Sample preservation: Because H_2S oxidizes rapidly, analyze samples and standards without delay. To preserve samples, add 4 drops 2M zinc acetate to 100 mL sample and adjust pH to >9 with 6M NaOH, then cool to 4°C. Samples are distilled into a trapping solution resulting in 0.25M NaOH matrix.

Also see Section 4500-S²⁻.A, Section 4500-S²⁻.B, and Section 4500-S²⁻.E, and Section 4130, Flow Injection Analysis (FIA).

c. Interferences: This method measures total sulfide, which is defined as the acid-soluble sulfide fraction of a sample. Total sulfide includes both acid-soluble sulfides such as H_2S , and acid-soluble metal sulfides present in suspended matter. This method does not measure acid-insoluble sulfides such as CuS.

Most nonvolatile interferences are eliminated by distillation. Strong reducing agents inhibit color formation at concentrations of several hundred milligrams per liter. Iodide interferes at concentrations greater than 2 mg I/L.

Also see Section 4500-S²⁻.A and Section 4500-S²⁻.B.

2. Apparatus

a. Distillation apparatus consisting of a glass or polypropylene micro-distillation device*(87) capable of distilling 6 mL or more of sample into a 0.25M NaOH final concentration trapping solution.

b. Flow injection analysis equipment consisting of:

- 1) *FIA injection valve* with sample loop or equivalent.
- 2) *Multichannel proportioning pump*.
- 3) *FIA manifold* (Figure 4500-S²⁻:4) with cation exchange column and flow cell. Relative flow rates only are shown in Figure 4500-S²⁻:4. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.
- 4) *Absorbance detector*, 660 nm, 10-nm bandpass.
- 5) *Injection valve control and data acquisition system*.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min.

a. Sodium hydroxide carrier and diluent, NaOH, 0.25M: In a 2-L volumetric flask, dissolve 20 g NaOH in approximately 1800 mL water. Dilute to mark and mix with a magnetic stirrer

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until dissolved. Store in a plastic container.

b. *Hydrochloric acid, HCl, 3M*: To a tared 1-L container, add 752 g water and then slowly add 295 g conc HCl. Invert to mix.

c. *Hydrochloric acid, HCl, 0.20M*: To a tared 1-L container, add 983.5 g water. Then add 19.7 g conc HCl. Invert to mix.

d. *N,N-dimethyl-p-phenylenediamine*: In a 1-L volumetric flask dissolve 1.0 g *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride, $(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{NH}_2 \cdot 2\text{HCl}$, in about 800 mL 3M HCl (§ 3b). Dilute to mark and invert to mix. If solution appears dark, it is likely that the *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride is decomposed; discard, and use fresh reagent.

e. *Ferric chloride*: In a 500-mL volumetric flask dissolve 6.65 g ferric chloride hexahydrate, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, in about 450 mL 0.20M HCl (§ 3c). Dilute to mark with water and invert to mix.

f. *Stock sulfide standard, 100 mg S²⁻/L*: In a 1-L volumetric flask dissolve 0.7491 g sodium sulfide nonahydrate, $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, in approximately 900 mL NaOH diluent (§ 3a). Dilute to mark and invert to mix.

g. *Standard solutions*: Prepare sulfide standards in desired concentration range, using stock standard (§ 3 f), and diluting with NaOH diluent (§ 3a).

h. *Sulfuric acid distillation releasing solution, H₂SO₄, 9M*: To a tared 500-mL container, add 150.0 g water, then add slowly while swirling, in increments of 40 g, 276 g conc H₂SO₄. CAUTION: Solution will become very hot. Allow to cool before using.

4. Procedure

a. *Distillation*: This procedure is designed for the determination of sulfides in aqueous solutions, solid waste materials, or effluents. To preserve and remove sulfide from interfering substances, distill samples immediately after collection.

Follow manufacturer's instructions for use of distillation apparatus. Add sufficient 9M H₂SO₄ (§ 3h) to sample to dissolve ZnS (s), digest total sulfides, and release the sulfide as hydrogen sulfide gas. Immediately place sample on-line with the receiving vessel or collector tube and distill hydrogen sulfide and water in the sample into a 0.25M trapping solution.

b. *Flow injection analysis*: Set up a manifold equivalent to that in Figure 4500-S²⁻:4 and follow method supplied by the manufacturer or laboratory standard operating procedure. The carrier concentration should be identical to the final concentration of NaOH in the trapping solution from the distillation procedure (§ 4a). Follow quality control protocols outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus sulfide concentration.

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6. Precision and Bias

a. *MDL*: A 200- μ L sample loop was used in the method described above. Using a published method,¹ analysts ran 21 replicates of a 10.0-mg S^{2-} /L standard. These gave a mean of 9.0 mg S^{2-} /L, a standard deviation of 0.23 mg S^{2-} /L, and MDL of 0.58 mg S^{2-} /L. A higher MDL may be obtained by decreasing sample loop volume.

b. *Precision*: Ten injections of a distilled 50-mg S^{2-} /L standard gave a mean of 49.4 mg S^{2-} /L, a standard deviation of 0.27 mg S^{2-} /L, and percent relative standard deviation of 0.54.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-SO₃²⁻ SULFITE*(88)

4500-SO₃²⁻ A. Introduction

1. Occurrence

Sulfite ions (SO_3^{2-}) may occur in boilers and boiler feedwaters treated with sulfite for dissolved oxygen control, in natural waters or wastewaters as a result of industrial pollution, and in treatment plant effluents dechlorinated with sulfur dioxide (SO_2). Excess sulfite ion in boiler waters is deleterious because it lowers the pH and promotes corrosion. Control of sulfite ion in wastewater treatment and discharge may be important environmentally, principally because of its toxicity to fish and other aquatic life and its rapid oxygen demand.

2. Selection of Method

The iodometric titration method is suitable for relatively clean waters with concentrations above 2 mg SO_3^{2-} /L. The phenanthroline colorimetric determination, following evolution of sulfite from the sample matrix as SO_2 , is preferred for low levels of sulfite.

4500-SO₃²⁻ B. Iodometric Method

1. General Discussion

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a. Principle: An acidified sample containing sulfite (SO_3^{2-}) is titrated with a standardized potassium iodide-iodate titrant. Free iodine, liberated by the iodide-iodate reagent, reacts with SO_3^{2-} . The titration endpoint is signalled by the blue color resulting from the first excess of iodine reacting with a starch indicator.

b. Interferences: The presence of other oxidizable materials, such as sulfide, thiosulfate, and Fe^{2+} ions, can cause apparently high results for sulfite. Some metal ions, such as Cu^{2+} , may catalyze the oxidation of SO_3^{2-} to SO_4^{2-} when the sample is exposed to air, thus leading to low results. NO_2^- will react with SO_3^{2-} in the acidic reaction medium and lead to low sulfite results unless sulfamic acid is added to destroy nitrite. Addition of EDTA as a complexing agent at the time of sample collection inhibits Cu^{2+} catalysis and promotes oxidation of ferrous to ferric iron before analysis. Sulfide and thiosulfate ions normally would be expected only in samples containing certain industrial discharges, but must be accounted for if present. Sulfide may be removed by adding about 0.5 g zinc acetate and analyzing the supernatant of the settled sample. However, thiosulfate may have to be determined by an independent method (e.g., the formaldehyde/iodometric method¹), and then the sulfite determined by difference.

c. Minimum detectable concentration: 2 mg SO_3^{2-} /L.

2. Reagents

a. Sulfuric acid: H_2SO_4 , 1 + 1.

b. Standard potassium iodide-iodate titrant, 0.002083M: Dissolve 0.4458 g primary-grade anhydrous KIO_3 (dried for 4 h at 120°C), 4.35 g KI, and 310 mg sodium bicarbonate (NaHCO_3) in distilled water and dilute to 1000 mL; 1.00 mL = 500 μg SO_3^{2-} .

c. Sulfamic acid, $\text{NH}_2\text{SO}_3\text{H}$, crystalline.

d. EDTA reagent: Dissolve 2.5 g disodium EDTA in 100 mL distilled water.

e. Starch indicator: To 5 g starch (potato, arrowroot, or soluble) in a mortar, add a little cold distilled water and grind to a paste. Add mixture to 1 L boiling distilled water, stir, and let settle overnight. Use clear supernatant. Preserve by adding either 1.3 g salicylic acid, 4 g ZnCl_2 , or a combination of 4 g sodium propionate and 2 g sodium azide to 1 L starch solution.

3. Procedure

a. Sample collection: Collect a fresh sample, taking care to minimize contact with air. Fix cooled samples ($<50^\circ\text{C}$) immediately by adding 1 mL EDTA solution/100 mL sample. Cool hot samples to 50°C or below. Do not filter.

b. Titration: Add 1 mL H_2SO_4 and 0.1 g $\text{NH}_2\text{SO}_3\text{H}$ crystals to a 250-mL erlenmeyer flask or other suitable titration vessel. Accurately measure 50 to 100 mL EDTA-stabilized sample into flask, keeping pipet tip below liquid surface. Add 1 mL starch indicator solution. Titrate

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immediately with standard KI-KIO₃ titrant, while swirling flask, until a faint permanent blue color develops. Analyze a reagent blank using distilled water instead of sample.

4. Calculation

$$\text{mg SO}_3^{2-}/\text{L} = \frac{(A - B) \times M \times 6 \times 40\,000}{\text{mL sample}}$$

where:

A = mL titrant for sample,

B = mL titrant for blank, and

M = molarity of KI-KIO₃ titrant.

5. Precision and Bias

Three laboratories analyzed five replicate portions of a standard sulfite solution and of secondary treated wastewater effluent to which sulfite was added. The data are summarized below. Individual analyst's precision ranged from 0.7 to 3.6% standard deviation (*N* = 45).

Sample	$\bar{x}$ mg/L	Standard Deviation, σ mg/L	Relative Standard Deviation %
Standard, 6.3 mg SO ₃ ²⁻ /L	4.5	0.25	5.5
Secondary effluent with 2.0 mg SO ₃ ²⁻ /L	2.1	0.28	13.4
Secondary effluent with 4.0 mg SO ₃ ²⁻ /L	3.6	0.17	4.8

6. Reference

1. KURTENACKER, A. 1924. The aldehyde-bisulfite reaction in mass analysis. *Z. Anal. Chem.* 64:56.

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4500-SO₃²⁻ C. Phenanthroline Method

1. General Discussion

a. Principle: An acidified sample is purged with nitrogen gas and the liberated SO₂ is trapped in an absorbing solution containing ferric ion and 1,10-phenanthroline. Ferric iron is reduced to the ferrous state by SO₂, producing the orange tris(1,10-phenanthroline) iron(II) complex. After excess ferric iron is removed with ammonium bifluoride, the phenanthroline complex is measured colorimetrically at 510 nm.¹

b. Interferences: See Section 4500-SO₃²⁻.B..

c. Minimum detectable concentration: 0.01 mg SO₃²⁻/L.

2. Apparatus

a. Apparatus for evolution of SO₂: Figure 4500-SO₃²⁻:1 shows the following components:

- 1) *Gas flow meter*, with a capacity to measure 2 L/min of pure nitrogen gas.
- 2) *Gas washing bottle*, 250-mL, with coarse-porosity, 12- mm-diam fritted cylinder gas dispersion tube.
- 3) *Tubing connectors*, quick-disconnect, polypropylene.
- 4) *Tubing*, flexible PVC, for use in all connections.
- 5) *Nessler tube*, 100-mL.

b. Colorimetric equipment: One of the following is required:

- 1) *Spectrophotometer*, for use at 510 nm, providing a light path of 1 cm or longer.
- 2) *Filter photometer*, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 510 nm.

3. Reagents

a. 1,10-phenanthroline solution, 0.03M: Dissolve 5.95 g 1,10-phenanthroline in 100 mL 95% ethanol. Dilute to 1 L with distilled water. Discard if solution becomes colored.

b. Ferric ammonium sulfate solution, 0.01M: Dissolve 4.82 g NH₄Fe(SO₄)₂·12H₂O in 1 L distilled water to which has been added 1 mL conc H₂SO₄ to suppress ferric hydrolysis. Filter through a glass fiber filter if insoluble matter is visible. If necessary, adjust volume of acid so that a mixture of 10 parts of phenanthroline solution and one part of ferric ammonium sulfate solution will have a pH between 5 and 6.

c. Ammonium bifluoride, 5%: Dissolve 25 g NH₄HF₂ in 500 mL distilled water. Store in a polyethylene bottle and dispense with a plastic pipet.

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d. Potassium tetrachloromercurate, (TCM), K_2HgCl_4 , 0.04M: Dissolve 10.86 g $HgCl_2$, 5.96 g KCl, and 0.066 g disodium EDTA in distilled water and dilute to 1 L. Adjust pH to 5.2. This reagent normally is stable for 6 months, but discard if a precipitate forms.²

e. Dilute TCM-stabilized sulfite standard: Dissolve 0.5 g Na_2SO_3 in 500 mL distilled water. Standardize on the day of preparation, but wait at least 30 min to allow the rate of oxidation to slow. Determine molarity by titrating with standard 0.0125M potassium iodide-iodate titrant using starch indicator (see Section 4500- SO_3^{2-} .B). Calculate molarity of working standard as follows:

$$\text{Molarity of } SO_3^{2-} \text{ standard} = \frac{(A - B) \times M}{\text{mL sample}}$$

where:

A = titrant for sample, mL,

B = titrant for blank, mL,

M = molarity of potassium iodide-iodate titrant.

Because stock Na_2SO_3 solution is unstable, immediately after standardization, pipet 10 mL into a 500-mL volumetric flask partially filled with TCM and dilute to mark with TCM. Calculate the concentration of this dilute sulfite solution by multiplying the stock solution concentration by 0.02. This TCM-stabilized standard is stable for 30 d if stored at 5°C. Discard as soon as any precipitate is noticed at the bottom.

f. Standard potassium iodide-iodate titrant, 0.0125M: See Section 4500- SO_3^{2-} .B..

g. Hydrochloric acid, 1 + 1.

h. Octyl alcohol, reagent-grade.

i. Sulfamic acid, 10%: Dissolve 10 g NH_2SO_3H in 100 mL distilled water. This reagent can be kept for a few days if protected from air.

j. EDTA reagent: See Section 4500- SO_3^{2-} .B..

4. Procedure

a. Sample collection: Collect a fresh sample taking care to minimize contact with air. Fix cooled samples (<50°C) immediately by adding 1 mL EDTA solution for each 100 mL sample.

b. SO_2 evolution: Prepare the absorbing solution by adding 5 mL 1,10-phenanthroline solution, 0.5 mL ferric ammonium sulfate solution, 25 mL distilled water, and 5 drops octyl alcohol (to act as defoamer) to a 100-mL nessler tube; insert a gas dispersion tube. Add 1 mL

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sulfamic acid solution to the gas washing bottle and 100 mL of sample or a portion containing less than 100 $\mu\text{g SO}_3^{2-}$ diluted to 100 mL. Add 10 mL 1 + 1 HCl and immediately connect the gas washing bottle to the gas train as shown in Figure 4500-SO₃²⁻:1. Place a spring or rubber band on the gas washing bottle to keep the top securely closed during gas flow. Adjust nitrogen flow to 2.0 L/min and purge for 60 min.

c. Colorimetric measurement: After exactly 60 min, turn off nitrogen flow, disconnect nessler tube, and immediately add 1 mL ammonium bifluoride solution. Remove gas dispersion tube after rinsing it with distilled water into the tube and forcing the rinse water into the nessler tube with a rubber bulb. Dilute to 50 mL in the nessler tube and mix by rapidly moving the tube in a circular motion. Do not let rubber stoppers or PVC tubing come in contact with the absorbing solution. After at least 5 min from the time of adding ammonium bifluoride, read the absorbance versus distilled water at 510 nm using either a 5-cm cell for a range of 0 to 30 $\mu\text{g SO}_3^{2-}$ per portion or a 1-cm cell for a range of 0 to 100 $\mu\text{g SO}_3^{2-}$. Avoid transferring octyl alcohol into the cell by letting it rise to the surface of the absorbing solution and transferring the clear lower solution to the cell with a pipet. Make a calibration curve by analyzing a procedure blank and at least three standards. Run at least one standard with each set of samples. For maximum accuracy hold samples and standards at the same temperature and keep the time interval from start of purging to the addition of ammonium bifluoride constant. This is easier to do if several gas trains are used simultaneously in parallel. If ambient temperatures are subject to frequent fluctuation, a water bath may be used to control color development at a fixed temperature.

5. Calculation

$$\text{mg SO}_3^{2-}/\text{L} = \frac{\mu\text{g SO}_3^{2-} \text{ from calibration curve}}{\text{mL sample}}$$

6. Precision and Bias

Three laboratories analyzed five replicate portions of a standard sulfite solution and of secondary treated wastewater effluent to which sulfite was added. The data are summarized below. Individual analyst's precision ranged from 4.1 to 10.5% standard deviation ($N = 45$).

Sample	$\bar{x}$ mg/L	Standard Deviation, σ mg/L	Relative Standard Deviation %
Standard, 4.7 mg SO ₃ ²⁻ /L	3.7	0.78	21

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Sample	$\bar{x}$ mg/L	Standard Deviation, σ mg/L	Relative Standard Deviation %
Secondary effluent with 0.12 mg SO_3^{2-} /L	0.12	0.03	25
Secondary effluent with 4.0 mg SO_3^{2-} /L	3.7	0.30	8.0

7. References

1. STEPHENS, B.G. & F. LINDSTROM. 1964. Spectrophotometric determination of sulfur dioxide suitable for atmospheric analysis. *Anal. Chem.* 36:1308.
2. WEST, P. W. & G.C. GAEKE. 1956. Fixation of sulfur dioxide as sulfitomercurate and subsequent colorimetric determination. *Anal. Chem.* 28:1816.

4500-SO₄²⁻ SULFATE*#(89)

4500-SO₄²⁻ A. Introduction

1. Occurrence

Sulfate (SO_4^{2-}) is widely distributed in nature and may be present in natural waters in concentrations ranging from a few to several thousand milligrams per liter. Mine drainage wastes may contribute large amounts of SO_4^{2-} through pyrite oxidation. Sodium and magnesium sulfate exert a cathartic action.

2. Selection of Method

The ion chromatographic method (4110) and capillary ion electrophoresis (CIE—see Section 4140) are suitable for sulfate concentrations above 0.1 mg/L. The gravimetric methods (C and D) are suitable for SO_4^{2-} concentrations above 10 mg/L. The turbidimetric method (E) is applicable in the range of 1 to 40 mg SO_4^{2-} /L. The automated methylthymol blue methods (F and G) are the procedures for analyzing large numbers of samples for sulfate alone when the equipment is available; over 30 samples can be analyzed per hour. Methods C, D, F, G, 4110, or

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CIE (4140) are preferred for accurate results.

3. Sampling and Storage

In the presence of organic matter certain bacteria may reduce SO_4^{2-} to S^{2-} . To avoid this, store samples at 4°C.

4500-SO₄²⁻ B. (Reserved)

4500-SO₄²⁻ C. Gravimetric Method with Ignition of Residue

1. General Discussion

a. Principle: Sulfate is precipitated in a hydrochloric acid (HCl) solution as barium sulfate (BaSO_4) by the addition of barium chloride (BaCl_2).

The precipitation is carried out near the boiling temperature, and after a period of digestion the precipitate is filtered, washed with water until free of Cl^- , ignited or dried, and weighed as BaSO_4 .

b. Interference: The gravimetric determination of SO_4^{2-} is subject to many errors, both positive and negative. In potable waters where the mineral concentration is low, these may be of minor importance.

1) Interferences leading to high results—Suspended matter, silica, BaCl_2 precipitant, NO_3^- , SO_3^{2-} and occluded mother liquor in the precipitate are the principal factors in positive errors. Suspended matter may be present in both the sample and the precipitating solution; soluble silicate may be rendered insoluble and SO_3^{2-} may be oxidized to SO_4^{2-} during analysis. Barium nitrate [$\text{Ba}(\text{NO}_3)_2$], BaCl_2 , and water are occluded to some extent with the BaSO_4 although water is driven off if the temperature of ignition is sufficiently high.

2) Interferences leading to low results—Alkali metal sulfates frequently yield low results. This is true especially of alkali hydrogen sulfates. Occlusion of alkali sulfate with BaSO_4 causes substitution of an element of lower atomic weight than barium in the precipitate. Hydrogen sulfates of alkali metals act similarly and, in addition, decompose on being heated. Heavy metals, such as chromium and iron, cause low results by interfering with the complete precipitation of SO_4^{2-} and by formation of heavy metal sulfates. BaSO_4 has small but significant solubility, which is increased in the presence of acid. Although an acid medium is necessary to prevent precipitation of barium carbonate and phosphate, it is important to limit its concentration to minimize the solution effect.

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2. Apparatus

- a. *Steam bath.*
- b. *Drying oven*, equipped with thermostatic control.
- c. *Muffle furnace*, with temperature indicator.
- d. *Desiccator.*
- e. *Analytical balance*, capable of weighing to 0.1 mg.
- f. *Filter*: Use one of the following:
 - 1) *Filter paper*, acid-washed, ashless hard-finish, sufficiently retentive for fine precipitates.
 - 2) *Membrane filter*, with a pore size of about 0.45 μm .
- g. *Filtering apparatus*, appropriate to the type of filter selected. (Coat membrane filter holder with silicone fluid to prevent precipitate from adhering.)

3. Reagents

- a. *Methyl red indicator solution*: Dissolve 100 mg methyl red sodium salt in distilled water and dilute to 100 mL.
- b. *Hydrochloric acid*, HCl, 1 + 1.
- c. *Barium chloride solution*: Dissolve 100 g $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ in 1 L distilled water. Filter through a membrane filter or hard-finish filter paper before use; 1 mL is capable of precipitating approximately 40 mg SO_4^{2-} .
- d. *Silver nitrate-nitric acid reagent*: Dissolve 8.5 g AgNO_3 and 0.5 mL conc HNO_3 in 500 mL distilled water.
- e. *Silicone fluid*. *#(90)

4. Procedure

- a. *Removal of silica*: If the silica concentration exceeds 25 mg/L, evaporate sample nearly to dryness in a platinum dish on a steam bath. Add 1 mL HCl, tilt, and rotate dish until the acid comes in complete contact with the residue. Continue evaporation to dryness. Complete drying in an oven at 180°C and if organic matter is present, char over flame of a burner. Moisten residue with 2 mL distilled water and 1 mL HCl, and evaporate to dryness on a steam bath. Add 2 mL HCl, take up soluble residue in hot water, and filter. Wash insoluble silica with several small portions of hot distilled water. Combine filtrate and washings. Discard residue.
- b. *Precipitation of barium sulfate*: Adjust volume of clarified sample to contain approximately 50 mg SO_4^{2-} in a 250-mL volume. Lower concentrations of SO_4^{2-} may be tolerated if it is impracticable to concentrate sample to the optimum level, but in such cases limit total volume to 150 mL. Adjust pH with HCl to pH 4.5 to 5.0, using a pH meter or the orange color of methyl red indicator. Add 1 to 2 mL HCl. Heat to boiling and, while stirring gently,

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slowly add warm BaCl_2 solution until precipitation appears to be complete; then add about 2 mL in excess. If amount of precipitate is small, add a total of 5 mL BaCl_2 solution. Digest precipitate at 80 to 90°C, preferably overnight but for not less than 2 h.

c. Filtration and weighing: Mix a small amount of ashless filter paper pulp with the BaSO_4 , quantitatively transfer to a filter, and filter at room temperature. The pulp aids filtration and reduces the tendency of the precipitate to creep. Wash precipitate with small portions of warm distilled water until washings are free of Cl^- as indicated by testing with $\text{AgNO}_3\text{-HNO}_3$ reagent. Place filter and precipitate in a weighed platinum crucible and ignite at 800°C for 1 h. Do not let filter paper flame. Cool in desiccator and weigh.

5. Calculation

$$\text{mg SO}_4^{2-}/\text{L} = \frac{\text{mg BaSO}_4 \times 411.6}{\text{mL sample}}$$

6. Precision and Bias

A synthetic sample containing 259 mg $\text{SO}_4^{2-}/\text{L}$, 108 mg Ca^{2+}/L , 82 mg Mg^{2+}/L , 3.1 mg K^+/L , 19.9 mg Na^+/L , 241 mg Cl^-/L , 0.250 mg $\text{NO}_2^-/\text{N/L}$, 1.1 mg $\text{NO}_3^-/\text{N/L}$, and 42.5 mg total alkalinity/L (contributed by NaHCO_3) was analyzed in 32 laboratories by the gravimetric method, with a relative standard deviation of 4.7% and a relative error of 1.9%.

7. Bibliography

HILLEBRAND, W.F. et al. 1953. Applied Inorganic Analysis, 2nd ed. John Wiley & Sons, New York, N.Y.

KOLTHOFF, I.M., E.J. MEEHAN, E.B. SANDELL & S. BRUCKENSTEIN. 1969. Quantitative Chemical Analysis, 4th ed. Macmillan Co., New York, N.Y.

4500-SO₄²⁻ D. Gravimetric Method with Drying of Residue

1. General Discussion

See Method C, preceding.

2. Apparatus

With the exception of the filter paper, all of the apparatus cited in Section 4500-SO₄²⁻.C. is required, plus the following:

a. Filters: Use one of the following:

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1) *Fritted-glass filter*, fine (“F”) porosity, with a maximum pore size of 5 µm.

2) *Membrane filter*, with a pore size of about 0.45 µm.

b. *Vacuum oven*.

3. Reagents

All the reagents listed in Section 4500-SO₄²⁻.C. are required.

4. Procedure

a. *Removal of interference*: See Section 4500-SO₄²⁻.C..

b. *Precipitation of barium sulfate*: See Section 4500-SO₄²⁻.C..

c. *Preparation of filters*:

1) Fritted glass filter—Dry to constant weight in an oven maintained at 105°C or higher, cool in desiccator, and weigh.

2) Membrane filter—Place filter on a piece of filter paper or a watch glass and dry to constant weight*#(91) in a vacuum oven at 80°C, while maintaining a vacuum of at least 85 kPa or in a conventional oven at a temperature of 103 to 105°C. Cool in desiccator and weigh membrane only.

d. *Filtration and weighing*: Filter BaSO₄ at room temperature. Wash precipitate with several small portions of warm distilled water until washings are free of Cl⁻, as indicated by testing with AgNO₃-HNO₃ reagent. If a membrane filter is used add a few drops of silicone fluid to the suspension before filtering, to prevent adherence of precipitate to holder. Dry filter and precipitate by the same procedure used in preparing filter. Cool in a desiccator and weigh.

5. Calculation

$$\text{mg SO}_4^{2-}/\text{L} = \frac{\text{mg BaSO}_4 \times 411.6}{\text{mL sample}}$$

6. Bibliography

See Section 4500-SO₄²⁻.C.

4500-SO₄²⁻ E. Turbidimetric Method

1. General Discussion

a. *Principle*: Sulfate ion (SO₄²⁻) is precipitated in an acetic acid medium with barium

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chloride (BaCl_2) so as to form barium sulfate (BaSO_4) crystals of uniform size. Light absorbance of the BaSO_4 suspension is measured by a photometer and the SO_4^{2-} concentration is determined by comparison of the reading with a standard curve.

b. Interference: Color or suspended matter in large amounts will interfere. Some suspended matter may be removed by filtration. If both are small in comparison with the SO_4^{2-} concentration, correct for interference as indicated in ¶ 4d below. Silica in excess of 500 mg/L will interfere, and in waters containing large quantities of organic material it may not be possible to precipitate BaSO_4 satisfactorily.

In potable waters there are no ions other than SO_4^{2-} that will form insoluble compounds with barium under strongly acid conditions. Make determination at room temperature; variation over a range of 10°C will not cause appreciable error.

c. Minimum detectable concentration: Approximately 1 mg SO_4^{2-} /L.

2. Apparatus

a. Magnetic stirrer: Use a constant stirring speed. It is convenient to incorporate a fixed resistance in series with the motor operating the magnetic stirrer to regulate stirring speed. Use magnets of identical shape and size. The exact speed of stirring is not critical, but keep it constant for each run of samples and standards and adjust it to prevent splashing.

b. Photometer: One of the following is required, with preference in the order given:

1) *Nephelometer.*

2) *Spectrophotometer*, for use at 420 nm, providing a light path of 2.5 to 10 cm.

3) *Filter photometer*, equipped with a violet filter having maximum transmittance near 420 nm and providing a light path of 2.5 to 10 cm.

c. Stopwatch or electric timer.

d. Measuring spoon, capacity 0.2 to 0.3 mL.

3. Reagents

a. Buffer solution A: Dissolve 30 g magnesium chloride, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5 g sodium acetate, $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$, 1.0 g potassium nitrate, KNO_3 , and 20 mL acetic acid, CH_3COOH (99%), in 500 mL distilled water and make up to 1000 mL.

b. Buffer solution B (required when the sample SO_4^{2-} concentration is less than 10 mg/L): Dissolve 30 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5 g $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$, 1.0 g KNO_3 , 0.111 g sodium sulfate, Na_2SO_4 , and 20 mL acetic acid (99%) in 500 mL distilled water and make up to 1000 mL.

c. Barium chloride, BaCl_2 , crystals, 20 to 30 mesh. In standardization, uniform turbidity is produced with this mesh range and the appropriate buffer.

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d. Standard sulfate solution: Prepare a standard sulfate solution as described in 1) or 2) below; 1.00 mL = 100 $\mu\text{g SO}_4^{2-}$.

1) Dilute 10.4 mL standard 0.0200N H_2SO_4 titrant specified in Alkalinity, Section 2320B.3c, to 100 mL with distilled water.

2) Dissolve 0.1479 g anhydrous Na_2SO_4 in distilled water and dilute to 1000 mL.

4. Procedure

a. Formation of barium sulfate turbidity: Measure 100 mL sample, or a suitable portion made up to 100 mL, into a 250-mL erlenmeyer flask. Add 20 mL buffer solution and mix in stirring apparatus. While stirring, add a spoonful of BaCl_2 crystals and begin timing immediately. Stir for 60 ± 2 s at constant speed.

b. Measurement of barium sulfate turbidity: After stirring period has ended, pour solution into absorption cell of photometer and measure turbidity at 5 ± 0.5 min.

c. Preparation of calibration curve: Estimate SO_4^{2-} concentration in sample by comparing turbidity reading with a calibration curve prepared by carrying SO_4^{2-} standards through the entire procedure. Space standards at 5-mg/L increments in the 0- to 40-mg/L SO_4^{2-} range. Above 40 mg/L accuracy decreases and BaSO_4 suspensions lose stability. Check reliability of calibration curve by running a standard with every three or four samples.

d. Correction for sample color and turbidity: Correct for sample color and turbidity by running blanks to which BaCl_2 is not added.

5. Calculation

$$\text{mg SO}_4^{2-}/\text{L} = \frac{\text{mg SO}_4^{2-} \times 1000}{\text{mL sample}}$$

If buffer solution A was used, determine SO_4^{2-} concentration directly from the calibration curve after subtracting sample absorbance before adding BaCl_2 . If buffer solution B was used subtract SO_4^{2-} concentration of blank from apparent SO_4^{2-} concentration as determined above; because the calibration curve is not a straight line, this is not equivalent to subtracting blank absorbance from sample absorbance.

6. Precision and Bias

With a turbidimeter, #92 in a single laboratory with a sample having a mean of 7.45 mg $\text{SO}_4^{2-}/\text{L}$, a standard deviation of 0.13 mg/L and a coefficient of variation of 1.7% were obtained.

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Two samples dosed with sulfate gave recoveries of 85 and 91%.

7. Bibliography

SHEEN, R.T., H.L. KAHLER & E.M. ROSS. 1935. Turbidimetric determination of sulfate in water.

Ind. Eng. Chem., Anal. Ed. 7:262.

THOMAS, J.F. & J.E. COTTON. 1954. A turbidimetric sulfate determination. *Water Sewage Works* 101:462.

ROSSUM, J.R. & P. VILLARRUZ. 1961. Suggested methods for turbidimetric determination of sulfate in water. *J. Amer. Water Works Assoc.* 53: 873.

4500-SO₄²⁻ F. Automated Methylthymol Blue Method

1. General Discussion

a. Principle: Barium sulfate is formed by the reaction of the SO₄²⁻ with barium chloride (BaCl₂) at a low pH. At high pH excess barium reacts with methylthymol blue to produce a blue chelate. The uncomplexed methylthymol blue is gray. The amount of gray uncomplexed methylthymol blue indicates the concentration of SO₄²⁻.

b. Interferences: Because many cations interfere, use an ion-exchange column to remove interferences.

Molybdenum, often used to treat cooling waters, has been shown to cause a strong positive bias with this method, even with as little as 1 mg Mo/L.

c. Application: This method is applicable to potable, ground, surface, and saline waters as well as domestic and industrial wastewaters over a range from about 10 to 300 mg SO₄²⁻/L.

2. Apparatus

a. Automated analytical equipment: An example of the required continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-SO₄²⁻:1.

b. Ion-exchange column: Fill a piece of 2-mm-ID glass tubing about 20 cm long with the ion-exchange resin.*#(93) To simplify filling column put resin in distilled water and aspirate it into the tubing, which contains a glass-wool plug. After filling, plug other end of tube with glass wool. Avoid trapped air in the column.

3. Reagents

a. Barium chloride solution: Dissolve 1.526 g BaCl₂·2H₂O in 500 mL distilled water and dilute to 1 L. Store in a polyethylene bottle.

b. Methylthymol blue reagent: Dissolve 118.2 mg methylthymol blue†#(94) in 25 mL BaCl₂ solution. Add 4 mL 1N HCl and 71 mL distilled water and dilute to 500 mL with 95% ethanol.

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Store in a brown glass bottle. Prepare fresh daily.

c. *Buffer solution*, pH 10.1: Dissolve 6.75 g NH_4Cl in 500 mL distilled water. Add 57 mL conc NH_4OH and dilute to 1 L with distilled water. Adjust pH to 10.1 and store in a polyethylene bottle. Prepare fresh monthly.

d. *EDTA reagent*: Dissolve 40 g tetrasodium ethylenediaminetetraacetate in 500 mL pH 10.1 buffer solution. Dilute to 1 L with pH 10.1 buffer solution and store in a polyethylene bottle.

e. *Sodium hydroxide solution*, 0.36N: Dissolve 7.2 g NaOH in 250 mL distilled water. Cool and make up to 500 mL with distilled water.

f. *Stock sulfate solution*: Dissolve 1.479 g anhydrous Na_2SO_4 in 500 mL distilled water and dilute to 1000 mL; 1.00 mL = 1.00 mg SO_4^{2-} .

g. *Standard sulfate solutions*: Prepare in appropriate concentrations from 10 to 300 mg SO_4^{2-} /L, using the stock sulfate solution.

4. Procedure

Set up the manifold as shown in Figure 4500- SO_4^{2-} :1 and follow the general procedure described by the manufacturer.

After use, rinse methylthymol blue and NaOH reagent lines in water for a few minutes, rinse them in the EDTA solution for 10 min, and then rinse in distilled water.

5. Calculation

Prepare standard curves by plotting peak heights of standards processed through the manifold against SO_4^{2-} concentrations in standards. Compute sample SO_4^{2-} concentration by comparing sample peak height with standard curve.

6. Precision and Bias

In a single laboratory a sample with an average concentration of about 28 mg SO_4^{2-} /L had a standard deviation of 0.68 mg/L and a coefficient of variation of 2.4%. In two samples with added SO_4^{2-} , recoveries were 91% and 100%.

7. Bibliography

- LAZRUS, A.L., K.C. HILL & J.P. LODGE. 1965. A new colorimetric microdetermination of sulfate ion. *In Automation in Analytical Chemistry*. Technicon Symposium.
- COLOROS, E., M. R. PANESAR & F.P. PERRY. 1976. Linearizing the calibration curve in determination of sulfate by the methylthymol blue method. *Anal. Chem.* 48:1693.

4500- SO_4^{2-} G. Methylthymol Blue Flow Injection Analysis (PROPOSED)

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1. General Discussion

a. *Principle:* At pH 13.0 barium forms a blue complex with methylthymol blue (MTB). This gives a dark blue base line. The sample is injected into a low, but known, concentration of sulfate. The sulfate from the sample then reacts with the ethanolic barium-MTB solution and displaces the MTB from the barium to give barium sulfate and uncomplexed MTB. Uncomplexed MTB has a grayish color. The pH is raised with NaOH and the color of the gray uncomplexed MTB is measured at 460 nm. The intensity of gray color is proportional to the sulfate concentration.

Also see Section 4500-SO₄²⁻.A and Section 4500-SO₄²⁻.F, and Section 4130, Flow Injection Analysis (FIA).

b. *Interferences:* Remove large or fibrous particulates by filtering sample through glass wool. Guard against nitrate and nitrite contamination from reagents, water, glassware, and the sample preservation process.

A cation-exchange column removes multivalent cations such as Ca²⁺ and Mg²⁺. A midrange sulfate standard containing a typical level of hardness as CaCO₃ can be run periodically to check the performance of the column. Any decrease in peak height from that of a sulfate standard without added CaCO₃ indicates the need to regenerate or replace the resin.

Neutralize samples that have pH less than 2. High acid concentrations can displace multivalent cations from the column.

Orthophosphate forms a precipitate with barium at high pH. If samples are known to be high in orthophosphate, make a recovery study using added amounts of sulfate, or run a sample blank containing only the orthophosphate matrix.

Also see Section 4500-SO₄²⁻.F..

2. Apparatus

Flow injection analysis equipment consisting of:

a. *FIA injection valve* with sample loop or equivalent.

b. *Multichannel proportioning pump.*

c. *FIA manifold* (Figure 4500-SO₄²⁻:2) with cation-exchange column and flow cell. Relative flow rates only are shown in Figure 4500-SO₄²⁻:2. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

d. *Absorbance detector*, 460 nm, 10-nm bandpass.

e. *Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas

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carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. *Carrier solution*, 0.30 mg SO_4^{2-} /L: To a tared 1-L container, add 0.30 g 1000 mg/L stock sulfate standard (¶ 3h) and 999.7 g water. Shake or stir to mix. Degas with helium.

b. *Barium chloride solution*, 6.24mM: To a tared 1-L container, add 1.526 g barium chloride dihydrate, $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$, and 995 g water. Shake or stir until dissolved. Degas with helium.

c. *Hydrochloric acid*, HCl, 1.0M: To a tared 1-L container, add 913 g water and 99.6 g conc HCl (specific gravity 1.20, 37%). CAUTION: *Fumes*. Shake or stir to mix well. Degas with helium.

d. *Barium—MTB color reagent*: NOTE: The purity of the methylthymol blue and the denaturants in the alcohol are critical. Use the sources specified below, or test the material from alternative sources for suitability before using.

To a tared 500-mL dry brown plastic bottle, place 0.236 g methylthymol blue, 3,3'-bis[*N,N*-di(carboxymethyl)amino-methyl]-thymol-sulfonephthalein, pentasodium salt. Add 50 g barium chloride solution (¶ 3b), which may be used to aid in transfer of the dye. Swirl to dissolve. Add 4.0 g of 1.0M HCl (¶ 3c) and mix. The solution should turn orange. Add 71 g water and 321 g ethanol (ethyl alcohol, specially denatured anhydrous alcohol*#(95)). Stir or shake to mix well. The pH should be 2.5. Prepare solution the day before use and store, refrigerated, in a brown plastic bottle. Let warm to room temperature before using, then degas with helium.

e. *Stock sodium hydroxide solution*, NaOH, 50% (w/v) solution: To a glass 1-L container add 500 g water and 500.0 g NaOH. Dilute to 1 L. CAUTION: *The solution becomes very hot*. Shake or stir until dissolved. Cool to ambient. Store in a plastic bottle.

f. *Working sodium hydroxide solution*, NaOH, 0.18M: To a tared plastic 1-L container add 982 g water and 19.8 g stock NaOH solution (¶ 3e). Shake or stir to mix. Degas with helium.

g. *Cation exchange column preparation*: Prepare approximately 0.5 g ion exchange resin,†#(96) 50 to 100 mesh, by mixing with sufficient water to make a slurry. Remove one end fitting from the threaded glass column. Fill column with water and aspirate slurry into column or let it settle into column by gravity. Take care to avoid trapping air bubbles in column or fittings at this point and during all subsequent operations. When resin has settled, replace end fitting. To ensure a good seal, remove any resin particles from the threads of glass, column end, and end fitting. To store column, join ends of the TFE tubing.

To test column effectiveness, make up two midrange standards, one of only sodium sulfate and the other with an identical concentration of sodium sulfate but with hardness typical of the samples. If the column is depleted, the standard with hardness will give a lower response because the divalent Mg^{2+} and Ca^{2+} cations are complexing with the free MTB. If depletion has occurred, repack column with fresh resin.

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h. Stock sulfate standard, 1000 mg SO₄²⁻/L: Dry approximately 2 g sodium sulfate, Na₂SO₄, at 105° overnight. Cool in a desiccator. In a 1-L volumetric flask, add 1.479 g of dried sodium sulfate to about 800 mL water. Dissolve by swirling, dilute to mark, and mix by inversion.

i. Standard solutions: Prepare sulfate standards in desired concentration range, using the stock standard (§ 3h), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-SO₄²⁻:2 and follow method supplied by manufacturer or laboratory standard operating procedure. Follow quality control protocols outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting adsorbance of standards processed through the manifold versus sulfate concentration.

6. Precision and Bias

a. Recovery and relative standard deviation: Table 4500-SO₄²⁻:I gives results of single-laboratory studies.

b. MDL: A 180-μL sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 5.00-mg SO₄²⁻/L standard. These gave a mean of 4.80 mg SO₄²⁻/L, a standard deviation of 0.69 mg SO₄²⁻/L, and MDL of 1.8 mg SO₄²⁻/L.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

Figures

Figure 4110:1. Typical inorganic anion separation. Eluent: 1.7 mM NaHCO₃, 1.8 mM Na₂CO₃; sample loop: 50 μL; flow: 2.0 mL/min; column: Dionex AG4A plus AS4A.

Anion	Time	Conc mg/L
Fluoride	1.15	2
Chloride	1.72	20

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Anion	Time	Conc mg/L
Nitrite	2.03	10
Bromide	2.93	5
Nitrate	3.20	10
Orthophosphate	5.10	10
Sulfate	6.82	20

Figure 4110:2. Typical inorganic anion separation. Eluent: borate/gluconate; flow rate: 1.5 mL/min; injection volume: 100 μ L.

Anion	Time min	Conc mg/L
1. System—peak	—	—
2. Cl ⁻	2.3	40
3. NO ₂ ⁻	3.1	10
4. Br ⁻	4.2	40
5. NO ₃ ⁻	5.3	20
6. PO ₄ ³⁻	5.7	40
7. SO ₄ ²⁻	8.3	80

Figure 4120:1 Schematic of a segmented flow analyzer.

Figure 4140:1. Electropherogram of the inorganic anions and typically found organic acids using capillary ion electrophoresis and chromate electrolyte. Electrolyte: 4.7 mM Na₂CrO₄/4.0 mM TTAOH/10 mM CHES/0.1 mM calcium gluconate; capillary: 75- μ m-ID $\times$ 375- μ m-OD $\times$ 60-cm length, uncoated silica; voltage: 15 kV using a negative power supply; current: 14 $\pm$ 1 μ A; sampling: hydrostatic at 10 cm for 30 s; detection: indirect UV with Hg lamp and 254-nm filter.

Anion	Conc mg/L	Migration Time min	Migration Time Ratio to Cl	Peak Area	Time-Cor Peak Area
Chloride	2.0	3.200	1.000	1204	376.04
Bromide	4.0	3.296	1.030	1147	348.05

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Anion	Conc mg/L	Migration Time min	Migration Time Ratio to Cl	Peak Area	Time-Cor Peak Area
Nitrite	4.0	3.343	1.045	2012	601.72
Sulfate	4.0	3.465	1.083	1948	562.05
Nitrate	4.0	3.583	1.120	1805	503.69
Oxalate	5.0	3.684	1.151	3102	842.14
Fluoride	1.0	2.823	1.195	1708	446.65
Formate	5.0	3.873	1.210	1420	366.61
o-Phosphate	4.0	4.004	1.251	2924	730.25
Carbonate & bicarbonate		4.281	1.338		
Acetate	5.0	4.560	1.425	3958	868.01

Figure 4140:2. Electropherogram of 0.1 mg/L inorganic anions at minimum detection level. Seven replicates of 0.1 mg/L inorganic anion standard used to calculate minimum detection levels, as mg/L, using analytical protocol described in Section 1030E.

Chloride = 0.046
 Nitrate = 0.084
 Bromide = 0.090
 Fluoride = 0.020
 Nitrite = 0.072
 Phosphate = 0.041
 Sulfate = 0.032

Figure 4140:3. Representative electropherograms of Youden anion standards. For composition of standards, see Table 4140:I.

Figure 4140:4. Linearity calibration curve for chloride, bromide, and sulfate. Three data points were used per concentration; based on Youden pair design.

Figure 4140:5. Linearity calibration curve for fluoride and o-phosphate. Three data points were used per concentration; based on Youden pair design.

Figure 4140:6. Linearity calibration curve for nitrite and nitrate. Three data points were used per concentration; based on Youden pair design.

Figure 4140:7. Electropherogram of typical drinking water.

Chloride = 24.72 mg/L
 Sulfate = 7.99 mg/L
 Nitrate = 0.36 mg/L
 Fluoride < 0.10 mg/L
 Carbonate & bicarbonate = natural

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Figure 4140:8. Electropherogram of typical municipal wastewater discharge, undiluted.

Chloride = 93.3 mg/L
Nitrate = 0.46 mg/L
Sulfate = 60.3 mg/L
Nitrate < 40.8 mg/L
Carbonate & bicarbonate = natural

Figure 4140:9. Electropherogram of typical industrial wastewater discharge, undiluted.

Chloride = 2.0 mg/L
Nitrite = 1.6 mg/L
Sulfate = 34.7 mg/L
Nitrate = 16.5 mg/L
Formate < 0.05 mg/L
Phosphate = 12.3 mg/L
Carbonate & bicarbonate = natural

Figure 4500-Br⁻:1. FIA bromide manifold.

Figure 4500-CO₂:1. Nomograph for evaluation of hydroxide ion concentration. To use:

Align temperature (Scale 1) and total dissolved solids (Scale 5); pivot on Line 2 to proper pH (Scale 3); read hydroxide ion concentration, as mg CaCO₃/L, on Scale 4. (Example: For 13°C temperature, 240 mg total dissolved solids/L, pH 9.8, the hydroxide ion concentration is found to be 1.4 mg as CaCO₃/L.)

Figure 4500-CO₂:2. Nomograph for evaluation of bicarbonate alkalinity. To use: Align temperature (Scale 1) and total dissolved solids (Scale 3); pivot on Line 2 to proper pH (Scale 4) and read constant on Scale 5; locate constant on Scale 6 and align with nonhydroxide alkalinity (found with aid of Figure 4500-CO₂:1) on Scale 7; read bicarbonate alkalinity on Scale 8. (Example: For 13°C temperature, 240 mg total dissolved solids/L, pH 9.8, and 140 mg alkalinity/L, the bicarbonate content is found to be 90 mg as CaCO₃/L.)

Figure 4500-CO₂:3. Nomograph for evaluation of carbonate alkalinity. To use: Align temperature (Scale 1) and total dissolved solids (Scale 3); pivot on Line 2 to proper pH (Scale 4) and read constant on Scale 5; locate constant on Scale 6 and align with nonhydroxide alkalinity (found with aid of Figure 4500-CO₂:1) on Scale 7; read carbonate alkalinity on Scale 8. (Example: For 13°C temperature, 240 mg total dissolved solids/L, pH 9.8, and 140 mg alkalinity/L, the carbonate content is found to be 50 mg as CaCO₃/L.)

Figure 4500-CO₂:4. Nomograph for evaluation of free carbon dioxide content. To use:

Align temperature (Scale 1) and total dissolved solids (Scale 3), which determines Point P₁ on Line 2; align pH (Scale 4) and bicarbonate alkalinity (Scale 7), which determines Point P₂ on Line 6; align P₁ and P₂ and read free carbon dioxide on Scale 5. (Example: For 13°C temperature, 560 mg total dissolved solids/L, pH 7.4, and 320 mg alkalinity/L, the free

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carbon dioxide content is found to be 28 mg/L.)

Figure 4500-CN⁻:1. Cyanide distillation apparatus.

Figure 4500-CN⁻:2. FIA cyanide manifold.

Figure 4500-CN⁻:3. FIA in-line total and WAD cyanide manifold.

Figure 4500-Cl⁻:1. Example of differential titration curve (end point is 25.5 mL).

Figure 4500-Cl⁻:2. Flow scheme for automated chloride analysis.

Figure 4500-Cl⁻:3. FIA chloride manifold.

Figure 4500-ClO₂:1. Chlorine dioxide generation and absorption system.

Figure 4500-F⁻:1. Direct distillation apparatus for fluoride.

Figure 4500-F⁻:2. Fluoride manifold.

Figure 4500-F⁻:3. FIA fluoride manifold.

Figure 4500-H⁺:1. Electrode potential vs. pH. Intercept control shifts response curve laterally.

Figure 4500-H⁺:2. Typical pH electrode response as a function of temperature.

Figure 4500-N:1. FIA in-line total nitrogen manifold.

Figure 4500-NH₃:1. Ammonia manifold.

Figure 4500-NH₃:2. FIA ammonia manifold.

Figure 4500-NO₃⁻:1. Reduction column.

Figure 4500-NO₃⁻:2. Nitrate-nitrite manifold.

Figure 4500-NO₃⁻:3. Nitrate-nitrite manifold.

Figure 4500-NO₃⁻:4. FIA nitrate + nitrite manifold.

Figure 4500-N_{org}:1. Micro-kjeldahl distillation apparatus.

Figure 4500-N_{org}:2. FIA total kjeldahl nitrogen manifold.

Figure 4500-O:1. DO and BOD sampler assembly.

Figure 4500-O:2. Effect of temperature on electrode sensitivity.

Figure 4500-O:3. The salting-out effect at different temperatures.

Figure 4500-O:4. Typical trend of effect of stirring on electrode response.

Figure 4500-P:1. Steps for analysis of phosphate fractions.

* Direct determination of phosphorus on the membrane filter containing suspended matter will be required where greater precision than that obtained by difference is desired. Digest filter with HNO₃ and follow by perchloric acid. Then perform colorimetry.

† Total phosphorus measurements on highly saline samples may be difficult because of

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precipitation of large quantities of salt as a result of digestion techniques that drastically reduce sample volume. For total phosphorus analyses on such samples, directly determine total dissolved phosphorus and total suspended phosphorus and add the results.

‡ In determination of total dissolved or total suspended reactive phosphorus, anomalous results may be obtained on samples containing large amounts of suspended sediments. Very often results depend largely on the degree of agitation and mixing to which samples are subjected during analysis because of a time-dependent desorption of orthophosphate from the suspended particles.

Figure 4500-P:2. Phosphate manifold for automated analytical system.

Figure 4500-P:3. FIA orthophosphate manifold.

Figure 4500-P:4. FIA total phosphorus manifold.

Figure 4500-P:5. FIA in-line total phosphorus manifold.

Figure 4500-P:6. Correlation between manual and in-line total phosphorus methods.

Figure 4500-SiO₂:1. Silica manifold.

Figure 4500-SiO₂:2. FIA manifold.

Figure 4500-S²⁻:1. Analytical flow paths for sulfide determinations.

Figure 4500-S²⁻:2. Sulfide manifold.

Figure 4500-S²⁻:3. Proportions of H₂S and HS⁻ in dissolved sulfide.

Figure 4500-S²⁻:4. FIA sulfide manifold.

Figure 4500-SO₃²⁻:1. Apparatus for evolution of SO₂ from samples for colorimetric analysis.

Figure 4500-SO₄²⁻:1. Sulfate manifold.

Figure 4500-SO₄²⁻:2. FIA manifold.

Tables

TABLE 4110:I. DETERMINATION OF BIAS FOR FLUORIDE

	Amount Added mg/L	Amount Found mg/L	S _t	S ₀	Bias %
Water					

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Water	Amount Added mg/L	Amount Found mg/L	S_t	S₀	Bias %
Reagent	0.26	0.25	0.08	0.11	-3.8
	0.34	0.29	0.11		-14.7
	2.12	2.12	0.07	0.12	0.0
	2.55	2.48	0.14		-2.7
	6.79	6.76	0.20	0.19	-0.4
	8.49	8.46	0.30		-0.4
Drinking	0.26	0.24	0.08	0.05	-7.7
	0.34	0.34	0.11		0.0
	2.12	2.09	0.18	0.06	-1.4
	2.55	2.55	0.16		0.0
	6.79	6.84	0.54	0.25	+0.7
	8.49	8.37	0.75		-1.4
Waste	0.26	0.25	0.15	0.06	-3.8
	0.34	0.32	0.08		-5.9
	2.12	2.13	0.22	0.15	+0.5
	2.55	2.48	0.16		-2.7
	6.79	6.65	0.41	0.20	-2.1
	8.49	8.27	0.36		-2.6

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4110:II. DETERMINATION OF BIAS FOR CHLORIDE

Water	Amount Added mg/L	Amount Found mg/L	S_t	S₀	Bias %
Reagent	0.78	0.79	0.17	0.29	+1.3
	1.04	1.12	0.46		+7.7
	6.50	6.31	0.27	0.14	-2.9
	7.80	7.76	0.39		-0.5
	20.8	20.7	0.54	0.62	-0.5
	26.0	25.9	0.58		-0.4

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Water	Amount Added mg/L	Amount Found mg/L	S_t	S₀	Bias %
Drinking	0.78	0.54	0.35	0.20	-30.8
	1.04	0.51	0.38		-51.0
	6.50	5.24	1.35	1.48	-19.4
	7.80	6.02	1.90		-22.8
	20.8	20.0	2.26	1.14	-3.8
	26.0	24.0	2.65		-7.7
Waste	0.78	0.43	0.32	0.39	-44.9
	1.04	0.65	0.48		-37.5
	6.50	4.59	1.82	0.83	-29.4
	7.80	5.45	2.02		-30.1
	20.8	18.3	2.41	1.57	-11.8
	26.0	23.0	2.50		-11.5

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4110:III. DETERMINATION OF BIAS FOR NITRITE NITROGEN

Water	Amount Added mg/L	Amount Found mg/L	S_t	S₀	Bias %
Reagent	0.36	0.37	0.04	0.04	+2.8
	0.48	0.48	0.06		0.0
	3.00	3.18	0.12	0.06	+6.0
	3.60	3.83	0.12		+6.4
	9.60	9.84	0.36	0.26	+2.5
	12.0	12.1	0.27		+0.6
Drinking	0.36	0.30	0.13	0.03	-16.7
	0.48	0.40	0.14		-16.7
	3.00	3.02	0.23	0.12	+0.7
	3.60	3.62	0.22		+0.6
	9.60	9.59	0.44	0.28	-0.1
	12.0	11.6	0.59		-3.1

Standard Methods for the Examination of Water and Wastewater

Water	Amount Added mg/L	Amount Found mg/L	S_t	S₀	Bias %
Waste	0.36	0.34	0.06	0.04	-5.6
	0.48	0.46	0.07		-4.2
	3.00	3.18	0.13	0.10	+6.0
	3.60	3.76	0.18		+4.4
	9.60	9.74	0.49	0.26	+1.5
	12.0	12.0	0.56		+0.3

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4110:IV. DETERMINATION OF BIAS FOR BROMIDE

Water	Amount Added mg/L	Amount Found mg/L	S_t	S₀	Bias %
Water	0.63	0.69	0.11	0.05	+9.5
	0.84	0.85	0.12		+1.2
	5.24	5.21	0.22	0.21	-0.6
	6.29	6.17	0.35		-1.9
	16.8	17.1	0.70	0.36	+1.6
	21.0	21.3	0.93		+1.5
Drinking	0.63	0.63	0.13	0.04	0.0
	0.84	0.81	0.13		-3.6
	5.24	5.11	0.23	0.13	-2.5
	6.29	6.18	0.30		-1.7
	16.8	17.0	0.55	0.57	+0.9
	21.0	20.9	0.65		-0.4
Waste	0.63	0.63	0.15	0.09	0.0
	0.84	0.85	0.15		+1.2
	5.24	5.23	0.36	0.11	-0.2
	6.29	6.27	0.46		-0.3
	16.8	16.6	0.69	0.43	-1.0
	21.0	21.1	0.63		+0.3

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

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TABLE 4110:V. DETERMINATION OF BIAS FOR NITRATE NITROGEN

Water	Amount Added mg/L	Amount Found mg/L	S_t	S₀	Bias %
Reagent	0.42	0.42	0.04	0.02	0.0
	0.56	0.56	0.06		0.0
	3.51	3.34	0.15	0.08	-4.8
	4.21	4.05	0.28		-3.8
	11.2	11.1	0.47	0.34	-1.1
	14.0	14.4	0.61		+2.6
Drinking	0.42	0.46	0.08	0.03	+9.5
	0.56	0.58	0.09		+3.6
	3.51	3.45	0.27	0.10	-1.7
	4.21	4.21	0.38		0.0
	11.2	11.5	0.50	0.48	+2.3
	14.0	14.2	0.70		+1.6
Waste	0.42	0.36	0.07	0.06	-14.6
	0.56	0.40	0.16		-28.6
	3.51	3.19	0.31	0.07	-9.1
	4.21	3.84	0.28		-8.8
	11.2	10.9	0.35	0.51	-3.0
	14.0	14.1	0.74		+0.4

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4110:VI. DETERMINATION OF BIAS FOR ORTHOPHOSPHATE

Water	Amount Added mg/L	Amount Found mg/L	S_t	S₀	Bias %
Reagent	0.69	0.69	0.06	0.06	0.0
	0.92	0.98	0.15		+6.5
	5.77	5.72	0.36	0.18	-0.9
	6.92	6.78	0.42		-2.0
	18.4	18.8	1.04	0.63	+2.1
	23.1	23.2	0.35		+0.4

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Water	Amount Added mg/L	Amount Found mg/L	S_t	S₀	Bias %
Drinking	0.69	0.70	0.17	0.17	+1.4
	0.92	0.96	0.20		+4.3
	5.77	5.43	0.52	0.40	-5.9
	6.92	6.29	0.72		-9.1
	18.4	18.0	0.68	0.59	-2.2
	23.1	22.6	1.07		-2.0
Waste	0.68	0.64	0.26	0.09	-7.2
	0.92	0.82	0.28		-10.9
	5.77	5.18	0.66	0.34	-10.2
	6.92	6.24	0.74		-9.8
	18.4	17.6	2.08	1.27	-4.1
	23.1	22.4	0.87		-3.0

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4110:VII. DETERMINATION OF BIAS FOR SULFATE

Water	Amount Added mg/L	Amount Found mg/L	S_t	S₀	Bias %
Reagent	2.85	2.83	0.32	0.52	-0.7
	3.80	3.83	0.92		+0.8
	23.8	24.0	1.67	0.68	+0.8
	28.5	28.5	1.56		-0.1
	76.0	76.8	3.42	2.33	+1.1
	95.0	95.7	3.59		+0.7
Drinking	2.85	1.12	0.37	0.41	-60.7
	3.80	2.26	0.97		-40.3
	23.8	21.8	1.26	0.51	-8.4
	28.5	25.9	2.48		-9.1
	76.0	74.5	4.63	2.70	-2.0
	95.0	92.3	5.19		-2.8

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Water	Amount Added mg/L	Amount Found mg/L	S _t	S ₀	Bias %
Waste	2.85	1.89	0.37	0.24	-33.7
	3.80	2.10	1.25		-44.7
	23.8	20.3	3.19	0.58	-14.7
	28.5	24.5	3.24		-14.0
	76.0	71.4	5.65	3.39	-6.1
	95.0	90.3	6.80		-5.0

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4110:VIII. DETECTION LIMITS FOR ANIONS IN REAGENT WATER*

Anion	Retention Time min	MDL† ¹ mg/L
Cl ⁻	2.3	0.035
NO ₂ ⁻	3.1	0.022
Br ⁻	4.2	0.110
NO ₃ ⁻	5.3	0.035
PO ₄ ³⁻	5.7	0.110
SO ₄ ²⁻	8.3	0.350

* Standard conditions as defined in text.

† MDL calculated from the peak height in mm taken from chart recorder.

TABLE 4110:IX. SINGLE-COLUMN CHROMATOGRAPHY SINGLE-OPERATOR PRECISION AND BIAS*

Anion	Sample Type†	Amount Added mg/L	Mean Recovery %	SD mg/L	Anion	Sample Type†	Amount Added mg/L	Mean Recovery %
Cl ⁻	RW	16	105	1.6	NO ₃ +—	RW	8	103
	DW	16	98	1.9		DW	8	87
NO ₂ +—	RW	4	101	0.10	PO ₄ +3—	RW	16	113
	DW	4	101	0.43		DW	16	110
Br ⁻	RW	16	104	0.75	SO ₄ +2—	RW	32	101
	DW	16	98	2.3		DW	32	94

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*Data provided by EPA/EMSL, Cincinnati, Ohio 95268. Seven replicates were analyzed for each anion and sample type.

†RW = reagent water; DW = drinking water.

TABLE 4140:I. COLLABORATIVE DESIGN AS FOUR YODEN PAIR SETS

Anion	Anion Concentration in Individual Youden Pair Standards mg/L							
	1	2	3	4	5	6	7	8
Cl ⁻	0.7	2.0	3.0	15.0	40.0	20.0	50.0	0.5
Br ⁻	2.0	3.0	15.0	40.0	20.0	50.0	0.7	0.5
NO ₂ ⁻	3.0	40.0	20.0	15.0	50.0	0.5	2.0	0.7
SO ₄ ²⁻	40.0	50.0	0.5	0.7	2.0	3.0	15.0	20.0
NO ₃ ⁻	15.0	20.0	40.0	50.0	0.5	0.7	2.0	3.0
F ⁻	2.0	0.7	0.5	3.0	10.0	7.0	20.0	25.0
PO ₄ ³⁻	50.0	40.0	20.0	0.5	3.0	2.0	0.7	15.0

* The collaborative design is intended to demonstrate performance between 0.1 and 50 mg anion/L, except for fluoride between 0.1 and 25 mg/L. The concentrations among anions are varied so as not to have any one standard at all low or all high anion concentrations.

TABLE 4140:II. ANION MIGRATION TIME REPRODUCIBILITY FROM YODEN PAIR STANDARDS

Youden Standard	Anion Midpoint Migration Time, Average of Triplicate Samplings min						
	Cl ⁻	Br ⁻	NO ₂ ⁻	SO ₄ ²⁻	NO ₃ ⁻	F ⁻	PO ₄ ³⁻
1	3.132	3.226	3.275	3.405	3.502	3.761	3.906
2	3.147	3.239	3.298	3.431	3.517	3.779	3.931
3	3.138	3.231	3.283	3.411	3.497	3.771	3.925
4	3.158	3.244	3.307	3.434	3.510	3.781	3.963
5	3.184	3.271	3.331	3.435	3.551	3.787	3.981
6	3.171	3.260	3.312	3.418	3.537	3.776	3.964
7	3.191	3.272	3.315	3.437	3.544	3.773	3.978
8	3.152	3.248	3.294	3.418	3.526	3.739	3.954
SD	0.021	0.015	0.018	0.012	0.20	0.015	0.027
%RSD	0.67%	0.46%	0.55%	0.36%	0.56%	0.40%	0.68%

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Youden Standard	Anion Midpoint Migration Time, Average of Triplicate Samplings <i>min</i>						
	Cl ⁻	Br ⁻	NO ₂ ⁻	SO ₄ ²⁻	NO ₃ ⁻	F ⁻	PO ₄ ³⁻

* Average SD = 0.018 min = 1.1 s; average %RSD = 0.53%.

TABLE 4140:III. COMPARISON OF CAPILLARY ION ELECTROPHORESIS AND OTHER METHODS

Source	Statistic	Value for Given Anion <i>mg/L</i>					
		Cl ⁻	NO ₂ ⁻	SO ₄ ²⁻	NO ₃ ⁻	F ⁻	PO ₄ ³⁻
Performance evaluation standard	True value	43.00	1.77	37.20	15.37	2.69	6.29
Wet chemical and ion chromatography methods	Measured mean	43.30	1.77	37.00	15.42	2.75	6.38
	Measured SD	3.09	0.07	2.24	1.15	0.26	0.21
CIE using chromate electrolyte‡	Average (<i>n</i> = 18)	43.34	1.64	37.11	14.41	2.64	6.34
	CIE/mean	1.003	0.927	1.003	0.935	0.959	0.993
	CIE/true value	1.008	0.927	0.996	0.938	0.981	1.008

* Purchased from APG Laboratories, June 1996; diluted 1:100 with deionized water.

† Measured result is the average from numerous laboratories using approved *Standard Methods* and EPA wet chemistry and ion chromatography methods.

‡ CIE results determined in July 1996 with proposed EPA and ASTM method, operationally identical to 4140; they are the average from four laboratories using the Youdenpair standards for quantitation. These data can be considered known addition of the performance evaluation standard in reagent water; they conform to quality control acceptance limits given in Section 1020.

TABLE 4140:IV. CAPILLARY ION ELECTROPHORESIS REPRODUCIBILITY AND PRECISION*

Laboratory No.	Anion Concentration <i>mg/L</i>				
	Cl ⁻	NO ₂ ⁻	SO ₄ ²⁻	NO ₃ ⁻	F ⁻
1 (<i>n</i> = 5)	43.22 ± 0.22	1.58 ± 0.09	36.39 ± 0.33	14.57 ± 0.12	2.54 ±
2 (<i>n</i> = 5)	43.68 ± 0.61	1.58 ± 0.08	37.01 ± 0.37	13.94 ± 0.09	2.69 ±
3 (<i>n</i> = 5)	43.93 ± 0.39	1.60 ± 0.06	37.68 ± 0.24	15.05 ± 0.11	2.69 ±

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Laboratory No.	Anion Concentration mg/L				
	Cl ⁻	NO ₂ ⁻	SO ₄ ²⁻	NO ₃ ⁻	F ⁻
4 (<i>n</i> = 3)	42.51 ± 0.22	1.78 ± 0.06	37.34 ± 0.19	14.06 ± 0.07	2.69 ±
Average (mean ± SD)	43.34 ± 0.36	1.64 ± 0.07	37.11 ± 0.28	14.41 ± 0.10	2.64 ±
%RSD	0.83%	4.5%	0.77%	0.67%	1.6%

* Results from four laboratories analyzing the performance evaluation standard using the Youden pair standards for quantitation. Only one laboratory reported results for PO₄³⁻ as 6.34 ± 0.02 mg/L on triplicate samplings yielding %RSD of 0.07%. Calculated replicate reproducibility and precision conform to the quality control acceptance limits given in Section 1020.

TABLE 4140:V. CAPILLARY ION ELECTROPHORESIS KNOWN-ADDITION RECOVERY AND PRECISION OF PERFORMANCE EVALUATION STANDARD WITH DRINKING WATER

Variable	Value for Given Anion				
	Cl ⁻	NO ₂ ⁻	SO ₄ ²⁻	NO ₃ ⁻	F ⁻
Milford drinking water (MDW) <i>n</i> = 3, concentration as mg/L	24.72 ± 0.18	Not detected	7.99 ± 0.07	0.36 ± 0.05	Not detected
%RSD	0.73	—	0.91	13.3	—
Performance evaluation standard (PES), concentration as mg/L	43.00	1.77	37.20	15.37	2.69
MDW + PES,* <i>n</i> = 3, concentration as mg/L	66.57 ± 0.34	1.74 ± 0.03	45.19 ± 0.17	15.42 ± 0.12	2.62 ± 0.07
%RSD	0.51%	1.85%	0.38%	0.79%	2.69%
% Recovery	97.9%	98.3%	100.2%	98.1%	97.4%

* Performance evaluation standard diluted 1:100 with Milford drinking water. Calculated analyte recovery and precision conform to the quality control acceptance limits given in Section 1020.

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TABLE 4140:VI. COMPARISON OF CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE WITH OTHER METHODS FOR THE DETERMINATION OF ANIONS

Anion	Matrix	Sample No.	Concentration by Given Method* <i>mg/L</i>		
			Wet Chemical or Other Method†	IC‡	CIE
Chloride	Effluent	1	—	149	147
		2	—	162	161
		3	—	152	151
		4	—	139	139
		5	—	111	110
		6	—	109	107
		7	—	3.6	3.5
	Drinking water	1	5.5	5.1	5.0
		2	5.5	5.0	5.0
		3	5.3	5.2	5.2
		4	5.5	5.1	5.1
		5	5.3	5.0	5.1
		6	5.3	4.9	4.9
		7	5.5	4.9	4.9
	Landfill leachate	1	0.1	<0.1	ND
		2	230	245	240
Fluoride	Effluent	1	1.7	1.2	1.5
		2	0.9	0.6	0.6
		3	0.8	0.5	0.6
		4	0.8	0.4	0.7
		5	0.9	0.5	0.8
		6	0.9	0.5	0.7
		7	<0.1	ND	<0.1

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Anion	Matrix	Sample No.	Concentration by Given Method* <i>mg/L</i>		
			Wet Chemical or Other Method†	IC‡	CIE
Sulfate	Drinking water	1	1.2	0.9	0.9
		2	1.3	0.9	0.9
		3	1.3	0.9	0.9
		4	1.3	0.9	0.9
		5	1.3	0.9	0.9
		6	0.9	0.6	0.6
		7	1.3	0.9	0.9
	Landfill leachate	1	<0.2	ND	ND
		2	16	10.6	10.9
	Effluent	1	98	87.5	86.4
		2	110	95.3	95.9
		3	130	118	115
		4	130	139	136
		5	110	113	110
		6	100	107	106
		7	6	5.6	5.8
	Drinking water	1	6	5.8	6.0
		2	6	5.8	6.0
		3	6	5.9	6.1
		4	6	5.9	6.1
		5	5	5.8	6.2
		6	4	3.0	3.4
		7	5	5.8	6.1
	Landfill leachate	1	<1	ND	ND

Standard Methods for the Examination of Water and Wastewater

Anion	Matrix	Sample No.	Concentration by Given Method* <i>mg/L</i>		
			Wet Chemical or Other Method†	IC‡	CIE
		2	190	211	201
Nitrite nitrate [§] (as N)	Effluent	1	0.3	ND	ND
		2	—	ND	ND
		3	—	ND	ND
		4	—	ND	0.5
		5	—	2.1	2.4
		6	2.4	1.9	2.2
		7	0.7	0.3	0.4
	Drinking water	1	0.6	0.3	0.4
		2	0.6	0.3	0.4
		3	0.4	0.3	0.4
		4	0.6	0.3	0.3
		5	0.6	0.3	0.4
		6	0.3	0.1	0.1
		7	0.5	0.3	0.4
	Landfill leachate	1	—	ND	ND
		2	—	ND	ND
Orthophosphate (as P)	Effluent	1	3.4	ND	2.8
		2	4.9	ND	4.4
		3	4.7	ND	4.5
		4	5.3	ND	4.2
		5	3.0	ND	3.0
		6	2.9	ND	2.3
		7	<0.1	ND	0.04

Standard Methods for the Examination of Water and Wastewater

Anion	Matrix	Sample No.	Concentration by Given Method* <i>mg/L</i>		
			Wet Chemical or Other Method†	IC‡	CIE
	Drinking water	1	<0.1	ND	ND
		2	<0.1	ND	ND
		3	—	ND	ND
		4	<0.1	ND	ND
		5	<0.1	ND	ND
		6	—	ND	ND
		7	—	ND	ND
	Landfill leachate	1	<0.1	ND	0.1
		2	2.2	1.6	1.4

* — = test not performed; ND = not detected.

† Methods used were: chloride—iodometric method (4500-Cl.C); fluoride—ion selective electrode method (4500-F.C); sulfate—turbidimetric method (4500-SO₄.E); nitrite+ nitrate (total)—cadmium reduction method (4500-NO₃.E); orthophosphate—ascorbic acid method (4500-P.E).

‡ Single-column ion chromatography with direct conductivity detection (4110C).

§ Each technique gave separate nitrite and nitrate values; because of their interconvertability, results were added for comparison purposes.

TABLE 4500-CN⁻:I. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition <i>mg CN⁻/L</i>	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	94	—
	Blank†	0.050	96	—
		0.10	96	—
		0	—	<0.5
	Site A‡	0.010	104	—
		0.020	104	—
		0	—	<0.5
		0.010	101	—

Standard Methods for the Examination of Water and Wastewater

Matrix	Sample/Blank Designation	Known Addition <i>mg CN⁻/L</i>	Recovery %	Relative Standard Deviation %
Wastewater treatment plant effluent	Site C‡	0.020	106	—
		0	—	<0.5
		0.010	103	—
		0.020	108	—
	Reference sample*	—	95	—
	Blank†	0.050	88	—
	Site A‡	0.10	95	—
		0	—	<0.5
		0.010	112	—
		0.020	106	—
	Site B‡	0	—	<0.5
		0.010	110	—
		0.020	105	—
		0	—	<0.5
	Site C‡	0.010	101	—
		0.020	106	—
		0	—	<0.5
Landfill leachate	Reference sample*	—	98	—
	Blank†	0.050	96	—
	Site A‡	0.10	98	—
		0	—	<0.5
		0.050	114	—
		0.10	106	—
	Site B‡	0	—	<0.5
		0.050	104	—
		0.10	104	—
		0	—	<0.5
	Site C‡	0.050	103	—
		0	—	<0.5

Standard Methods for the Examination of Water and Wastewater

Matrix	Sample/Blank Designation	Known Addition <i>mg CN⁻/L</i>	Recovery %	Relative Standard Deviation %
		0.10	107	—

* U.S. EPA QC sample, 0.498 mg CN⁻/L, diluted five-fold.

† Determined in duplicate.

‡ Samples diluted five-fold. Samples without known additions determined four times; sample with known additions determined in duplicate; typical relative differences between duplicates <0.5%.

TABLE 4500-Cl⁻:I. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition <i>mg Cl⁻/L</i>	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	101	—
	Blank†	10	104	—
		20	102	—
	Site A‡	0	—	0.4
		10	92	—
		20	101	—
	Site B‡	0	—	0.2
		10	97	—
		20	106	—
	Site C‡	0	—	0.4
		10	102	—
		20	102	—
Wastewater treatment plant effluent	Reference sample*	—	101	—
	Blank†	10	104	—
		20	102	—
	Site A‡	0	—	0.3
		10	98	—
		20	101	—
	Site B‡	0	—	0.2
		10	99	—
		20	103	—

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Matrix	Sample/Blank Designation	Known Addition <i>mg Cl⁻/L</i>	Recovery %	Relative Standard Deviation %
Landfill leachate	Site C‡	0	—	0.4
		10	91	—
		20	97	—
	Reference sample*	—	100	—
	Blank†	10	101	—
		20	100	—
	Site A§	0	—	0.3
		10	97	—
		20	103	—
	Site B§	0	—	0.2
		10	89	—
		20	103	—
	Site C§	0	—	0.5
		10	89	—
		20	103	—

* U.S. EPA nutrient QC sample, 51.7 mg Cl⁻/L.

† Determined in duplicate.

‡ Samples diluted 5-fold. Samples without known additions determined four times; samples with known additions determined in duplicate. Typical relative difference between duplicates 0.2%.

§ Sample from Site A diluted 50-fold, those from B and C 100-fold. Samples without known additions determined four times; samples with known additions determined in duplicate; typical relative difference between duplicates 0.5%.

TABLE 4500-CLO₂:I. EQUIVALENT WEIGHTS FOR CALCULATING CONCENTRATIONS ON THE BASIS OF MASS

pH	Species	Molecular Weight <i>mg/mol</i>	Electrons Transferred	Equivalent Weight <i>mg/eq</i>
7	Chlorine dioxide	67 452	1	67 452
2, 0.1	Chlorine dioxide	67 452	5	13 490
7, 2, 0.1	Chlorine	70 906	2	35 453
2, 0.1	Chlorite	67 452	4	16 863
0.1	Chlorate	83 451	6	13 909

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TABLE 4500-F⁻:I. CONCENTRATION OF SOME SUBSTANCES CAUSING 0.1-MG/L ERROR AT 1.0 MG F/L IN FLUORIDE METHODS

Substance	Method C (Electrode)		Method D (SPADNS)	
	Conc mg/L	Type of Error*	Conc mg/L	Type of Error*
Alkalinity (CaCO ₃)	7 000	+	5 000	—
Aluminum (Al ³⁺)	3.0	—	0.1†	—
Chloride (Cl ⁻)	20 000		7 000	+
Chlorine	5 000			Remove completely with arsenite
Color & turbidity				Remove or compensate for
Iron	200	—	10	—
Hexametaphosphate ([NaPO ₃])	50 000		1.0	+
Phosphate (PO ₄ ³⁻)	50 000		16	+
Sulfate (SO ₄ ²⁻)	50 000	—	200	—

* + denotes positive error

— denotes negative error

Blank denotes no measurable error.

† On immediate reading. Tolerance increases with time: after 2 h, 3.0; after 4 h, 30.

TABLE 4500-F⁻:II. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg F ⁻ /L	Recovery %	Relative Standard Deviation %
Wastewater treatment	Reference sample*	—	101	—

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Matrix	Sample/Blank Designation	Known Addition <i>mg F⁻/L</i>	Recovery %	Relative Standard Deviation %
plant influent	Blank†	1.0	91	—
		2.0	97	—
		0	—	4.8
	Site A‡	1.0	93	—
		2.0	82	—
		0	—	6.4
		1.0	96	—
		2.0	86	—
	Site C‡§	0	—	15
		1.0	99	—
		2.0	86	—
Wastewater treatment plant effluent	Reference sample*	—	103	—
Landfill leachate	Blank†	1.0	97	—
		2.0	97	—
		0	—	ND
	Site A‡	1.0	ND	—
		2.0	ND	—
		0	—	<0.1
		1.0	80	—
		2.0	78	—
	Site C‡	0	—	<0.1
		1.0	93	—
		2.0	91	—
Landfill leachate	Reference sample*	—	99	—
	Blank†	1.0	87	—
		2.0	88	—
	Site A‡	0	—	13

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Matrix	Sample/Blank Designation	Known Addition $\text{mg F}^-/\text{L}$	Recovery %	Relative Standard Deviation %
		1.0	74	—
		2.0	68	—
	Site B†	0	—	10
		1.0	68	—
		2.0	73	—
	Site C‡	0	—	32
		1.0	66	—
		2.0	79	—

ND = not detectable.

* U.S. EPA QC sample, 1.81 $\text{mg F}^-/\text{L}$.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate. Typical difference between duplicates for influent 5%, for effluent 6%.

§ Mean concentration 0.18 $\text{mg F}^-/\text{L}$.

|| All sites had mean concentration of <0.2 $\text{mg F}^-/\text{L}$.

TABLE 4500-H⁺:I. PREPARATION OF PH STANDARD SOLUTIONS³

Standard Solution (molality)	pH at 25°C	Weight of Chemicals Needed/1000 mL Aqueous Solution at 25°C
<i>Primary standards:</i>		
Potassium hydrogen tartrate (saturated at 25°C)	3.557	> 7 g $\text{KHC}_4\text{H}_4\text{O}_6^*$
0.05 potassium dihydrogen citrate	3.776	11.41 g $\text{KH}_2\text{C}_6\text{H}_5\text{O}_7$
0.05 potassium hydrogen phthalate	4.004	10.12 g $\text{KHC}_8\text{H}_4\text{O}_4$
0.025 potassium dihydrogen phosphate + 0.025 disodium hydrogen phosphate	6.863	3.387 g KH_2PO_4 + 3.533 g $\text{Na}_2\text{HPO}_4^\dagger$
0.008 695 potassium dihydrogen phosphate + 0.030 43 disodium hydrogen phosphate	7.415	1.179 g KH_2PO_4 + 4.303 g $\text{Na}_2\text{HPO}_4^\dagger$
0.01 sodium borate decahydrate (borax)	9.183	3.80 g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}^\dagger$

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Standard Solution (molality)	pH at 25°C	Weight of Chemicals Needed/1000 mL Aqueous Solution at 25°C
0.025 sodium bicarbonate + 0.025 sodium carbonate	10.014	2.092 g NaHCO ₃ + 2.640 g Na ₂ CO ₃
<i>Secondary standards:</i>		
0.05 potassium tetroxalate dihydrate	1.679	12.61 g KH ₃ C ₄ O ₈ ·2H ₂ O
Calcium hydroxide (saturated at 25°C)	12.454	> 2 g Ca(OH) ₂ *

* Approximate solubility.

† Prepare with freshly boiled and cooled distilled water (carbon-dioxide-free).

TABLE 4500-H⁺:II. STANDARD PH VALUES³

Temperature °C	Primary Standards						Bicarbonate- Carbonate (0.025M)	T
	Tartrate (Saturated)	Citrate (0.05M)	Phthalate (0.05M)	Phosphate (1:1)	Phosphate (1:3.5)	Borax (0.01M)		
0			4.003	6.982	7.534	9.460	10.321	
5			3.998	6.949	7.501	9.392	10.248	
10			3.996	6.921	7.472	9.331	10.181	
15			3.996	6.898	7.449	9.276	10.120	
20			3.999	6.878	7.430	9.227	10.064	
25	3.557	3.776	4.004	6.863	7.415	9.183	10.014	
30	3.552		4.011	6.851	7.403	9.143	9.968	
35	3.549		4.020	6.842	7.394	9.107	9.928	
37			4.024	6.839	7.392	9.093		
40	3.547		4.030	6.836	7.388	9.074	9.891	
45	3.547		4.042	6.832	7.385	9.044	9.859	
50	3.549		4.055	6.831	7.384	9.017	9.831	
55	3.554		4.070					
60	3.560		4.085					
70	3.580		4.12					
80	3.609		4.16					
90	3.650		4.19					
85	3.674		4.21					

TABLE 4500-N:I. RECOVERIES OF TOTAL NITROGEN

Compound	Mean Recovery%	
	10 mg N/L	4 mg N/L
Ammonium chloride	98.1	99.7

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Compound	Mean Recovery%	
	10 mg N/L	4 mg N/L
Sodium nitrite	100.5	101.8
Glycine	101.0	100.8
Glutamic acid	99.7	99.2
Ammonium <i>p</i> -toluenesulfonate	99.6	97.4
Glycine <i>p</i> -toluenesulfonate	101.4	102.3
Nicotinic acid	98.6	102.0
Urea	94.9	98.0
EDTA	89.4	89.4

TABLE 4500-N:II. PRECISION DATA FOR TOTAL NITROGEN, PERSULFATE METHOD, BASED ON TRIPPLICATE ANALYSES OF NICOTINIC ACID

Lab/ Analyst	Nicotinic Acid mg N/L	Recovery of N %	Standard Deviation mg/L	Relative Standard Deviation %
1/1	0.5	104	0.019	3.82
1/2	0.5	99.7	0.012	2.44
2/1	0.5	97.7	0.035	7.02
2/2	0.5	87.8	0.024	4.89
3/1	0.5	105	0.072	13.7
3/2	0.5	95.3	0.015	3.20
1/1	1.0	98.5	0.023	2.32
1/2	1.0	99.3	0.022	2.21
2/1	1.0	113	0.053	5.31
2/2	1.0	97.1	0.031	3.10
3/1	1.0	96.2	0.019	1.97
3/2	1.0	102	0.025	2.46
1/1	2.0	100	0.030	1.50
1/2	2.0	97.8	0.014	0.7
2/1	2.0	104	0.069	3.45
2/2	2.0	100	0.080	3.98
3/1	2.0	95.3	0.078	4.12
3/2	2.0	98.3	0.015	0.75

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TABLE 4500-NH₃:I. PRECISION AND BIAS OF AMMONIA-SELECTIVE ELECTRODE

Level mg/L	Matrix	Mean Recovery %	Precision	
			Overall % <i>RSD</i>	Single Operator % <i>RSD</i>
0.04	Distilled water	200	125	25
	Effluent water	100	75	0
0.10	Distilled water	180	50	10
	Effluent water	470	610	10
0.80	Distilled water	105	14	5
	Effluent water	105	38	7.5
20	Distilled water	95	10	5
	Effluent water	95	15	10
100	Distilled water	98	5	2
	Effluent water	97	—	—
750	Distilled water	97	10.4	1.6
	Effluent water	99	14.1	1.3

Source: AMERICAN SOCIETY FOR TESTING AND MATERIALS. Method 1426-79. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4500-NH₃:II. * VALUES OF *Q* VS. ΔE (59 MV SLOPE) FOR 10% VOLUME CHANGE

ΔE	<i>Q</i>	ΔE	<i>Q</i>	ΔE	<i>Q</i>	ΔE	<i>Q</i>	ΔE	<i>Q</i>
5.0	0.297	9.0	0.178	16.0	0.0952	24.0	0.0556	32.0	0.0354
5.1	0.293	9.1	0.176	16.2	0.0938	24.2	0.0549	32.2	0.0351
5.2	0.288	9.2	0.174	16.4	0.0924	24.4	0.0543	32.4	0.0347
5.3	0.284	9.3	0.173	16.6	0.0910	24.6	0.0536	32.6	0.0343
5.4	0.280	9.4	0.171	16.8	0.0897	24.8	0.0530	32.8	0.0340
5.5	0.276	9.5	0.169	17.0	0.0884	25.0	0.0523	33.0	0.0335
5.6	0.272	9.6	0.167	17.2	0.0871	25.2	0.0517	33.2	0.0333
5.7	0.268	9.7	0.165	17.4	0.0858	25.4	0.0511	33.4	0.0329
5.8	0.264	9.8	0.164	17.6	0.0846	25.6	0.0505	33.6	0.0326
5.9	0.260	9.9	0.162	17.8	0.0834	25.8	0.0499	33.8	0.0323

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ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q
6.0	0.257	10.0	0.160	18.0	0.0822	26.0	0.0494	34.0	0.0319
6.1	0.253	10.2	0.157	18.2	0.0811	26.2	0.0488	34.2	0.0316
6.2	0.250	10.4	0.154	18.4	0.0799	26.4	0.0482	34.4	0.0313
6.3	0.247	10.6	0.151	18.6	0.0788	26.6	0.0477	34.6	0.0310
6.4	0.243	10.8	0.148	18.8	0.0777	26.8	0.0471	34.8	0.0307
6.5	0.240	11.0	0.145	19.0	0.0767	27.0	0.0466	35.0	0.0304
6.6	0.237	11.2	0.143	19.2	0.0756	27.2	0.0461	36.0	0.0289
6.7	0.234	11.4	0.140	19.4	0.0746	27.4	0.0456	37.0	0.0275
6.8	0.231	11.6	0.137	19.6	0.0736	27.6	0.0450	38.0	0.0261
6.9	0.228	11.8	0.135	19.8	0.0726	27.8	0.0445	39.0	0.0249
7.0	0.225	12.0	0.133	20.0	0.0716	28.0	0.0440	40.0	0.0237
7.1	0.222	12.2	0.130	20.2	0.0707	28.2	0.0435	41.0	0.0226
7.2	0.219	12.4	0.128	20.4	0.0698	28.4	0.0431	42.0	0.0216
7.3	0.217	12.6	0.126	20.6	0.0689	28.6	0.0426	43.0	0.0206
7.4	0.214	12.8	0.123	20.8	0.0680	28.8	0.0421	44.0	0.0196
7.5	0.212	13.0	0.121	21.0	0.0671	29.0	0.0417	45.0	0.0187
7.6	0.209	13.2	0.119	21.2	0.0662	29.2	0.0412	46.0	0.0179
7.7	0.207	13.4	0.117	21.4	0.0654	29.4	0.0408	47.0	0.0171
7.8	0.204	13.6	0.115	21.6	0.0645	29.6	0.0403	48.0	0.0163
7.9	0.202	13.8	0.113	21.8	0.0637	29.8	0.0399	49.0	0.0156
8.0	0.199	14.0	0.112	22.0	0.0629	30.0	0.0394	50.0	0.0149
8.1	0.197	14.2	0.110	22.2	0.0621	30.2	0.0390	51.0	0.0143
8.2	0.195	14.4	0.108	22.4	0.0613	30.4	0.0386	52.0	0.0137
8.3	0.193	14.6	0.106	22.6	0.0606	30.6	0.0382	53.0	0.0131
8.4	0.190	14.8	0.105	22.8	0.0598	30.8	0.0378	54.0	0.0125
8.5	0.188	15.0	0.103	23.0	0.0591	31.0	0.0374	55.0	0.0120
8.6	0.186	15.2	0.1013	23.2	0.0584	31.2	0.0370	56.0	0.0115
8.7	0.184	15.4	0.0997	23.4	0.0576	31.4	0.0366	57.0	0.0110

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ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q
8.8	0.182	15.6	0.0982	23.6	0.0569	31.6	0.0362	58.0	0.0105
8.9	0.180	15.8	0.0967	23.8	0.0563	31.8	0.0358	59.0	0.0101

* Orion Research Inc. Instruction Manual, Ammonia Electrode, Model 95-12, Boston, MA. 02129.

TABLE 4500-NH₃:III. PRECISION DATA FOR MANUAL PHENATE METHOD BASED ON TRIPPLICATE ANALYSES OF AMMONIUM SULFATE

Lab/ Analyst	NH ₃ -N		Relative Standard Deviation %
	Concentration mg/L	Optical Density	
1/1	0.1	0.129	1.55
1/2	0.1	0.114	9.66
2/1	0.1	0.100	10.2
2/2	0.1	0.122	2.36
3/1	0.1	0.112	3.61
3/2	0.1	0.107	1.94
1/1	0.3	0.393	0.39
1/2	0.3	0.364	0.32
2/1	0.3	0.372	2.64
2/2	0.3	0.339	0.90
3/1	0.3	0.370	0.31
3/2	0.3	0.373	0.46
1/1	0.5	0.637	0.77
1/2	0.5	0.630	0.56
2/1	0.5	0.624	1.65
2/2	0.5	0.618	0.86
3/1	0.5	0.561	0.27
3/2	0.5	0.569	0.91

TABLE 4500-NH₃:IV. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg NH ₃ -N/L	Recovery %	Relative Standard Deviation %
Wastewater treatment	Reference sample*	—	104	—

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Matrix	Sample/Blank Designation	Known Addition <i>mg NH₃-NIL</i>	Recovery %	Relative Standard Deviation %
plant influent	Blank†	0.4	100	—
		0.8	102	—
	Site A‡§	0	—	0.5
		0.4	108	—
		0.8	105	—
	Site B‡§	0	—	0.5
		0.4	105	—
		0.8	105	—
	Site C‡§	0	—	1.1
		0.4	109	—
		0.8	107	—
Wastewater treatment plant effluent	Reference sample*	—	106	—
	Blank†	0.4	105	—
		0.8	105	—
	Site A‡	0	—	ND
		0.4	90	—
		0.8	88	—
	Site B‡	0	—	<0.1
		0.4	93	—
		0.8	94	—
	Site C‡	0	—	<0.1
		0.4	89	—
		0.8	91	—
Landfill leachate	Reference sample*	—	106	—
	Blank†	0.4	105	—
		0.8	106	—
	Site A‡#	0	—	1.9

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Matrix	Sample/Blank Designation	Known Addition <i>mg NH₃-N/L</i>	Recovery %	Relative Standard Deviation %
		0.4	125	—
		0.8	114	—
	Site B†‡	0	—	0.4
		0.4	96	—
		0.8	106	—
	Site C†‡	0	—	0.2
		0.4	102	—
		0.8	107	—

ND = not detectable.

* U.S. EPA QC sample, 1.98 mg N/L.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate.

§ Site A and C samples diluted 20-fold, Site B sample diluted 100-fold. Typical relative difference between duplicates 0.2%.

|| Samples not diluted. Typical relative difference between duplicates <1%.

Site A and C samples diluted 50-fold; Site B sample diluted 150-fold. Typical relative difference between duplicates 0.3%.

TABLE 4500-NO₃⁻:I. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition <i>mg NO₃⁻-N/L</i>	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	102	—
	Blank†	0.2	100	—
		0.4	100	—
	Site A‡	0	—	<1
		0.2	101	—
		0.4	96	—
	Site B‡	0	—	<1
		0.2	90	—
		0.4	88	—
	Site C‡	0	—	<1

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Matrix	Sample/Blank Designation	Known Addition <i>mg NO₃⁻</i> N/L	Recovery %	Relative Standard Deviation %
Wastewater treatment plant effluent	Reference sample*	0.2	95	—
		0.4	95	—
		—	102	—
	Blank†	0.2	100	—
		0.4	95	—
	Site A‡	0	—	0.9
		0.2	95	—
	Site B‡	0.4	102	—
		0	—	0.7
	Site C‡	0.2	91	—
		0.4	101	—
	Site C‡	0	—	0.5
		0.2	91	—
		0.4	96	—
Landfill leachate	Reference sample*	—	98	—
		0.2	100	—
		0.4	98	—
	Site A‡	0	—	<1
		0.2	104	—
	Site B‡	0.4	96	—
		0	—	<1
	Site C‡	0.2	95	—
		0.4	94	—
	Site C‡	0	—	<1
		0.2	91	—
		0.4	93	—

* U.S. EPA QC sample, 1.98 mg N/L.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate. Typical difference

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between duplicates: influent, 2%; effluent, 1%; leachate, <1%.

TABLE 4500-N_{org}:I. PRECISION DATA FOR KJELDAHL NITROGEN METHOD BASED ON MEAN OF TRIPPLICATE ANALYSES OF NICOTINIC ACID

Lab/ Analyst	Nicotinic Acid <i>mg N/L</i>	Recovery of N %	Standard Deviation <i>mg/L</i>	Relative Standard Deviation %
1/1	5	93.3	0.16	3.46
1/2	5	101	0.16	3.17
1/1	10	87.7	0.16	1.84
1/2	10	91.5	0.28	3.06
1/1	20	95.7	0.16	0.84
1/2	20	95.7	0.58	3.03
2/1	0.5	97.4	0.005	1.04
2/2	0.5	95.3	0.027	5.46
3/1	0.5	87.3	0.130	29.9
4/1	0.5	113	0.235	41.7
2/1	1.0	103	0.012	1.15
2/2	1.0	101	0.046	4.63
3/1	1.0	84.3	0.081	9.66
4/1	1.0	99.3	0.396	39.9
2/1	2.0	104	0	0
2/2	2.0	99.2	0.029	1.44
3/1	2.0	89.2	0.071	3.98
4/1	2.0	112	0.139	6.18

TABLE 4500-N_{ORG}:II. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition <i>mg NIL</i>	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	97	—
	Blank†	3.0	97	—
		6.0	99	—
	Site A‡§	0	—	3.3

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Matrix	Sample/Blank Designation	Known Addition <i>mg NIL</i>	Recovery %	Relative Standard Deviation %
Wastewater treatment plant effluent	Site B‡§	3.0	91	—
		6.0	95	—
		0	—	3.6
		3.0	115	—
		6.0	93	—
		0	—	5.1
	Site C‡§	3.0	97	—
		6.0	107	—
		—	92	—
		3.0	97	—
		6.0	100	—
		0	—	5.4
	Site A‡	3.0	94	—
		6.0	100	—
		0	—	4.1
		3.0	119	—
		6.0	81	—
		0	—	7.3
	Site B‡	3.0	93	—
		6.0	105	—
		—	96	—
		3.0	101	—
		6.0	99	—
		0	—	3.3
Landfill leachate	Site A‡#	3.0	95	—
		6.0	98	—
		0	—	4.4
		3.0	134	—
	Site B‡#	6.0	85	—
		—	—	—
		—	—	—
		—	—	—

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Matrix	Sample/Blank Designation	Known Addition mg N/L	Recovery %	Relative Standard Deviation %
	Site C†‡	0	—	3.8
		3.0	98	—
		6.0	105	—

* U.S. EPA nutrient QC sample, 1.52 mg N/L.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate.

§ Sample dilutions: A - 5-fold; B - 10-fold; C - 5-fold. Typical relative difference between duplicates 3%.

|| Sample dilutions: A - none; B - 2-fold; C - none. Typical relative difference between duplicates 1%.

Sample dilutions: A, B, and C - 25-fold. Typical relative difference between duplicates 4%.

TABLE 4500-O:I. SOLUBILITY OF OXYGEN IN WATER EXPOSED TO WATER-SATURATED AIR AT ATMOSPHERIC P_{RESSURE} (101.3 kPa)¹

Temperature °C	Oxygen Solubility mg/L					
	Chlorinity: 0	5.0	10.0	15.0	20.0	25.0
0.0	14.621	13.728	12.888	12.097	11.355	10.657
1.0	14.216	13.356	12.545	11.783	11.066	10.392
2.0	13.829	13.000	12.218	11.483	10.790	10.139
3.0	13.460	12.660	11.906	11.195	10.526	9.897
4.0	13.107	12.335	11.607	10.920	10.273	9.664
5.0	12.770	12.024	11.320	10.656	10.031	9.441
6.0	12.447	11.727	11.046	10.404	9.799	9.228
7.0	12.139	11.442	10.783	10.162	9.576	9.023
8.0	11.843	11.169	10.531	9.930	9.362	8.826
9.0	11.559	10.907	10.290	9.707	9.156	8.636
10.0	11.288	10.656	10.058	9.493	8.959	8.454
11.0	11.027	10.415	9.835	9.287	8.769	8.279
12.0	10.777	10.183	9.621	9.089	8.586	8.111
13.0	10.537	9.961	9.416	8.899	8.411	7.949
14.0	10.306	9.747	9.218	8.716	8.242	7.792
15.0	10.084	9.541	9.027	8.540	8.079	7.642
16.0	9.870	9.344	8.844	8.370	7.922	7.496
17.0	9.665	9.153	8.667	8.207	7.770	7.356

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Temperature °C	Oxygen Solubility <i>mg/L</i>					
	Chlorinity: 0	5.0	10.0	15.0	20.0	25.0
18.0	9.467	8.969	8.497	8.049	7.624	7.221
19.0	9.276	8.792	8.333	7.896	7.483	7.090
20.0	9.092	8.621	8.174	7.749	7.346	6.964
21.0	8.915	8.456	8.021	7.607	7.214	6.842
22.0	8.743	8.297	7.873	7.470	7.087	6.723
23.0	8.578	8.143	7.730	7.337	6.963	6.609
24.0	8.418	7.994	7.591	7.208	6.844	6.498
25.0	8.263	7.850	7.457	7.083	6.728	6.390
26.0	8.113	7.711	7.327	6.962	6.615	6.285
27.0	7.968	7.575	7.201	6.845	6.506	6.184
28.0	7.827	7.444	7.079	6.731	6.400	6.085
29.0	7.691	7.317	6.961	6.621	6.297	5.990
30.0	7.559	7.194	6.845	6.513	6.197	5.896
31.0	7.430	7.073	6.733	6.409	6.100	5.806
32.0	7.305	6.957	6.624	6.307	6.005	5.717
33.0	7.183	6.843	6.518	6.208	5.912	5.631
34.0	7.065	6.732	6.415	6.111	5.822	5.546
35.0	6.950	6.624	6.314	6.017	5.734	5.464
36.0	6.837	6.519	6.215	5.925	5.648	5.384
37.0	6.727	6.416	6.119	5.835	5.564	5.305
38.0	6.620	6.316	6.025	5.747	5.481	5.228
39.0	6.515	6.217	5.932	5.660	5.400	5.152
40.0	6.412	6.121	5.842	5.576	5.321	5.078
41.0	6.312	6.026	5.753	5.493	5.243	5.005
42.0	6.213	5.934	5.667	5.411	5.167	4.933
43.0	6.116	5.843	5.581	5.331	5.091	4.862
44.0	6.021	5.753	5.497	5.252	5.017	4.793
45.0	5.927	5.665	5.414	5.174	4.944	4.724
46.0	5.835	5.578	5.333	5.097	4.872	4.656
47.0	5.744	5.493	5.252	5.021	4.801	4.589
48.0	5.654	5.408	5.172	4.947	4.730	4.523

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Temperature °C	Oxygen Solubility mg/L					
	Chlorinity: 0	5.0	10.0	15.0	20.0	25.0
49.0	5.565	5.324	5.094	4.872	4.660	4.457
50.0	5.477	5.242	5.016	4.799	4.591	4.392

NOTE:

1. The table provides three decimal places to aid interpolation. When computing saturation values to be used with measured values, such as in computing DO deficit in a receiving water, precision of measured values will control choice of decimal places to be used.
2. Equations are available to compute DO concentration in fresh water¹⁻³ and in seawater¹ at equilibrium with water-saturated air. Figures and tables also are available.³

Calculate the equilibrium oxygen concentration, C^* , from equation:

$$\ln C^* = -139.344 \text{ } 11 + (1.575 \text{ } 701 \times 10^5/T) - (6.642 \text{ } 308 \times 10^7/T^2) \\ + (1.243 \text{ } 800 \times 10^{10}/T^3) - (8.621 \text{ } 949 \times 10^{11}/T^4) \\ - \text{Chl} [(3.1929) \times 10^{-2}] - (1.9428 \times 10^1/T) \\ + (3.8673 \times 10^3/T^2)]$$

where:

C^* = equilibrium oxygen concentration at 101.325 kPa, mg/L,
 T = temperature (°K) = °C + 273.150, (°C is between 0.0 and 40.0 in the equation; the table is accurate up to 50.0), and
 Chl = Chlorinity (see definition in Note 4, below).

Example 1: At 20°C and 0.000 Chl, $\ln C^* = 2.207 \text{ } 442$ and $C^* = 9.092 \text{ mg/L}$;

Example 2: At 20°C and 15.000 Chl,
 $\ln C^* = (2.207 \text{ } 442) - 15.000 (0.010 \text{ } 657)$
 $= 2.0476$ and $C^* = 7.749 \text{ mg/L}$.

When salinity is used, replace the chlorinity term ($-\text{Chl}[\dots]$) by:
 $-S(1.7674 \times 10^{-2}) - (1.0754 \times 10^1/T) + (2.1407 \times 10^3/T^2)$

where:

S = salinity (see definition in Note 4, below).

$$C_p = C^*P \left[\frac{(1 - P_{wv}/P)(1 - \theta P)}{(1 - P_{wv})(1 - \theta)} \right]$$

3. For nonstandard conditions of pressure:

where:

C_p = equilibrium oxygen concentration at nonstandard pressure, mg/L,
 C^* = equilibrium oxygen concentration at standard pressure of 1 atm, mg/L.
 P = nonstandard pressure, atm,

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P_{ww} = partial pressure of water vapor, atm, computed from:

$$\ln P_{ww} = 11.8571 - (3840.70/T) - (216\,961/T^2),$$

T = temperature, °K,

$\theta = 0.000\,975 - (1.426 \times 10^{-5}t) + (6.436 \times 10^{-8}t^2)$, and

t = temperature, °C.

N.B.: Although not explicit in the above, the quantity in brackets in the equation for C_p has dimensions of atm^{-1} per Reference 4, so that P multiplied by this quantity is dimensionless.

Also, the equation for $\ln P_{ww}$ is strictly valid for fresh water only, but for practical purposes no error is made by neglecting the effect of salinity. An equation for P_{ww} that includes the salinity factor may be found in Reference 1.

Example 3: At 20°C, 0.000 Chl, and 0.700 atm,
 $C_p = C \cdot P (0.990\,092) = 6.30 \text{ mg/L.}$

4. Definitions:

Salinity: Although salinity has been defined traditionally as the total solids in water after all carbonates have been converted to oxides, all bromide and iodide have been replaced by chloride, and all organic matter has been oxidized (see Section 2520), the new scale used to define salinity is based on the electrical conductivity of seawater relative to a specified solution of KCl in water.⁵ The scale is dimensionless and the traditional dimension of parts per thousand (i.e., g/kg of solution) no longer applies.

Chlorinity: Chlorinity is defined in relation to salinity as follows:

$$\text{Salinity} = 1.806\,55 \times \text{chlorinity}$$

Although chlorinity is not equivalent to chloride concentration, the factor for converting a chloride concentration in seawater to include bromide, for example, is only 1.0045 (based on the relative molecular weights and amounts of the two ions). Therefore, for practical purposes, chloride concentration (in g/kg of solution) is nearly equal to chlorinity in seawater. For wastewater, it is necessary to know the ions responsible for the solution's electrical conductivity to correct for their effect on oxygen solubility and use of the tabular value. If this is not done, the equation is inappropriate unless the relative composition of the wastewater is similar to that of seawater.

TABLE 4500-P:I. PRECISION AND BIAS DATA FOR MANUAL PHOSPHORUS METHODS

Method	Phosphorus Concentration			No. of Laboratories	Relative Standard Deviation	Relative Error
	Ortho-phosphate µg/L	Poly-phosphate µg/L	Total µg/L		%	%
Vanadomolybdophosphoric acid	100			45	75.2	21.6
	600			43	19.6	10.8
	7000			44	8.6	5.4
Stannous chloride	100			45	25.5	28.7
	600			44	14.2	8.0
	7000			45	7.6	4.3

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Method	Phosphorus Concentration			No. of Laboratories	Relative Standard Deviation	Relative Error
	Ortho-phosphate µg/L	Poly-phosphate µg/L	Total µg/L		%	%
Ascorbic acid	100			3	9.1	10.0
	600			3	4.0	4.4
	7000			3	5.2	4.9
Acid hydrolysis + vanadomolybdophosphoric acid		80		37	106.8	7.4
		300		38	66.5	14.0
		3000		37	36.1	23.5
Acid hydrolysis + stannous chloride		80		39	60.1	12.5
		300		36	47.6	21.7
		3000		38	37.4	22.8
Persulfate + vanadomolybdophosphoric acid			210	32	55.8	1.6
			990	32	23.9	2.3
			10 230	31	6.5	0.3
Sulfuric-nitric acids + vanadomolybdophosphoric acid			210	23	65.6	20.9
			990	22	47.3	0.6
			10 230	20	7.0	0.4
Perchloric acid + vanadomolybdophosphoric acid			210	4	33.5	45.2
			990	5	20.3	2.6
			10 230	6	11.7	2.2
Persulfate + stannous chloride			210	29	28.1	9.2
			990	30	14.9	12.3
			10 230	29	11.5	4.3
Sulfuric-nitric acids + stannous chloride			210	20	20.8	1.2
			990	17	8.8	3.2
			10 230	19	7.5	0.4

TABLE 4500-P:II. COMPARISON OF PRECISION AND BIAS OF ASCORBIC ACID METHODS

Relative Standard Deviation %	Relative Error %

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Method	phosphate µg/L	phosphate µg/L	total µg/L	NO. OF Laboratories	Deviation %	Error %

TABLE 4500-P:II. COMPARISON OF PRECISION AND BIAS OF ASCORBIC ACID METHODS

Ascorbic Acid Method	Phosphorus Concentration, Dissolved Orthophosphate µg/L	No. of Labora- tories	Relative Standard Deviation %		Relative Error %	
			Distilled Water	River Water	Distilled Water	River Water
13th Edition ¹	228	8	3.87	2.17	4.01	2.08
Current method ²	228	8	3.03	1.75	2.38	1.39

TABLE 4500-P:III. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg P/L	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	101	—
	Blank†	0.05	96	—
		0.1	95	—
		0	—	0.7
	Site A‡§	0.05	98	—
		0.1	101	—
		0	—	5
	Site B‡§	0.05	75	—
		0.1	91	—
		0	—	0.6
	Site C‡§	0.05	88	—
		0.1	97	—

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Matrix	Sample/Blank Designation	Known Addition <i>mg P/L</i>	Recovery %	Relative Standard Deviation %
Wastewater treatment plant effluent	Reference sample*	—	100	—
	Blank†	0.05	96	—
		0.1	96	—
	Site A‡	0	—	0.7
		0.05	94	—
		0.1	96	—
	Site B‡	0	—	0.3
		0.05	94	—
		0.1	99	—
	Site C‡	0	—	0.5
		0.05	109	—
		0.1	107	—
Landfill leachate	Reference sample*	—	98	—
	Blank†	0.05	94	—
		0.1	95	—
	Site A‡#	0	—	0.9
		0.05	105	—
		0.1	106	—
	Site B‡#	0	—	6.7
		0.05	89	—
		0.1	94	—
	Site C‡#	0	—	0.9
		0.05	110	—
		0.1	109	—

* U.S. EPA QC sample, 0.109 mg P/L.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate.

§ Sample dilutions: A - 5-fold; B - 100-fold; C - 10-fold. Typical relative difference between duplicates 0.5%.

|| Sample dilutions: A - 5-fold; B - 20-fold; C - 10-fold. Typical relative difference between duplicates 0.3%.

Sample dilutions: A - 20-fold; B - 10-fold; C - 20-fold. Typical relative difference between duplicates 1%.

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TABLE 4500-P:IV. RECOVERIES OF TOTAL PHOSPHORUS

Compound	Known Concentration <i>mg PIL</i>	Mean Concentration Recovered <i>mg PIL</i>	Recovery %	Relative Standard Deviation %
Sodium	10	8.99	90.3	0.5
pyrophosphate	2	1.81	90.2	0.6
	0.2	0.19	93.4	1.0
Phenylphosphate	10	10.10	101.5	0.2
	2	2.12	105.0	5.6
	0.2	0.20	101.3	1.0
Trimethylphosphate	10	8.99	90.3	0.2
	2	1.86	92.7	0.3
	0.2	0.18	95.3	1.1
Sodium	10	10.61	106.7	1.0
tripolyphosphate	2	2.14	106.6	0.2
	0.2	0.22	108.9	0.9

TABLE 4500-P:V. COMPARISON OF MANUAL AND IN-LINE TOTAL PHOSPHORUS METHODS

Samples	Concentration by Manual Persulfate Digestion Method <i>mg PIL</i>	Concentration by In-Line Digestion Method <i>mg PIL</i>	Relative Difference %
Influent (I2)	5.93	5.52	-6.9
Influent (I3)	5.03	4.50	-10.5
Influent (I5)	2.14	2.11	-1.4
Influent (I6)	1.88	1.71	-9.0
Effluent (E1)	3.42	2.87	-16.1
Effluent (E2)	3.62	3.55	-1.9
Effluent (E3)	3.26	3.34	+2.4
Effluent (E4)	8.36	8.16	-2.4

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Samples	Concentration by Manual Persulfate Digestion Method <i>mg PIL</i>	Concentration by In-Line Digestion Method <i>mg PIL</i>	Relative Difference %
Effluent (E5)	0.65	0.71	+9.2
Effluent (E6)	0.74	0.81	+9.5
Phenylphosphate	1.95	1.91	-2.1
Trimethylphosphate	1.87	1.80	-3.7
Sodium pyrophosphate	1.90	1.73	-8.9
Sodium tripolyphosphate	1.84	1.73	-6.0

TABLE 4500-SiO₂:I. SELECTION OF LIGHT PATH LENGTH FOR VARIOUS SILICA CONCENTRATIONS

Light Path <i>cm</i>	Method C	Method D	
	Silica in 55 mL Final Volume <i>μg</i>	Silica in 55 mL Final Volume <i>μg</i>	
		650 nm Wavelength	815 nm Wavelength
1	200–1300	40–300	20–100
2	100–700	20–150	10–50
5	4–250	7–50	4–20
10	20–130	4–30	2–10

TABLE 4500-SiO₂:II. PREPARATION OF PERMANENT COLOR STANDARDS FOR VISUAL DETERMINATION OF SILICA

Values in Silica <i>μg</i>	Potassium Chromate Solution <i>mL</i>	Borax Solution <i>mL</i>	Water <i>mL</i>

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Values in Silica μg	Potassium Chromate Solution mL	Borax Solution mL	Water mL
0	0.0	25	30
100	1.0	25	29
200	2.0	25	28
400	4.0	25	26
500	5.0	25	25
750	7.5	25	22
1000	10.0	25	20

TABLE 4500-S²⁻:I. DILUTION OF SULFIDE STOCK SOLUTION FOR PREPARATION OF STANDARDS (100 mL TOTAL VOLUME)

Dilution	Alkaline Antioxidant Reagent mL	Sulfide Solution	Sulfide Solution mL	1M Zinc Acetate mL
1:10	45	Stock	10	0.15
1:100	50	Stock	1	0.15
1:1 000	45	1:100	10	0.14
1:10 000	50	1:100	1	0.15

TABLE 4500-SO₄²⁻:I. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg SO ₄ ²⁻ /L	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	99	—
	Blank†	10.0	99	—
		20.0	99	—
	Site A‡	0	—	0.7
		10.0	109	—

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Matrix	Sample/Blank Designation	Known Addition <i>mg</i> $\text{SO}_4^{2-}/\text{L}$	Recovery %	Relative Standard Deviation %
Wastewater treatment plant effluent	Site B‡	20.0	110	—
		0	—	0.7
		10.0	106	—
	Site C‡	20.0	112	—
		0	—	1.9
		10.0	104	—
	Reference sample*	20.0	107	—
		—	99	—
		10.0	95	—
	Blank†	20.0	99	—
		0	—	0.9
		10.0	108	—
	Site A‡	20.0	108	—
		0	—	2.4
		10.0	107	—
	Site B‡	20.0	107	—
		0	—	0.6
		10.0	97	—
	Site C‡	20.0	104	—
		—	100	—
Landfill leachate	Reference sample*	10.0	100	—
		20.0	99	—
		0	—	0.7
	Blank†	10.0	106	—
		20.0	110	—
		0	—	0.5
	Site A‡	10.0	106	—
		20.0	110	—
		0	—	0.5
	Site B‡	10.0	106	—
		20.0	107	—

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Matrix	Sample/Blank Designation	Known Addition <i>mg</i> $\text{SO}_4^{2-}/\text{L}$	Recovery %	Relative Standard Deviation %
	Site C‡	0	—	0.9
		10.0	101	—
		20.0	103	—

* U.S. EPA QC sample, 20.0 mg $\text{SO}_4^{2-}/\text{L}$.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate. Typical relative difference between duplicates 1%. Sample dilutions: Influent and effluent, all sites - 5-fold; leachate A - 100-fold; B - 50-fold; C - 10-fold.

Part 5000 AGGREGATE ORGANIC CONSTITUENTS

5010 INTRODUCTION

5010 A. General Discussion

Analyses for organic matter in water and wastewater can be classified into two general types of measurements: those that quantify an aggregate amount of organic matter comprising organic constituents with a common characteristic and those that quantify individual organic compounds. The latter can be found in Part 6000. The former, described here in Part 5000, have been grouped into four categories: oxygen-demanding substances, organically bound elements, classes of compounds, and formation potentials.

Methods for total organic carbon and chemical oxygen demand are used to assess the total amount of organics present. Gross fractions of the organic matter can be identified analytically, as in the measurement of BOD, which is an index of the biodegradable organics present, oil and grease, which represents material extractable from a sample by a nonpolar solvent, or dissolved organic halide (DOX), which measures organically bound halogens. Trihalomethane formation potential is an aggregate measure of the total concentration of trihalomethanes formed upon chlorination of a water sample.

Analyses of organics are made to assess the concentration and general composition of organic matter in raw water supplies, wastewaters, treated effluents, and receiving waters; and to

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determine the efficiency of treatment processes.

5010 B. Sample Collection and Preservation

The sampling, field treatment, preservation, and storage of samples taken for organic matter analysis are covered in detail in the individual introductions to the methods. If possible, analyze samples immediately because preservatives often interfere with the tests. Otherwise, store at a low temperature (4°C) immediately after collection to preserve most samples. Use chemical preservatives only when they are shown not to interfere with the examinations to be made (see Section 1060). Never use preservatives for samples to be analyzed for BOD. When preservatives are used, add them to the sample bottle initially so that all portions are preserved as soon as collected. No single method of preservation is entirely satisfactory; choose the preservative with due regard to the determinations that are to be made. All methods of preservation may be inadequate when applied to samples containing significant amounts of suspended matter.

5020 QUALITY ASSURANCE/QUALITY CONTROL

Part 1000 contains important information relevant to analyses included in Part 5000. Give particular attention to Section 1020B (Quality Control), Section 1060 (Collection and Preservation of Samples), Section 1080 (Reagent Water), and Section 1090 (Laboratory Occupational Health and Safety), all of which are critical for many of the Part 5000 methods.

Take special precautions when analyses are performed by independent laboratories. Reliable use of independent laboratories deserves the same quality assurance procedures observed for in-house analyses: replicate samples, samples with known additions, and blanks.

Preparation of samples with known additions may not be feasible for certain analyses. In such cases, consider using a mixture, in varying ratios, of several samples. Use the reported concentrations in the samples and the proportions in which they were mixed to calculate the expected concentration in the mixture. Examine laboratory performance using externally prepared standards and check samples (see Section 1020B).

Reagent water (Section 1080) should give satisfactory results for most of the analyses in Part 5000, but additional purification steps may be needed for certain methods, such as dissolved organic halogen (DOX) and disinfection by-product formation potential (DBPFP).

5210 BIOCHEMICAL OXYGEN DEMAND (BOD)*#(97)

5210 A. Introduction

1. General Discussion

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The biochemical oxygen demand (BOD) determination is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of wastewaters, effluents, and polluted waters. The test has its widest application in measuring waste loadings to treatment plants and in evaluating the BOD-removal efficiency of such treatment systems. The test measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron. It also may measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. The seeding and dilution procedures provide an estimate of the BOD at pH 6.5 to 7.5.

Measurements of oxygen consumed in a 5-d test period (5-d BOD or BOD₅, Section 5210B), oxygen consumed after 60 to 90 d of incubation (ultimate BOD or UBOD, Section 5210C), and continuous oxygen uptake (respirometric method, Section 5210D) are described here. Many other variations of oxygen demand measurements exist, including using shorter and longer incubation periods and tests to determine rates of oxygen uptake. Alternative seeding, dilution, and incubation conditions can be chosen to mimic receiving-water conditions, thereby providing an estimate of the environmental effects of wastewaters and effluents.

The UBOD measures the oxygen required for the total degradation of organic material (ultimate carbonaceous demand) and/or the oxygen to oxidize reduced nitrogen compounds (ultimate nitrogenous demand). UBOD values and appropriate kinetic descriptions are needed in water quality modeling studies such as UBOD: BOD₅ ratios for relating stream assimilative capacity to regulatory requirements; definition of river, estuary, or lake deoxygenation kinetics; and instream ultimate carbonaceous BOD (UCBOD) values for model calibration.

2. Carbonaceous Versus Nitrogenous BOD

A number of factors, for example, soluble versus particulate organics, settleable and floatable solids, oxidation of reduced iron and sulfur compounds, or lack of mixing may affect the accuracy and precision of BOD measurements. Presently, there is no way to include adjustments or corrections to account for the effect of these factors.

Oxidation of reduced forms of nitrogen, such as ammonia and organic nitrogen, can be mediated by microorganisms and exert nitrogenous demand. Nitrogenous demand historically has been considered an interference in the determination of BOD, as clearly evidenced by the inclusion of ammonia in the dilution water. The interference from nitrogenous demand can now be prevented by an inhibitory chemical.¹ If an inhibiting chemical is not used, the oxygen demand measured is the sum of carbonaceous and nitrogenous demands.

Measurements that include nitrogenous demand generally are not useful for assessing the oxygen demand associated with organic material. Nitrogenous demand can be estimated directly from ammonia nitrogen (Section 4500-NH₃); and carbonaceous demand can be estimated by subtracting the theoretical equivalent of the reduced nitrogen oxidation from uninhibited test results. However, this method is cumbersome and is subject to considerable error. Chemical

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inhibition of nitrogenous demand provides a more direct and more reliable measure of carbonaceous demand.

The extent of oxidation of nitrogenous compounds during the 5-d incubation period depends on the concentration and type of microorganisms capable of carrying out this oxidation. Such organisms usually are not present in raw or settled primary sewage in sufficient numbers to oxidize sufficient quantities of reduced nitrogen forms in the 5-d BOD test. Many biological treatment plant effluents contain sufficient numbers of nitrifying organisms to cause nitrification in BOD tests. Because oxidation of nitrogenous compounds can occur in such samples, inhibition of nitrification as directed in Section 5210B.4e6) is recommended for samples of secondary effluent, for samples seeded with secondary effluent, and for samples of polluted waters.

Report results as carbonaceous biochemical oxygen demand (CBOD₅) when inhibiting the nitrogenous oxygen demand. When nitrification is not inhibited, report results as BOD₅.

3. Dilution Requirements

The BOD concentration in most wastewaters exceeds the concentration of dissolved oxygen (DO) available in an air-saturated sample. Therefore, it is necessary to dilute the sample before incubation to bring the oxygen demand and supply into appropriate balance. Because bacterial growth requires nutrients such as nitrogen, phosphorus, and trace metals, these are added to the dilution water, which is buffered to ensure that the pH of the incubated sample remains in a range suitable for bacterial growth. Complete stabilization of a sample may require a period of incubation too long for practical purposes; therefore, 5 d has been accepted as the standard incubation period.

If the dilution water is of poor quality, the BOD of the dilution water will appear as sample BOD. This effect will be amplified by the dilution factor. A positive bias will result. The methods included below (Section 5210B and Section 5210C) contain both a dilution-water check and a dilution-water blank. Seeded dilution waters are checked further for acceptable quality by measuring their consumption of oxygen from a known organic mixture, usually glucose and glutamic acid.

The source of dilution water is not restricted and may be distilled, tap, or receiving-stream water free of biodegradable organics and bioinhibitory substances such as chlorine or heavy metals. Distilled water may contain ammonia or volatile organics; deionized waters often are contaminated with soluble organics leached from the resin bed. Use of copper-lined stills or copper fittings attached to distilled water lines may produce water containing excessive amounts of copper (see Section 3500-Cu).

4. Reference

1. YOUNG, J.C. 1973. Chemical methods for nitrification control. *J. Water Pollut. Control Fed.* 45:637.

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- THERIAULT, E.J., P.D. MCNAMEE & C.T. BUTTERFIELD. 1931. Selection of dilution water for use in oxygen demand tests. *Pub. Health Rep.* 46:1084.
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5210 B. 5-Day BOD Test

1. General Discussion

a. Principle: The method consists of filling with sample, to overflowing, an airtight bottle of the specified size and incubating it at the specified temperature for 5 d. Dissolved oxygen is measured initially and after incubation, and the BOD is computed from the difference between initial and final DO. Because the initial DO is determined shortly after the dilution is made, all oxygen uptake occurring after this measurement is included in the BOD measurement.

b. Sampling and storage: Samples for BOD analysis may degrade significantly during storage between collection and analysis, resulting in low BOD values. Minimize reduction of BOD by analyzing sample promptly or by cooling it to near-freezing temperature during storage. However, even at low temperature, keep holding time to a minimum. Warm chilled samples to $20 \pm 3^{\circ}\text{C}$ before analysis.

1) Grab samples—If analysis is begun within 2 h of collection, cold storage is unnecessary. If analysis is not started within 2 h of sample collection, keep sample at or below 4°C from the time of collection. Begin analysis within 6 h of collection; when this is not possible because the sampling site is distant from the laboratory, store at or below 4°C and report length and temperature of storage with the results. In no case start analysis more than 24 h after grab sample collection. When samples are to be used for regulatory purposes make every effort to deliver samples for analysis within 6 h of collection.

2) Composite samples—Keep samples at or below 4°C during compositing. Limit compositing period to 24 h. Use the same criteria as for storage of grab samples, starting the measurement of holding time from end of compositing period. State storage time and conditions as part of the results.

2. Apparatus

a. Incubation bottles: Use glass bottles having 60 mL or greater capacity (300-mL bottles

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having a ground-glass stopper and a flared mouth are preferred). Clean bottles with a detergent, rinse thoroughly, and drain before use. As a precaution against drawing air into the dilution bottle during incubation, use a water seal. Obtain satisfactory water seals by inverting bottles in a water bath or by adding water to the flared mouth of special BOD bottles. Place a paper or plastic cup or foil cap over flared mouth of bottle to reduce evaporation of the water seal during incubation.

b. Air incubator or water bath, thermostatically controlled at $20 \pm 1^\circ\text{C}$. Exclude all light to prevent possibility of photosynthetic production of DO.

3. Reagents

Prepare reagents in advance but discard if there is any sign of precipitation or biological growth in the stock bottles. Commercial equivalents of these reagents are acceptable and different stock concentrations may be used if doses are adjusted proportionally.

a. Phosphate buffer solution: Dissolve 8.5 g KH_2PO_4 , 21.75 g K_2HPO_4 , 33.4 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, and 1.7 g NH_4Cl in about 500 mL distilled water and dilute to 1 L. The pH should be 7.2 without further adjustment. Alternatively, dissolve 42.5 g KH_2PO_4 or 54.3 g K_2HPO_4 in about 700 mL distilled water. Adjust pH to 7.2 with 30% NaOH and dilute to 1 L.

b. Magnesium sulfate solution: Dissolve 22.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in distilled water and dilute to 1 L.

c. Calcium chloride solution: Dissolve 27.5 g CaCl_2 in distilled water and dilute to 1 L.

d. Ferric chloride solution: Dissolve 0.25 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in distilled water and dilute to 1 L.

e. Acid and alkali solutions, 1N, for neutralization of caustic or acidic waste samples.

1) Acid—Slowly and while stirring, add 28 mL conc sulfuric acid to distilled water. Dilute to 1 L.

2) Alkali—Dissolve 40 g sodium hydroxide in distilled water. Dilute to 1 L.

f. Sodium sulfite solution: Dissolve 1.575 g Na_2SO_3 in 1000 mL distilled water. This solution is not stable; prepare daily.

g. Nitrification inhibitor, 2-chloro-6-(trichloromethyl) pyridine.*#(98)

h. Glucose-glutamic acid solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 h. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1 L. Prepare fresh immediately before use.

i. Ammonium chloride solution: Dissolve 1.15 g NH_4Cl in about 500 mL distilled water, adjust pH to 7.2 with NaOH solution, and dilute to 1 L. Solution contains 0.3 mg N/mL.

j. Dilution water: Use demineralized, distilled, tap, or natural water for making sample dilutions.

4. Procedure

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a. Preparation of dilution water: Place desired volume of water (¶ 3 j) in a suitable bottle and add 1 mL each of phosphate buffer, MgSO_4 , CaCl_2 , and FeCl_3 solutions/L of water. Seed dilution water, if desired, as described in ¶ 4d. Test dilution water as described in ¶ 4h so that water of assured quality always is on hand.

Before use bring dilution water temperature to $20 \pm 3^\circ\text{C}$. Saturate with DO by shaking in a partially filled bottle or by aerating with organic-free filtered air. Alternatively, store in cotton-plugged bottles long enough for water to become saturated with DO. Protect water quality by using clean glassware, tubing, and bottles.

b. Dilution water storage: Source water (¶ 3 j) may be stored before use as long as the prepared dilution water meets quality control criteria in the dilution water blank (¶ 4h). Such storage may improve the quality of some source waters but may allow biological growth to cause deterioration in others. Preferably do not store prepared dilution water for more than 24 h after adding nutrients, minerals, and buffer unless dilution water blanks consistently meet quality control limits. Discard stored source water if dilution water blank shows more than 0.2 mg/L DO depletion in 5 d.

c. Glucose-glutamic acid check: Because the BOD test is a bioassay its results can be influenced greatly by the presence of toxicants or by use of a poor seeding material. Distilled waters frequently are contaminated with copper; some sewage seeds are relatively inactive. Low results always are obtained with such seeds and waters. Periodically check dilution water quality, seed effectiveness, and analytical technique by making BOD measurements on a mixture of 150 mg glucose/L and 150 mg glutamic acid/L as a “standard” check solution. Glucose has an exceptionally high and variable oxidation rate but when it is used with glutamic acid, the oxidation rate is stabilized and is similar to that obtained with many municipal wastes. Alternatively, if a particular wastewater contains an identifiable major constituent that contributes to the BOD, use this compound in place of the glucose-glutamic acid.

Determine the 5-d 20°C BOD of a 2% dilution of the glucose-glutamic acid standard check solution using the techniques outlined in ¶s 4d-j. Adjust concentrations of commercial mixtures to give 3 mg/L glucose and 3 mg/L glutamic acid in each GGA test bottle. Evaluate data as described in ¶ 6, Precision and Bias.

d. Seeding:

1) Seed source—It is necessary to have present a population of microorganisms capable of oxidizing the biodegradable organic matter in the sample. Domestic wastewater, unchlorinated or otherwise-undisinfected effluents from biological waste treatment plants, and surface waters receiving wastewater discharges contain satisfactory microbial populations. Some samples do not contain a sufficient microbial population (for example, some untreated industrial wastes, disinfected wastes, high-temperature wastes, or wastes with extreme pH values). For such wastes seed the dilution water or sample by adding a population of microorganisms. The preferred seed is effluent or mixed liquor from a biological treatment system processing the waste. Where such seed is not available, use supernatant from domestic wastewater after settling at room

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temperature for at least 1 h but no longer than 36 h. When effluent or mixed liquor from a biological treatment process is used, inhibition of nitrification is recommended.

Some samples may contain materials not degraded at normal rates by the microorganisms in settled domestic wastewater. Seed such samples with an adapted microbial population obtained from the undisinfected effluent or mixed liquor of a biological process treating the waste. In the absence of such a facility, obtain seed from the receiving water below (preferably 3 to 8 km) the point of discharge. When such seed sources also are not available, develop an adapted seed in the laboratory by continuously aerating a sample of settled domestic wastewater and adding small daily increments of waste. Optionally use a soil suspension or activated sludge, or a commercial seed preparation to obtain the initial microbial population. Determine the existence of a satisfactory population by testing the performance of the seed in BOD tests on the sample. BOD values that increase with time of adaptation to a steady high value indicate successful seed adaptation.

2) Seed control—Determine BOD of the seeding material as for any other sample. This is the *seed control*. From the value of the seed control and a knowledge of the seeding material dilution (in the dilution water) determine seed DO uptake. Ideally, make dilutions of seed such that the largest quantity results in at least 50% DO depletion. A plot of DO depletion, in milligrams per liter, versus milliliters of seed for all bottles having a 2-mg/L depletion and a 1.0-mg/L minimum residual DO should present a straight line for which the slope indicates DO depletion per milliliter of seed. The DO-axis intercept is oxygen depletion caused by the dilution water and should be less than 0.1 mg/L (§ 4h). Alternatively, divide DO depletion by volume of seed in milliliters for each seed control bottle having a 2-mg/L depletion and a 1.0-mg/L residual DO. Average the results for all bottles meeting minimum depletion and residual DO criteria. The DO uptake attributable to the seed added to each bottle should be between 0.6 and 1.0 mg/L, but the amount of seed added should be adjusted from this range to that required to provide glucose-glutamic acid check results in the range of 198 ± 30.5 mg/L. To determine DO uptake for a test bottle, subtract DO uptake attributable to the seed from total DO uptake (see § 5).

Techniques for adding seeding material to dilution water are described for two sample dilution methods (§ 4 f).

e. *Sample pretreatment*: Check pH of all samples before testing unless previous experience indicates that pH is within the acceptable range.

1) Samples containing caustic alkalinity (pH >8.5) or acidity (pH <6.0)—Neutralize samples to pH 6.5 to 7.5 with a solution of sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH) of such strength that the quantity of reagent does not dilute the sample by more than 0.5%. The pH of dilution water should not be affected by the lowest sample dilution. Always seed samples that have been pH-adjusted.

2) Samples containing residual chlorine compounds—If possible, avoid samples containing residual chlorine by sampling ahead of chlorination processes. If the sample has been chlorinated but no detectable chlorine residual is present, seed the dilution water. If residual chlorine is present, dechlorinate sample and seed the dilution water (§ 4 f). Do not test

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chlorinated/dechlorinated samples without seeding the dilution water. In some samples chlorine will dissipate within 1 to 2 h of standing in the light. This often occurs during sample transport and handling. For samples in which chlorine residual does not dissipate in a reasonably short time, destroy chlorine residual by adding Na_2SO_3 solution. Determine required volume of Na_2SO_3 solution on a 100- to 1000-mL portion of neutralized sample by adding 10 mL of 1 + 1 acetic acid or 1 + 50 H_2SO_4 , 10 mL potassium iodide (KI) solution (10 g/100 mL) per 1000 mL portion, and titrating with Na_2SO_3 solution to the starch-iodine end point for residual. Add to neutralized sample the relative volume of Na_2SO_3 solution determined by the above test, mix, and after 10 to 20 min check sample for residual chlorine. (NOTE: Excess Na_2SO_3 exerts an oxygen demand and reacts slowly with certain organic chloramine compounds that may be present in chlorinated samples.)

3) Samples containing other toxic substances—Certain industrial wastes, for example, plating wastes, contain toxic metals. Such samples often require special study and treatment.

4) Samples supersaturated with DO—Samples containing more than 9 mg DO/L at 20°C may be encountered in cold waters or in water where photosynthesis occurs. To prevent loss of oxygen during incubation of such samples, reduce DO to saturation at 20°C by bringing sample to about 20°C in partially filled bottle while agitating by vigorous shaking or by aerating with clean, filtered compressed air.

5) Sample temperature adjustment—Bring samples to $20 \pm 1^\circ\text{C}$ before making dilutions.

6) Nitrification inhibition—If nitrification inhibition is desired add 3 mg 2-chloro-6-(trichloro methyl) pyridine (TCMP) to each 300-mL bottle before capping or add sufficient amounts to the dilution water to make a final concentration of 10 mg/L. (NOTE: Pure TCMP may dissolve slowly and can float on top of the sample. Some commercial formulations dissolve more readily but are not 100% TCMP; adjust dosage accordingly.) Samples that may require nitrification inhibition include, but are not limited to, biologically treated effluents, samples seeded with biologically treated effluents, and river waters. Note the use of nitrogen inhibition in reporting results.

f. Dilution technique: Make several dilutions of sample that will result in a residual DO of at least 1 mg/L and a DO uptake of at least 2 mg/L after a 5-d incubation. Five dilutions are recommended unless experience with a particular sample shows that use of a smaller number of dilutions produces at least two bottles giving acceptable minimum DO depletion and residual limits. A more rapid analysis, such as COD, may be correlated approximately with BOD and serve as a guide in selecting dilutions. In the absence of prior knowledge, use the following dilutions: 0.0 to 1.0% for strong industrial wastes, 1 to 5% for raw and settled wastewater, 5 to 25% for biologically treated effluent, and 25 to 100% for polluted river waters.

Prepare dilutions either in graduated cylinders or volumetric glassware, and then transfer to BOD bottles or prepare directly in BOD bottles. Either dilution method can be combined with any DO measurement technique. The number of bottles to be prepared for each dilution depends

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on the DO technique and the number of replicates desired.

When using graduated cylinders or volumetric flasks to prepare dilutions, and when seeding is necessary, add seed either directly to dilution water or to individual cylinders or flasks before dilution. Seeding of individual cylinders or flasks avoids a declining ratio of seed to sample as increasing dilutions are made. When dilutions are prepared directly in BOD bottles and when seeding is necessary, add seed directly to dilution water or directly to the BOD bottles. When a bottle contains more than 67% of the sample after dilution, nutrients may be limited in the diluted sample and subsequently reduce biological activity. In such samples, add the nutrient, mineral, and buffer solutions (§ 3a through e) directly to individual BOD bottles at a rate of 1 mL/L (0.33 mL/300-mL bottle) or use commercially prepared solutions designed to dose the appropriate bottle size.

1) Dilutions prepared in graduated cylinders or volumetric flasks—If the azide modification of the titrimetric iodometric method (Section 4500-O.C) is used, carefully siphon dilution water, seeded if necessary, into a 1- to 2-L-capacity flask or cylinder. Fill half full without entraining air. Add desired quantity of carefully mixed sample and dilute to appropriate level with dilution water. Mix well with a plunger-type mixing rod; avoid entraining air. Siphon mixed dilution into two BOD bottles. Determine initial DO on one of these bottles. Stopper the second bottle tightly, water-seal, and incubate for 5 d at 20°C. If the membrane electrode method is used for DO measurement, siphon dilution mixture into one BOD bottle. Determine initial DO on this bottle and replace any displaced contents with sample dilution to fill the bottle. Stopper tightly, water-seal, and incubate for 5 d at 20°C.

2) Dilutions prepared directly in BOD bottles—Using a wide-tip volumetric pipet, add the desired sample volume to individual BOD bottles of known capacity. Add appropriate amounts of seed material either to the individual BOD bottles or to the dilution water. Fill bottles with enough dilution water, seeded if necessary, so that insertion of stopper will displace all air, leaving no bubbles. For dilutions greater than 1:100 make a primary dilution in a graduated cylinder before making final dilution in the bottle. When using titrimetric iodometric methods for DO measurement, prepare two bottles at each dilution. Determine initial DO on one bottle. Stopper second bottle tightly, water-seal, and incubate for 5 d at 20°C. If the membrane electrode method is used for DO measurement, prepare only one BOD bottle for each dilution. Determine initial DO on this bottle and replace any displaced contents with dilution water to fill the bottle. Stopper tightly, water-seal, and incubate for 5 d at 20°C. Rinse DO electrode between determinations to prevent cross-contamination of samples.

Use the azide modification of the iodometric method (Section 4500-O.C) or the membrane electrode method (Section 4500-O.G) to determine initial DO on all sample dilutions, dilution water blanks, and where appropriate, seed controls.

If the membrane electrode method is used, the azide modification of the iodometric method (Method 4500-O.C) is recommended for calibrating the DO probe.

g. Determination of initial DO: If the sample contains materials that react rapidly with DO, determine initial DO immediately after filling BOD bottle with diluted sample. If rapid initial

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DO uptake is insignificant, the time period between preparing dilution and measuring initial DO is not critical but should not exceed 30 min.

h. Dilution water blank: Use a dilution water blank as a rough check on quality of unseeded dilution water and cleanliness of incubation bottles. Together with each batch of samples incubate a bottle of unseeded dilution water. Determine initial and final DO as in ¶s 4g and j. The DO uptake should not be more than 0.2 mg/L and preferably not more than 0.1 mg/L. Discard all dilution water having a DO uptake greater than 0.2 mg/L and either eliminate source of contamination or select an alternate dilution water source..

i. Incubation: Incubate at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ BOD bottles containing desired dilutions, seed controls, dilution water blanks, and glucose-glutamic acid checks. Water-seal bottles as described in ¶ 4 f.

j. Determination of final DO: After 5 d incubation determine DO in sample dilutions, blanks, and checks as in ¶ 4g.

5. Calculation

For each test bottle meeting the 2.0-mg/L minimum DO depletion and the 1.0-mg/L residual DO, calculate BOD_5 as follows:

When dilution water is not seeded:

$$\text{BOD}_5, \text{ mg/L} = \frac{D_1 - D_2}{P}$$

When dilution water is seeded:

$$\text{BOD}_5, \text{ mg/L} = \frac{D_1 - D_2}{P}$$

where:

D_1 = DO of diluted sample immediately after preparation, mg/L,

D_2 = DO of diluted sample after 5 d incubation at 20°C , mg/L,

P = decimal volumetric fraction of sample used,

B_1 = DO of seed control before incubation, mg/L (¶ 4d),

B_2 = DO of seed control after incubation mg/L (¶ 4d), and

f = ratio of seed in diluted sample to seed in seed control = (% seed in diluted sample)/(% seed in seed control).

If seed material is added directly to sample or to seed control bottles:

$$f = (\text{volume of seed in diluted sample})/(\text{volume of seed in seed control})$$

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Report results as CBOD₅ if nitrification is inhibited.

If more than one sample dilution meets the criteria of a residual DO of at least 1 mg/L and a DO depletion of at least 2 mg/L and there is no evidence of toxicity at higher sample concentrations or the existence of an obvious anomaly, average results in the acceptable range.

In these calculations, do not make corrections for DO uptake by the dilution water blank during incubation. This correction is unnecessary if dilution water meets the blank criteria stipulated above. If the dilution water does not meet these criteria, proper corrections are difficult ; do not record results or, as a minimum, mark them as not meeting quality control criteria.

6. Precision and Bias

There is no measurement for establishing bias of the BOD procedure. The glucose-glutamic acid check prescribed in ¶ 4c is intended to be a reference point for evaluation of dilution water quality, seed effectiveness, and analytical technique. Single-laboratory tests using a 300-mg/L mixed glucose-glutamic acid solution provided the following results:

Number of months: 14
Number of triplicates: 421
Average monthly recovery: 204 mg/L
Average monthly standard deviation: 10.4 mg/L

In a series of interlaboratory studies,¹ each involving 2 to 112 laboratories (and as many analysts and seed sources), 5-d BOD measurements were made on synthetic water samples containing a 1:1 mixture of glucose and glutamic acid in the total concentration range of 3.3 to 231 mg/L. The regression equations for mean value, $\bar{X}$, and standard deviation, S , from these studies were:

$$\begin{aligned}\bar{X} &= 0.658 (\text{added level, mg/L}) + 0.280 \text{ mg/L} \\ S &= 0.100 (\text{added level, mg/L}) + 0.547 \text{ mg/L}\end{aligned}$$

For the 300-mg/L mixed primary standard, the average 5-d BOD would be 198 mg/L with a standard deviation of 30.5 mg/L. When nitrification inhibitors are used, GGA test results falling outside the 198 ± 30.5 control limit quite often indicate use of incorrect amounts of seed. Adjust amount of seed added to the GGA test to achieve results falling within this range.

a. *Control limits:* Because of many factors affecting BOD tests in multilaboratory studies and the resulting extreme variability in test results, one standard deviation, as determined by interlaboratory tests, is recommended as a control limit for individual laboratories. Alternatively, for each laboratory, establish its control limits by performing a minimum of 25 glucose-glutamic acid checks (¶ 4c) over a period of several weeks or months and calculating the mean and standard deviation. Use the mean ± 3 standard deviations as the control limit for future glucose-glutamic acid checks. Compare calculated control limits to the single-laboratory tests

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presented above and to interlaboratory results. If control limits are outside the range of 198 ± 30.5 , re-evaluate the control limits and investigate source of the problem. If measured BOD for a glucose-glutamic acid check is outside the accepted control limit range, reject tests made with that seed and dilution water.

b. *Working range and detection limit:* The working range is equal to the difference between the maximum initial DO (7 to 9 mg/L) and minimum DO residual of 1 mg/L multiplied by the dilution factor. A lower detection limit of 2 mg/L is established by the requirement for a minimum DO depletion of 2 mg/L.

7. Reference

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5210 C. Ultimate BOD Test (PROPOSED)

1. General Discussion

The ultimate BOD test is an extension of the 5-d dilution BOD test as described in 5210B but with a number of specific test requirements and differences in application. The user should be familiar with the 5210B procedure before conducting tests for UBOD.

a. *Principle:* The method consists of placing a single sample dilution in full, airtight bottles and incubating under specified conditions for an extended period depending on wastewater, effluent, river, or estuary quality.¹ Dissolved oxygen (DO) is measured (with probes) initially and intermittently during the test. From the DO versus time series, UBOD is calculated by an appropriate statistical technique. For improved accuracy, run tests in triplicate.

Bottle size and incubation time are flexible to accommodate individual sample characteristics and laboratory limitations. Incubation temperature, however, is 20°C. Most effluents and some naturally occurring surface waters contain materials with oxygen demands exceeding the DO available in air-saturated water. Therefore, it is necessary either to dilute the sample or to monitor DO frequently to ensure that low DO or anaerobic conditions do not occur. When DO concentrations approach 2 mg/L, the sample should be reaerated.

Because bacterial growth requires nutrients such as nitrogen, phosphorus, and trace metals, the necessary amounts may be added to the dilution water together with buffer to ensure that pH

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remains in a range suitable for bacterial growth and seed to provide an adequate bacterial population. However, if the result is being used to estimate the rate of oxidation of naturally occurring surface waters, addition of nutrients and seed probably accelerates the decay rate and produces misleading results. If only UBOD is desired, it may be advantageous to add supplemental nutrients that accelerate decay and reduce the test duration. When nutrients are used, they also should be used in the dilution water blank. Because of the wide range of water and wastewater characteristics and varied applications of UBOD data, no specific nutrient or buffer formulations are included.

The extent of oxidation of nitrogenous compounds during the prescribed incubation period depends on the presence of microorganisms capable of carrying out this oxidation. Such organisms may not be present in wastewaters in sufficient numbers to oxidize significant quantities of reduced nitrogen. This situation may be reversed in naturally occurring surface waters. Erratic results may be obtained when a nitrification inhibitor is used;² therefore, the specified method precludes use of a nitrogen inhibitor unless prior experimental evidence on the particular sample suggests that it is acceptable.*#(99) Monitor NO_2^- -N and NO_3^- -N to compute the oxygen equivalency of the nitrification reaction. When these values are subtracted from the DO vs. time series, the carbonaceous BOD time series can be constructed.³ *b. Sampling and storage:* See Section 5210B.1b.

2. Apparatus

a. Incubation bottles: Glass bottles with ground-glass stoppers,†#(100) 2-L (or larger) capacity. Glass serum bottles of 4- to 10-L capacity are available. Alternatively use nonground-glass bottles with nonbiodegradable plastic caps as a plug insert. Do not reuse the plugs because discoloration occurs with continued use. Replace plugs every 7 to 14 d. Do not use rubber stoppers that may exert an oxygen demand. Clean bottles with a detergent and wash with dilute HCl (3N) to remove surface films and precipitated inorganic salts; rinse thoroughly with DI water before use. Cover top of bottles with paper after rinsing to prevent dust from collecting. To prevent drawing air into the sample bottle during incubation, use a water seal. If the bottle does not have a flared mouth, construct a water seal by making a watertight dam around the stopper (or plug) and fill with water from the reservoir as necessary. Cover dam with clean aluminum foil to retard evaporation. If a 2-L BOD bottle is used, fill reservoir with sample and cover with a polyethylene cap before incubation.

Place a clean magnetic stirring bar in each bottle to mix contents before making DO measurement or taking a subsample. Do not remove the magnets until the test is complete.

Alternatively use a series of 300-mL BOD bottles as described in 5210B, if larger bottles are not available or incubation space is limited.

b. Reservoir bottle: 4-L or larger glass bottle. Close with screw plastic cap or non-rubber plug.

c. Incubator or water bath, thermostatically controlled at $20 \pm 1^\circ\text{C}$. Exclude all light to prevent the possibility of photosynthetic production of DO.

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d. Oxygen-sensitive membrane electrode: See Section 4500-O.G.2.

3. Procedure

a. River water samples: Preferably fill large BOD bottle (>2 L, or alternatively 6 or more 300-mL BOD bottles) with sample at 20°C. Add no nutrients, seed, or nitrification inhibitor if in-bottle decay rates will be used to estimate in-stream rates. Do not dilute sample unless it is known by pretesting or by experience to have a high ultimate BOD (>20 mg/L).

Measure DO in each bottle, stopper, and make an airtight seal. Incubate at 20°C in the dark.

Measure DO in each bottle at intervals of at least 2 to 5 d over a period of 30 to 60 d (minimum of 6 to 8 readings) or longer under special circumstances. To avoid oxygen depletion in samples containing $\text{NH}_3\text{-N}$, measure DO more frequently until nitrification has taken place. If DO falls to about 2 mg/L, reaerate as directed below. Replace sample lost by the cap and DO probe displacement by adding 1 to 2 mL sample from the reservoir bottle.

When DO approaches 2 mg/L, reaerate. Pour a small amount of sample into a clean vessel and reaerate the remainder directly in the bottle by vigorous shaking or bubbling with purified air (medical grade). Refill bottle from the storage reservoir and measure DO. This concentration becomes the initial DO for the next measurement. If using 300-mL BOD bottles, pour all of the sample from the several bottles used into a clean vessel, reaerate, and refill the small bottles.

Analyze for nitrate plus nitrite nitrogen ($\text{NO}_3^- \text{-N} + \text{NO}_2^- \text{-N}$) (see Section 4500- NO_2^- and Section 4500- NO_3^-) on Days 0, 5, 10, 15, 20, and 30. Alternatively, determine $\text{NO}_2^- \text{-N}$ and $\text{NO}_3^- \text{-N}$ each time DO is determined, thereby producing corresponding BOD and nitrogen determinations. If the ultimate demand occurs at a time greater than 30 d, make additional analyses at 30-d intervals. Remove 10 to 20 mL from the bottle for these analyses. Refill bottle as necessary from the reservoir bottle. Preserve $\text{NO}_2^- \text{-N} + \text{NO}_3^- \text{-N}$ subsample with H_2SO_4 to pH <2 and refrigerate. If the purpose of the UBOD test is to assess the UBOD and not to provide data for rate calculations, measure nitrate nitrogen concentration only at Day 0 and on the last day of the test (kinetic rate estimates are not useful when the nitrification reaction is not followed).

Calculate oxygen consumption during each time interval and make appropriate corrections for nitrogenous oxygen demand. Correct by using $3.43 \times$ the $\text{NH}_3\text{-N}$ to $\text{NO}_2^- \text{-N}$ conversion plus $1.14 \times$ the $\text{NO}_2^- \text{-N}$ to $\text{NO}_3^- \text{-N}$ conversion to reflect the stoichiometry of the oxidation of NH_4^+ to NO_2^- or NO_3^- .

When using a dilution water blank, subtract DO uptake of the blank from the total DO consumed. High-quality reagent water without nutrients typically will consume a maximum of 1 mg DO/L in a 30- to 90-d period. If DO uptake of the dilution water is greater than 0.5 mg/L for a 20-d period, or 1 mg/L for a 90-d period, report the magnitude of the correction and try to obtain higher-quality dilution water for use with subsequent UBOD tests.

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When the weekly DO consumption drops below 1 to 2% of the total accumulative consumption, calculate the ultimate BOD using a nonlinear regression method.

b. Wastewater treatment plant samples: Use high-quality reagent water (see Section 1080) for dilution water. Add no nitrification inhibitors if decay rates are desired. If seed and nutrients are necessary, add the same amounts of each to the dilution water blank. Use minimal sample dilution. As a rule of thumb, the ultimate BOD of the diluted sample should be in the range of 20 to 30 mg/L. Dilution to this level probably will require two or three sample reaerations during the incubation period to avoid having dissolved oxygen concentrations fall below 2 mg/L.

Use 2-L or larger BOD bottles (alternatively, multiple 300-mL BOD bottles) for each dilution. Add desired volume of sample to each bottle and fill with dilution water.

Fill a BOD bottle with dilution water to serve as a dilution water blank. Treat blank the same as all samples. Follow procedure given in ¶ 3a) and incubate for at least as long as UBOD test.

4. Calculations

An example of results obtained for a wastewater sample, undiluted, without seed and nutrients, is given in Table 5210:I.

UBOD can be estimated by using a first-order model described as follows:

$$BOD_t = UBOD (1 - e^{-kt})$$

where:

BOD_t = oxygen uptake measured at time t , mg/L, and

k = first-order oxygen uptake rate.

The data in Table 5210:I were analyzed with a nonlinear regression technique applied to the above first-order model.⁴ However, a first-order kinetic model may not always be the best choice. Significantly better statistical fits usually are obtained with alternative kinetic models including sum of two first-order and logistic function models.^{1,3-8}

5. Precision and Bias

The precision of the ultimate BOD test was assessed with a series of replicate tests in a single laboratory. Interlaboratory studies have not been conducted.

Reference	Replicate No.	UBOD mg/L	Precision Summary*
2	1	154	$\mu = 151 \text{ mg/L}$
	2	154	
	3	145	CV = 3.5%

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Reference	Replicate No.	UBOD mg/L	Precision Summary*
5	1	10.3	μ = 10.0 mg/L CV = 5.8%
	2	11.1	
	3	9.6	
	4	9.9	
	5	9.8	
	6	9.6	
6	1	12.8	μ = 12.4 mg/L CV = 4.4%
	2	12.6	
	3	12.6	
	4	11.6	

* μ = mean, CV = coefficient of variation.

Bias was assessed by determining the BOD of a known concentration of glucose (150 mg/L) and glutamic acid (150 mg/L). This solution has a UBOD of 321 mg/L to 308 mg/L, depending on extent of nitrification. The results of the study conducted in triplicate were:¹

Estimated* UBOD mg/L	Theoretical BOD mg/L	Percent Difference
276	308/321	-10/-14
310	308/321	+1/-3
303	308/321	-2/-6

*By statistical model.

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5210 D. **Respirometric Method (PROPOSED)**

1. General Discussion

a. Principle: Respirometric methods provide direct measurement of the oxygen consumed by microorganisms from an air or oxygen-enriched environment in a closed vessel under conditions of constant temperature and agitation.

b. Uses: Respirometry measures oxygen uptake more or less continuously over time. Respirometric methods are useful for assessing: biodegradation of specific chemicals; treatability of organic industrial wastes; the effect of known amounts of toxic compounds on the oxygen-uptake reaction of a test wastewater or organic chemical; the concentration at which a pollutant or a wastewater measurably inhibits biological degradation; the effect of various treatments such as disinfection, nutrient addition, and pH adjustment on oxidation rates; the oxygen requirement for essentially complete oxidation of biologically oxidizable matter; the need for using adapted seed in other biochemical oxygen-uptake measurements, such as the dilution BOD test; and stability of sludges.

Respirometric data typically will be used comparatively, that is, in a direct comparison between oxygen uptakes from two test samples or from a test sample and a control. Because of inherent differences among uses, among seed cultures, among applications of results, and among instruments, a single procedure for respirometric tests applicable to all cases cannot be defined. Therefore, only basic recommendations and guidelines for overall test setup and procedure are given. Follow manufacturer's instructions for operating details for specific commercial instruments.

c. Types of respirometers: Four principal types of commercial respirometers are available. Manometric respirometers relate oxygen uptake to the change in pressure caused by oxygen

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consumption while maintaining a constant volume. Volumetric respirometers measure oxygen uptake in incremental changes in gas volume while maintaining a constant pressure at the time of reading. Electrolytic respirometers monitor the amount of oxygen produced by electrolysis of water to maintain a constant oxygen pressure within the reaction vessel. Direct-input respirometers deliver oxygen to the sample from a pure oxygen supply through metering on demand as detected by minute pressure differences. Most respirometers have been instrumented to permit data collection and processing by computer. Reaction-vessel contents are mixed by using a magnetic or mechanical stirring device or by bubbling the gaseous phase within the reaction vessel through the liquid phase. All respirometers remove carbon dioxide produced during biological growth by suspending a concentrated adsorbent (granular or solution) within the closed reaction chamber or by recirculating the gas phase through an external scrubber.

d. Interferences: Evolution of gases other than CO_2 may introduce errors in pressure or volume measurements; this is uncommon in the presence of dissolved oxygen. Incomplete CO_2 absorption will introduce errors if appropriate amounts and concentrations of alkaline absorbent are not used. Temperature fluctuations or inadequate mixing will introduce error. Fluctuations in barometric pressure can cause errors with some respirometers. Become familiar with the limits of the instrument to be used.

e. Minimum detectable concentration: Most commercial respirometers can detect oxygen demand in increments as small as 0.1 mg but test precision depends on the total amount of oxygen consumed at the time of reading, the precision of pressure or volume measurement, and the effect of temperature and barometric pressure changes. Upper limits of oxygen uptake rate are determined by the ability to transfer oxygen into the solution from the gas phase, which typically is related to mixing intensity. Transfer limits typically range from less than 10 mg $\text{O}_2/\text{L/h}$ for low-intensity mixing to above 100 mg $\text{O}_2/\text{L/h}$ for high-intensity mixing.

f. Relationship to dilution BOD: Variations in waste composition, substrate concentration, mixing, and oxygen concentrations from one wastewater source to another generally preclude use of a general relationship between oxygen uptake by respirometers and the 5-d, 20°C, BOD (see 5210B, above). Reasonably accurate correlations may be possible for a specific wastewater. The incubation period for respirometric measurements need not be 5 d because equally valid correlations can be made between the 5-d dilution BOD and respirometric oxygen uptake at any time after 2 d.^{1,2} The point of common dilution and respirometric BOD seems to occur at about 2 to 3 d incubation for municipal wastewaters. Correlations between respirometric measurements and 5-d BOD for industrial wastes and specific chemicals are less certain. Respirometric measurements also can provide an indication of the ultimate biochemical oxygen demand (UBOD) (see Section 5210C). In many cases, it is reasonable to consider that the 28-to 30-d oxygen uptake is essentially equal to the UBOD.³

More commonly, respirometers are used as a diagnostic tool. The continuous readout of oxygen consumption in respirometric measurements indicates lag, toxicity, or any abnormalities in the biodegradation reaction. The change in the normal shape of an oxygen-uptake curve in the

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first few hours may help to identify the effect of toxic or unusual wastes entering a treatment plant in time to make operating corrections.

g. Relationship to other test methods and protocols: This method supports most of the protocols and guidelines established by the European Organization for Economic Co-operation and Development³ (OECD) that require measurement of oxygen uptake.

h. Sampling and storage:

1) Grab samples—If analysis is begun within 2 h of sample collection, cold storage is unnecessary. Otherwise, keep sample at or below 4°C from the time of collection. Begin analysis within 6 h of collection; when this is not possible, store at or below 4°C and report length and temperature of storage. Never start analysis more than 24 h after grab sample collection.

2) Composite samples—Keep samples at or below 4°C during compositing. Limit compositing period to 24 h. Use the same criteria as for storage of grab samples, starting the measurement of holding time from the end of the compositing period. State storage time and conditions with results.

2. Apparatus

a. Respirometer system: Use commercial apparatus and check manufacturer's instructions for specific system requirements, reaction vessel type and volume, and instrument operating characteristics.

b. Incubator or water bath: Use a constant-temperature room, incubator chamber, or water bath to control temperature to $\pm 1^\circ\text{C}$. Exclude all light to prevent oxygen formation by algae in the sample. Use red, actinic-coated bottles for analysis outside of a darkened incubator.

3. Reagents

Formulations of reagent solutions are given for 1-L volumes, but smaller or larger volumes may be prepared according to need. Discard any reagent showing signs of biological growth or chemical precipitation. Stock solutions can be sterilized by autoclaving to provide longer shelf life.

a. Distilled water: Use only high-quality water distilled from a block tin or all-glass still (see Section 1080). Deionized water may be used but often contains high bacterial counts. The water must contain less than 0.01 mg heavy metals/L and be free of chlorine, chloramines, caustic alkalinity, organic material, or acids. Make all reagents with this water. When other waters are required for special-purpose testing, state clearly their source and quality characteristics.

b. Phosphate buffer solution, 1.5N: Dissolve 207 g sodium dihydrogen phosphate, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, in water. Neutralize to pH 7.2 with 6N KOH (¶ 3g below) and dilute to 1 L.

c. Ammonium chloride solution, 0.71N: Dissolve 38.2 g ammonium chloride, NH_4Cl , in water. Neutralize to pH 7.0 with KOH. Dilute to 1.0 L; 1 mL = 10 mg N.

d. Calcium chloride solution, 0.25N: Dissolve 27.7 g CaCl_2 in water and dilute to 1 L; 1 mL = 10 mg Ca.

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e. *Magnesium sulfate solution, 0.41N*: Dissolve 101 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in water and dilute to 1 L; 1 mL = 10 mg Mg.

f. *Ferric chloride solution, 0.018N*: Dissolve 4.84 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in water and dilute to 1 L; 1 mL = 1.0 mg Fe.

g. *Potassium hydroxide solution, 6N*: Dissolve 336 g KOH in about 700 mL water and dilute to 1 L. CAUTION: Add KOH to water slowly and use constant mixing to prevent excessive heat buildup. Alternately, use commercial solutions containing 30 to 50% KOH by weight.

h. *Acid solutions, 1N*: Add 28 mL conc H_2SO_4 or 83 mL conc HCl to about 700 mL water. Dilute to 1 L.

i. *Alkali solution, 1N*: Add 40 g NaOH to 700 mL water. Dilute to 1 L.

j. *Nitrification inhibitor*: Reagent-grade 2-chloro-6-(trichloromethyl) pyridine (TCMP) or equivalent.³*(101)

k. *Glucose-glutamic acid solution*: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 h. Add 15.0 g glucose and 15.0 g glutamic acid to distilled water and dilute to 1 L. Neutralize to pH 7.0 using 6N potassium hydroxide (¶ 3g). This solution may be stored for up to 1 week at 4°C.

l. *Electrolyte solution* (for electrolytic respirometers): Use manufacturer's recommended solution.

m. *Sodium sulfite solution, 0.025N*: Dissolve 1.575 g Na_2SO_3 in about 800 mL water. Dilute to 1 L. This solution is not stable; prepare daily or as needed.

n. *Trace element solution*: Dissolve 40 mg $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 57 mg H_3BO_3 , 43 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 35 mg $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, and 100 mg Fe-chelate (FeCl_3 -EDTA) in about 800 mL water. Dilute to 1 L. Sterilize at 120°C and 200 kPa (2 atm) pressure for 20 min.

o. *Yeast extract solution*:³ Add 15 mg laboratory- or pharmaceutical-grade brewer's yeast extract to 100 mL water. Make this solution fresh immediately before each test in which it is used.

p. *Nutrient solution*:³ Add 2.5 mL phosphate buffer solution (3b), 0.65 mL ammonium chloride solution (3c), 1.0 mL calcium chloride solution (3d), 0.22 mL magnesium sulfate solution (3a), 0.1 mL ferric chloride solution (3 f), 1 mL trace element solution (3n), and 1 mL yeast extract solution (3o) to about 900 mL water. Dilute to 1 L. This nutrient solution and those of ¶s n and o above are specifically formulated for use with the OECD method.³ (NOTE: A 10:1 concentrated nutrient solution can be made and diluted accordingly.)

4. Procedure

a. *Instrument operation*: Follow respirometer manufacturer's instructions for assembly, testing, calibration, and operation of the instrument. NOTE: The manufacturer's stated maximum

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and minimum limits of measurement are not always the same as the instrument output limits. Make sure that test conditions are within the limits of measurement.

b. Sample volume: Sample volume or concentration of organic chemicals to be added to test vessels is a function of expected oxygen uptake characteristics and oxygen transfer capability of the instrument. Small volumes or low concentrations may be required for high-strength wastes. Large volumes may be required for low-strength wastes to improve accuracy.

c. Data recording interval: Set instrument to give data readings at suitable intervals. Intervals of 15 min to 6 h typically are used.

d. Sample preparation:

1) Homogenization—If sample contains large settleable or floatable solids, homogenize it with a blender and transfer representative test portions while all solids are in suspension. If there is a concern for changing sample characteristics, skip this step.

2) pH adjustment—Neutralize samples to pH 7.0 with H_2SO_4 or NaOH of such strength (§s 3h and § 3i) that reagent quantity does not dilute the sample more than 0.5%.

3) Dechlorination—Avoid analyzing samples containing residual chlorine by collecting the samples ahead of chlorination processes. If residual chlorine is present, aerate as described in § 5) below or let stand in light for 1 to 2 h. If a chlorine residual persists, add Na_2SO_3 solution. Determine required volume of Na_2SO_3 solution by adding 10 mL 1 + 1 acetic acid or 1 + 50 H_2SO_4 and 10 mL potassium iodide solution (10 g/100 mL) to a portion of the sample. Titrate with 0.025N Na_2SO_3 solution to the starch-iodine end point (see Section 4500-Cl.B). Add to the neutralized sample a proportional volume of Na_2SO_3 solution determined above, mix, and after 10 to 20 min check for residual chlorine. Re-seed the sample (see § 4h below).

4) Samples containing toxic substances—Certain industrial wastes contain toxic metals or organic compounds. These often require special study and treatment.³

5) Initial oxygen concentration—If samples contain dissolved oxygen concentrations above or below the desired concentration, agitate or aerate with clean and filtered compressed air for about 1 h immediately before testing. Minimum and maximum actual DO concentrations will vary with test objectives. In some cases, pure oxygen may be added to respirometer vessels to increase oxygen levels above ambient.

6) Temperature adjustment—Bring samples and dilution water to desired test temperature ($\pm 1^\circ\text{C}$) before making dilutions or transferring to test vessels.

e. Sample dilution: Use distilled water or water from other appropriate sources free of organic matter. In some cases, receiving stream water may be used for dilution. Add desired sample volume to test vessels using a wide-tip volumetric pipet or other suitable volumetric glassware. Add dilution water to bring sample to about 80% of desired final volume. Add appropriate amounts of nutrients, minerals, buffer, nitrification inhibitor if desired, and seed culture as described in §§ 4 f and h below. Dilute sample to desired final volume. The number of

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test vessels to prepare for each dilution depends on test objectives and number of replicates desired.

f. Nutrients, minerals, and buffer: Add sufficient ammonia nitrogen to provide a COD:N:P ratio of 100:5:1 or a TOC:N:P ratio of 30:5:1. Add 2 mL each of calcium, magnesium, ferric chloride, and trace mineral solutions to each liter of diluted sample unless sufficient amounts of these minerals are present in the original sample. Phosphorus requirements will be met by the phosphate buffer if it is used (1 mL/50 mg/L COD or ultimate BOD of diluted sample usually is sufficient to maintain pH between 6.8 and 7.2). Be cautious in adding phosphate buffer to samples containing metal salts because metal phosphates may precipitate and show less toxic or beneficial effect than when phosphate is not present. For OECD-compatible tests, substitute the nutrient, mineral, and buffer amounts listed in ¶ 3 p for the above nutrient/ mineral/buffer quantities.

g. Nitrification inhibition: If nitrification inhibition is desired, add 10 mg 2-chloro-6-(trichloromethyl) pyridine (TCMP)/L sample in the test vessel. Samples that may nitrify readily include biologically treated effluents, samples seeded with biologically treated effluents, and river waters.⁴

h. Seeding: See 5210B.4d1) for seed preparation. Use sufficient amounts of seed culture to prevent major lags in the oxygen uptake reaction but not so much that the oxygen uptake of the seed exceeds about 10% of the oxygen uptake of the seeded sample.

Determine the oxygen uptake of the seeding material as for any other sample. This is the seed control. Typically, the seed volume in the seed control should be 10 times the volume used in seeded samples.

i. Incubation: Incubate samples at 20°C or other suitable temperature $\pm 1.0^\circ\text{C}$. Take care that the stirring device does not increase the temperature of the sample.

5. Calculations

To convert instrument readings to oxygen uptake, refer to manufacturer's procedures.

Correct oxygen uptake for seed and dilution by the following equation:

$$C = [A - B(S_A/S_B)](1000/N_A)$$

where:

- C = corrected oxygen uptake of sample, mg/L,
- A = measured oxygen uptake in seeded sample, mg,
- B = measured oxygen uptake in seed control, mg,
- S_A = volume of seed in Sample A, mL,
- S_B = volume of seed in Sample B, mL, and
- N_A = volume of undiluted sample in Sample A, mL.

6. Quality Control

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Periodically use the following procedure to check distilled water quality, instrument quality, instrument function, and analytical technique by making oxygen uptake measurements using a mixture of glucose and glutamic acid as a standard check solution.

Adjust water for sample formulation to test temperature and saturate with DO by aerating with clean, organic-free filtered air. Protect water quality by using clean glassware, tubing, and bottles.

Prepare a *test solution* by adding 10 mL glucose-glutamic acid solution (§ 3k); 6 mL phosphate buffer (§ 3b); 2 mL each of ammonium chloride (§ 3c), magnesium sulfate (§ 3e), calcium chloride (§ 3d), ferric chloride (§ 3 f), and trace element solution (§ 3n) to approximately 800 mL water. Add 10 mg nitrification inhibitor (TCMP)/L. Add sufficient seed from a suitable source as described in § 4h to give a lag time less than 6 h (usually 25 mL supernatant from settled primary effluent/L test solution is sufficient). Dilute to 1 L. Adjust temperature to $20 \pm 1^\circ\text{C}$.

Prepare a *seed blank* by diluting 500 mL or more of the seed solution to 800 mL with distilled water. Add the same amount of buffer, nutrients, and TCMP as in the test solution, and dilute to 1 L. Adjust temperature to $20 \pm 1^\circ\text{C}$.

Place test solution and seed blank solution in separate reaction vessels of respirometer and incubate for 5 d at 20°C . Run at least three replicates of each. The seed-corrected oxygen uptake after 5 d incubation should be 260 ± 30 mg/L. If the value of the check is outside this range, repeat the test using a fresh seed culture and seek the cause of the problem.

7. Precision and Bias

a. Precision: No standard is available to check the accuracy of respirometric oxygen uptake measurements. To obtain laboratory precision data, use a glucose-glutamic acid mixture (§ 6 above) having a known theoretical maximum oxygen uptake value. Tests with this and similar organic compound mixtures have shown that the standard deviation, expressed as the coefficient of variation, C_v , is approximately 5% for samples having total oxygen uptakes of 50 to 100 mg/L and 3% for more concentrated samples.^{1,2} Individual instruments have different readability limits that can affect precision. The minimum response or sensitivity of most commercial respirometers ranges from 0.05 to 1 mg oxygen. Check manufacturer's specifications for sensitivity of the instrument at hand.

b. Control limits: To establish laboratory control limits, perform a minimum of 25 glucose-glutamic acid checks over a period of several weeks or months and calculate mean and standard deviation. If measured oxygen uptake in 5 d at 20°C is outside the 260 ± 30 mg/L range, re-evaluate procedure to identify source of error. For other samples, use the mean ± 3 standard deviations as the control limit.

c. Working range and detection limits: The working range and detection limits are established by the limits of each commercial instrument. Refer to manufacturer's specifications.

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5220 CHEMICAL OXYGEN DEMAND (COD)*#(102)

5220 A. Introduction

Chemical oxygen demand (COD) is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence. Because of its unique chemical properties, the dichromate ion ($\text{Cr}_2\text{O}_7^{2-}$) is the specified oxidant in Methods Section 5220B, Section 5220C, and Section 5220D; it is reduced to the chromic ion (Cr^{3+}) in these tests. Both organic and inorganic components of a sample are subject to oxidation, but in most cases the organic component predominates and is of the greater interest. If it is desired to measure either organic or inorganic COD alone, additional steps not described here must be taken to distinguish one from the other.

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COD is a defined test; the extent of sample oxidation can be affected by digestion time, reagent strength, and sample COD concentration.

COD often is used as a measurement of pollutants in wastewater and natural waters. Other related analytical values are biochemical oxygen demand (BOD), total organic carbon (TOC), and total oxygen demand (TOD). In many cases it is possible to correlate two or more of these values for a given sample. BOD is a measure of oxygen consumed by microorganisms under specific conditions; TOC is a measure of organic carbon in a sample; TOD is a measure of the amount of oxygen consumed by all elements in a sample when complete (total) oxidation is achieved.

In a COD analysis, hazardous wastes of mercury, hexavalent chromium, sulfuric acid, silver, and acids are generated. Methods Section 5220C and Section 5220D reduce these waste problems but may be less accurate and less representative. (See ¶ 2 below.)

1. Selection of Method

The open reflux method (B) is suitable for a wide range of wastes where a large sample size is preferred. The closed reflux methods (C and D) are more economical in the use of metallic salt reagents and generate smaller quantities of hazardous waste, but require homogenization of samples containing suspended solids to obtain reproducible results. Ampules and culture tubes with premeasured reagents are available commercially. Measurements of sample volumes as well as reagent volumes and concentrations are critical. Consequently, obtain specifications as to limits of error for premixed reagents from manufacturer before use.

Determine COD values of >50 mg O_2/L by using procedures Section 5220B.4a, Section 5220C.4, or Section 5220D.4. Use procedure Section 5220B.4b to determine, with lesser accuracy, COD values from 5 to 50 mg O_2/L .

2. Interferences and Limitations

Oxidation of most organic compounds is 95 to 100% of the theoretical value. Pyridine and related compounds resist oxidation and volatile organic compounds will react in proportion to their contact with the oxidant. Straight-chain aliphatic compounds are oxidized more effectively in the presence of a silver sulfate catalyst.

The most common interferent is the chloride ion. Chloride reacts with silver ion to precipitate silver chloride, and thus inhibits the catalytic activity of silver. Bromide, iodide, and any other reagent that inactivates the silver ion can interfere similarly. Such interferences are negative in that they tend to restrict the oxidizing action of the dichromate ion itself. However, under the rigorous digestion procedures for COD analyses, chloride, bromide, or iodide can react with dichromate to produce the elemental form of the halogen and the chromic ion. Results then are in error on the high side. The difficulties caused by the presence of the chloride can be overcome largely, though not completely, by complexing with mercuric sulfate ($HgSO_4$) before the refluxing procedure. Although 1 g $HgSO_4$ is specified for 50 mL sample, a lesser amount may be used where sample chloride concentration is known to be less than 2000 mg/L, as long as

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a 10:1 weight ratio of $\text{HgSO}_4:\text{Cl}^-$ is maintained. Do not use the test for samples containing more than 2000 mg Cl^-/L . Techniques designed to measure COD in saline waters are available.^{1,2}

Halide interferences may be removed by precipitation with silver ion and filtration before digestion. This approach may introduce substantial errors due to the occlusion and carrydown of COD matter from heterogeneous samples.

Ammonia and its derivatives, in the waste or generated from nitrogen-containing organic matter, are not oxidized. However, elemental chlorine reacts with these compounds. Hence, corrections for chloride interferences are difficult.

Nitrite (NO_2^-) exerts a COD of 1.1 mg $\text{O}_2/\text{mg NO}_2^- \text{-N}$. Because concentrations of NO_2^- in waters rarely exceed 1 or 2 mg $\text{NO}_2^- \text{-N/L}$, the interference is considered insignificant and usually is ignored. To eliminate a significant interference due to NO_2^- , add 10 mg sulfamic acid for each mg $\text{NO}_2^- \text{-N}$ present in the sample volume used; add the same amount of sulfamic acid to the reflux vessel containing the distilled water blank.

Reduced inorganic species such as ferrous iron, sulfide, manganous manganese, etc., are oxidized quantitatively under the test conditions. For samples containing significant levels of these species, stoichiometric oxidation can be assumed from known initial concentration of the interfering species and corrections can be made to the COD value obtained.

The silver, hexavalent chromium, and mercury salts used in the COD determinations create hazardous wastes. The greatest problem is in the use of mercury. If the chloride contribution to COD is negligible, HgSO_4 can be omitted. Smaller sample sizes (see Section 5220C and Section 5220D) reduce the waste. Recovery of the waste material may be feasible if allowed by regulatory authority.³

3. Sampling and Storage

Preferably collect samples in glass bottles. Test unstable samples without delay. If delay before analysis is unavoidable, preserve sample by acidification to $\text{pH} \leq 2$ using conc H_2SO_4 . Blend (homogenize) all samples containing suspended solids before analysis. If COD is to be related to BOD, TOC, etc., ensure that all tests receive identical pretreatment. Make preliminary dilutions for wastes containing a high COD to reduce the error inherent in measuring small sample volumes.

4. References

1. BURNS, E.R. & C. MARSHALL. 1965. Correction for chloride interference in the chemical oxygen demand test. *J. Water Pollut. Control Fed.* 37:1716.
2. BAUMANN, F.I. 1974. Dichromate reflux chemical oxygen demand: A proposed method for chloride correction in highly saline waters. *Anal. Chem.* 46:1336.
3. HOLM, T.R. 1996. Treatment of Spent Chemical Oxygen Demand Solutions for Safe Disposal. Illinois State Water Survey, Champaign.

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4. AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1995. Standard Test Methods for Chemical Oxygen Demand. (Dichromate Oxygen Demand) of Water. D1252-95, American Soc. Testing & Materials, Philadelphia, Pa.

5220 B. Open Reflux Method

1. General Discussion

a. *Principle:* Most types of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfate to determine the amount of $K_2Cr_2O_7$ consumed and the oxidizable matter is calculated in terms of oxygen equivalent. Keep ratios of reagent weights, volumes, and strengths constant when sample volumes other than 50 mL are used. The standard 2-h reflux time may be reduced if it has been shown that a shorter period yields the same results. Some samples with very low COD or with highly heterogeneous solids content may need to be analyzed in replicate to yield the most reliable data. Results are further enhanced by reacting a maximum quantity of dichromate, provided that some residual dichromate remains.

2. Apparatus

- a. *Reflux apparatus*, consisting of 500- or 250-mL erlenmeyer flasks with ground-glass 24/40 neck and 300-mm jacket Liebig, West, or equivalent condenser with 24/40 ground-glass joint, and a hot plate having sufficient power to produce at least 1.4 W/cm^2 of heating surface, or equivalent.
- b. *Blender*.
- c. *Pipets*, Class A and wide-bore.

3. Reagents

- a. *Standard potassium dichromate solution, 0.04167M:* Dissolve 12.259 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, in distilled water and dilute to 1000 mL. This reagent undergoes a six-electron reduction reaction; the equivalent concentration is $6 \times 0.04167M$ or $0.2500N$.
- b. *Sulfuric acid reagent:* Add Ag_2SO_4 , reagent or technical grade, crystals or powder, to conc H_2SO_4 at the rate of 5.5 g Ag_2SO_4 /kg H_2SO_4 . Let stand 1 to 2 d to dissolve. Mix.
- c. *Ferriin indicator solution:* Dissolve 1.485 g 1,10-phenanthroline monohydrate and 695 mg $FeSO_4 \cdot 7H_2O$ in distilled water and dilute to 100 mL. This indicator solution may be purchased already prepared.*#(103)
- d. *Standard ferrous ammonium sulfate (FAS) titrant, approximately 0.25M:* Dissolve 98 g

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$\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ in distilled water. Add 20 mL conc H_2SO_4 , cool, and dilute to 1000 mL. Standardize this solution daily against standard $\text{K}_2\text{Cr}_2\text{O}_7$ solution as follows:

Dilute 25.00 mL standard $\text{K}_2\text{Cr}_2\text{O}_7$ to about 100 mL. Add 30 mL conc H_2SO_4 and cool. Titrate with FAS titrant using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator.

Molarity of FAS solution

$$= \frac{\text{Volume } 0.04167M \text{ K}_2\text{Cr}_2\text{O}_7 \text{ solution titrated, mL}}{\text{Volume FAS used in titration, mL}} \times 0.2500$$

e. *Mercuric sulfate*, HgSO_4 , crystals or powder.

f. *Sulfamic acid*: Required only if the interference of nitrites is to be eliminated (see Section 5220A.2 above).

g. *Potassium hydrogen phthalate (KHP) standard*, $\text{HOOC}_6\text{H}_4\text{COOK}$: Lightly crush and then dry KHP to constant weight at 110°C . Dissolve 425 mg in distilled water and dilute to 1000 mL. KHP has a theoretical COD¹ of 1.176 mg O_2/mg and this solution has a theoretical COD of 500 $\mu\text{g O}_2/\text{mL}$. This solution is stable when refrigerated, but not indefinitely. Be alert to development of visible biological growth. If practical, prepare and transfer solution under sterile conditions. Weekly preparation usually is satisfactory.

4. Procedure

a. *Treatment of samples with COD of $>50 \text{ mg O}_2/\text{L}$* : Blend sample if necessary and pipet 50.00 mL into a 500-mL refluxing flask. For samples with a COD of $>900 \text{ mg O}_2/\text{L}$, use a smaller portion diluted to 50.00 mL. Add 1 g HgSO_4 , several glass beads, and very slowly add 5.0 mL sulfuric acid reagent, with mixing to dissolve HgSO_4 . Cool while mixing to avoid possible loss of volatile materials. Add 25.00 mL 0.04167M $\text{K}_2\text{Cr}_2\text{O}_7$ solution and mix. Attach flask to condenser and turn on cooling water. Add remaining sulfuric acid reagent (70 mL) through open end of condenser. Continue swirling and mixing while adding sulfuric acid reagent. CAUTION: *Mix reflux mixture thoroughly before applying heat to prevent local heating of flask bottom and a possible blowout of flask contents.*

Cover open end of condenser with a small beaker to prevent foreign material from entering refluxing mixture and reflux for 2 h. Cool and wash down condenser with distilled water. Disconnect reflux condenser and dilute mixture to about twice its volume with distilled water. Cool to room temperature and titrate excess $\text{K}_2\text{Cr}_2\text{O}_7$ with FAS, using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator. Although the quantity of ferroin indicator is not critical, use the same

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volume for all titrations. Take as the end point of the titration the first sharp color change from blue-green to reddish brown that persists for 1 min or longer. Duplicate determinations should agree within 5% of their average. Samples with suspended solids or components that are slow to oxidize may require additional determinations. The blue-green may reappear. In the same manner, reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of sample.

b. Alternate procedure for low-COD samples: Follow procedure of ¶ 4a, with two exceptions: (i) use standard 0.004167M $K_2Cr_2O_7$, and (ii) titrate with standardized 0.025M FAS. Exercise extreme care with this procedure because even a trace of organic matter on the glassware or from the atmosphere may cause gross errors. If a further increase in sensitivity is required, concentrate a larger volume of sample before digesting under reflux as follows: Add all reagents to a sample larger than 50 mL and reduce total volume to 150 mL by boiling in the refluxing flask open to the atmosphere without the condenser attached. Compute amount of $HgSO_4$ to be added (before concentration) on the basis of a weight ratio of 10:1, $HgSO_4:Cl^-$, using the amount of Cl^- present in the original volume of sample. Carry a blank reagent through the same procedure. This technique has the advantage of concentrating the sample without significant losses of easily digested volatile materials. Hard-to-digest volatile materials such as volatile acids are lost, but an improvement is gained over ordinary evaporative concentration methods. Duplicate determinations are not expected to be as precise as in 5220B.4a.

c. Determination of standard solution: Evaluate the technique and quality of reagents by conducting the test on a standard potassium hydrogen phthalate solution.

5. Calculation

$$\text{COD as mg O}_2/\text{L} = \frac{(A - B) \times M \times 8000}{\text{mL sample}}$$

where:

A = mL FAS used for blank,

B = mL FAS used for sample,

M = molarity of FAS, and

8000 = milliequivalent weight of oxygen $\times$ 1000 mL/L.

6. Precision and Bias

A set of synthetic samples containing potassium hydrogen phthalate and NaCl was tested by 74 laboratories. At a COD of 200 mg O_2 /L in the absence of chloride, the standard deviation was ± 13 mg/L (coefficient of variation, 6.5%). At COD of 160 mg O_2 /L and 100 mg Cl^- /L, the standard deviation was ± 14 mg/L (coefficient of variation, 10.8%).

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7. Reference

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8. Bibliography

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5220 C. Closed Reflux, Titrimetric Method

1. General Discussion

a. *Principle:* See Section 5220B.1a.

b. *Interferences and limitations:* See Section 5220A.2. Volatile organic compounds are more completely oxidized in the closed system because of longer contact with the oxidant. Before each use inspect culture-tube caps for breaks in the TFE liner. Select culture-tube size according to block heater capacity and degree of sensitivity desired. Use the 25- × 150-mm tube for samples with low COD content because a larger volume sample can be treated.

This procedure is applicable to COD values between 40 and 400 mg/L. Obtain higher values by dilution. Alternatively, use higher concentrations of dichromate digestion solution to determine greater COD values. COD values of 100 mg/L or less can be obtained by using a more dilute dichromate digestion solution or a more dilute FAS titrant. Overall accuracy can be improved by using an FAS titrant which is less than the 0.10M solution specified below. Higher dichromate concentrations or reduced FAS concentrations probably require titrations to be done in a separate vessel, rather than in the digestion vessel, because of the volumes of titrant required.

2. Apparatus

a. *Digestion vessels:* Preferably use borosilicate culture tubes, 16- × 100-mm, 20- × 150-mm, or 25- × 150-mm, with TFE-lined screw caps. Alternatively, use borosilicate ampules, 10-mL capacity, 19- to 20-mm diam.

Digestion vessels with premixed reagents and other accessories are available from commercial suppliers. Contact supplier for specifications.*#(104)

b. *Block heater* or similar device to operate at $150 \pm 2^{\circ}\text{C}$, with holes to accommodate digestion vessels. Use of culture tubes probably requires the caps to be outside the vessel to

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protect caps from heat. CAUTION: *Do not use an oven because of the possibility of leaking samples generating a corrosive and possibly explosive atmosphere. Also, culture tube caps may not withstand the 150°C temperature in an oven.*

c. *Microburet.*

d. *Ampule sealer:* Use only a mechanical sealer to insure strong, consistent seals.

3. Reagents

a. *Standard potassium dichromate digestion solution, 0.01667M:* Add to about 500 mL distilled water 4.903 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, 167 mL conc H_2SO_4 , and 33.3 g $HgSO_4$. Dissolve, cool to room temperature, and dilute to 1000 mL.

b. *Sulfuric acid reagent:* See Section 5220B.3b.

c. *Ferrouin indicator solution:* See Section 5220B.3c. Dilute this reagent by a factor of 5 (1 + 4).

d. *Standard ferrous ammonium sulfate titrant (FAS), approximately 0.10M:* Dissolve 39.2 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Add 20 mL conc H_2SO_4 , cool, and dilute to 1000 mL. Standardize solution daily against standard $K_2Cr_2O_7$ digestion solution as follows:

Pipet 5.00 mL digestion solution into a small beaker. Add 10 mL reagent water to substitute for sample. Cool to room temperature. Add 1 to 2 drops diluted ferrouin indicator and titrate with FAS titrant.

Molarity of FAS solution

$$= \frac{\text{Volume 0.01667M } K_2Cr_2O_7 \text{ solution titrated, mL}}{\text{Volume FAS used in titration, mL}} \times 0.1000$$

e. *Sulfamic acid:* See Section 5220B.3 f.

f. *Potassium hydrogen phthalate standard:* See Section 5220B.3g.

4. Procedure

Wash culture tubes and caps with 20% H_2SO_4 before first use to prevent contamination.

Refer to Table 5220:I for proper sample and reagent volumes. Make volumetric measurements as accurate as practical; use Class A volumetric ware. The most critical volumes are of the sample and digestion solution. Use a microburet for titrations. Measure H_2SO_4 to ± 0.1 mL. The use of hand-held pipettors with non-wetting (polyethylene) pipet tips is practical and adequate. Place sample in culture tube or ampule and add digestion solution. Carefully run sulfuric acid reagent down inside of vessel so an acid layer is formed under the sample-digestion solution layer.

Tightly cap tubes or seal ampules, and invert each several times to mix completely. CAUTION: *Wear face shield and protect hands from heat produced when contents of vessels are mixed. Mix*

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thoroughly before applying heat to prevent local heating of vessel bottom and possible explosive reaction.

Place tubes or ampules in block digester preheated to 150°C and reflux for 2 h behind a protective shield. CAUTION: *These sealed vessels may be under pressure from gases generated during digestion. Wear face and hand protection when handling. If sulfuric acid is omitted or reduced in concentration, very high and dangerous pressures will be generated at 150°C.* Cool to room temperature and place vessels in test tube rack. Some mercuric sulfate may precipitate out but this will not affect the analysis. Remove culture tube caps and add small TFE-covered magnetic stirring bar. If ampules are used, transfer contents to a larger container for titrating. Add 0.05 to 0.10 mL (1 to 2 drops) ferroin indicator and stir rapidly on magnetic stirrer while titrating with standardized 0.10M FAS. The end point is a sharp color change from blue-green to reddish brown, although the blue-green may reappear within minutes. In the same manner reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of the sample.

5. Calculation

$$\text{COD as mg O}_2\text{/L} = \frac{(A - B) \times M \times 8000}{\text{mL sample}}$$

where:

A = mL FAS used for blank,

B = mL FAS used for sample,

M = molarity of FAS, and

8000 = milliequivalent weight of oxygen $\times$ 1000 mL/L.

Preferably analyze samples in duplicate because of small sample size. Samples that are inhomogeneous may require multiple determinations for accurate analysis. Results should agree within $\pm 5\%$ of their average unless the condition of the sample dictates otherwise.

6. Precision and Bias

Sixty synthetic samples containing potassium hydrogen phthalate and NaCl were tested by six laboratories. At an average COD of 195 mg O₂/L in the absence of chloride, the standard deviation was ± 11 mg O₂/L (coefficient of variation, 5.6%). At an average COD of 208 mg O₂/L and 100 mg Cl⁻/L, the standard deviation was ± 10 mg O₂/L (coefficient of variation, 4.8%).

5220 D. Closed Reflux, Colorimetric Method

1. General Discussion

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a. Principle: See Section 5220B.1a. When a sample is digested, the dichromate ion oxidizes COD material in the sample. This results in the change of chromium from the hexavalent (VI) state to the trivalent (III) state. Both of these chromium species are colored and absorb in the visible region of the spectrum. The dichromate ion ($\text{Cr}_2\text{O}_7^{2-}$) absorbs strongly in the 400-nm region, where the chromic ion (Cr^{3+}) absorption is much less. The chromic ion absorbs strongly in the 600-nm region, where the dichromate has nearly zero absorption. In 9M sulfuric acid solution, the approximate molar extinction coefficients for these chromium species are as follows: Cr^{3+} – 50 L/mole cm at 604 nm; $\text{Cr}_2\text{O}_7^{2-}$ – 380 L/mole cm at 444 nm; Cr^{3+} – 25 L/mole cm at 426 nm. The Cr^{3+} ion has a minimum in the region of 400 nm. Thus a working absorption maximum is at 420 nm.

For COD values between 100 and 900 mg/L, increase in Cr^{3+} in the 600-nm region is determined. Higher values can be obtained by sample dilution. COD values of 90 mg/L or less can be determined by following the decrease in $\text{Cr}_2\text{O}_7^{2-}$ at 420 nm. The corresponding generation of Cr^{3+} gives a small absorption increase at 420 nm, but this is compensated for in the calibration procedure.

b. Interferences and limitations: See Section 5220C.1b.

For this procedure to be applicable, all visible light-absorbing interferents must be absent or be compensated for. This includes insoluble suspended matter as well as colored components. If either type of interference occurs, the test is not necessarily lost because COD can be determined titrimetrically as in 5220C.

2. Apparatus

a. See Section 5220C.2. Ensure that reaction vessels are of optical quality. Other types of absorption cells with varying path lengths may be used. Use the extinction coefficients of the ions of interest for this approach.

b. Spectrophotometer, for use at 600 nm and/or 420 nm with access opening adapter for ampule or 16-, 20-, or 25-mm tubes. Verify that the instrument operates in the region of 420 nm and 600 nm. Values slightly different from these may be found, depending on the spectral bandpass of the instrument.

3. Reagents

a. Digestion solution, high range: Add to about 500 mL distilled water 10.216 g $\text{K}_2\text{Cr}_2\text{O}_7$, primary standard grade, previously dried at 150°C for 2 h, 167 mL conc H_2SO_4 , and 33.3 g HgSO_4 . Dissolve, cool to room temperature, and dilute to 1000 mL.

b. Digestion solution, low range: Prepare as in 3a, but use only 1.022 g potassium dichromate.

c. Sulfuric acid reagent: See Section 5220B.3b.

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d. *Sulfamic acid*: See Section 5220B.3 f.

e. *Potassium hydrogen phthalate standard*: See Section 5220B.3g.

4. Procedure

a. *Treatment of samples*: Measure suitable volume of sample and reagents into tube or ampule as indicated in Table 5220:I. Prepare, digest, and cool samples, blank, and one or more standards as directed in Section 5220C.4. *Note the safety precautions*. It is critical that the volume of each component be known and that the total volume be the same for each reaction vessel. If volumetric control is difficult, transfer digested sample, dilute to a known volume, and read. Premixed reagents in digestion tubes are available commercially.

b. *Measurement of dichromate reduction*: Cool sample to room temperature slowly to avoid precipitate formation. Once samples are cooled, vent, if necessary, to relieve any pressure generated during digestion. Mix contents of reaction vessels to combine condensed water and dislodge insoluble matter. Let suspended matter settle and ensure that optical path is clear. Measure absorption of each sample blank and standard at selected wavelength (420 nm or 600 nm). At 600 nm, use an undigested blank as reference solution. Analyze a digested blank to confirm good analytical reagents and to determine the blank COD; subtract blank COD from sample COD. Alternately, use digested blank as the reference solution once it is established that the blank has a low COD.

At 420 nm, use reagent water as a reference solution. Measure all samples, blanks, and standards against this solution. The absorption measurement of an undigested blank containing dichromate, with reagent water replacing sample, will give initial dichromate absorption. Any digested sample, blank, or standard that has a COD value will give lower absorbance because of the decrease in dichromate ion. Analyze a digested blank with reagent water replacing sample to ensure reagent quality and to determine the reagents' contribution to the decrease in absorbance during a given digestion. The difference between absorbances of a given digested sample and the digested blank is a measure of the sample COD. When standards are run, plot differences of digested blank absorbance and digested standard absorbance versus COD values for each standard.

c. *Preparation of calibration curve*: Prepare at least five standards from potassium hydrogen phthalate solution with COD equivalents to cover each concentration range. Make up to volume with reagent water; use same reagent volumes, tube, or ampule size, and digestion procedure as for samples. Prepare calibration curve for each new lot of tubes or ampules or when standards prepared in ¶ 4a differ by $\geq 5\%$ from calibration curve. Curves should be linear. However, some nonlinearity may occur, depending on instrument used and overall accuracy needed.

5. Calculation

If samples, standards, and blanks are run under same conditions of volume and optical path length, calculate COD as follows:

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$$\text{COD as mg O}_2/\text{L} = \frac{\text{mg O}_2 \text{ in final volume} \times 1000}{\text{mL sample}}$$

Preferably analyze samples in duplicate because of small sample size. Samples that are inhomogeneous may require multiple determinations for accurate analysis. These should not differ from their average by more than $\pm 5\%$ for the high-level COD test unless the condition of the sample dictates otherwise. In the low-level procedure, results below 25 mg/L may tend to be qualitative rather than quantitative.

6. Precision and Bias

Forty-eight synthetic samples containing potassium hydrogen phthalate and NaCl were tested by five laboratories. At an average COD of 193 mg O₂/L in the absence of chloride, the standard deviation was ± 17 mg O₂/L (coefficient of variation 8.7%). At an average COD of 212 mg O₂/L and 100 mg Cl⁻/L, the standard deviation was ± 20 mg O₂/L (coefficient of variation, 9.6%). Additional QA/QC data for both high- and low-level procedures may be found elsewhere.¹

7. Reference

1. AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1995. Standard test methods for chemical oxygen demand (dichromate oxygen demand) of water. D1252-95, ASTM Annual Book of Standards. American Soc. Testing & Materials, Philadelphia, Pa.

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5310 TOTAL ORGANIC CARBON (TOC)*#(105)

5310 A. Introduction

1. General Discussion

The organic carbon in water and wastewater is composed of a variety of organic compounds in various oxidation states. Some of these carbon compounds can be oxidized further by biological or chemical processes, and the biochemical oxygen demand (BOD), assimilable

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organic carbon (AOC), and chemical oxygen demand (COD) methods may be used to characterize these fractions. Total organic carbon (TOC) is a more convenient and direct expression of total organic content than either BOD, AOC, or COD, but does not provide the same kind of information. If a repeatable empirical relationship is established between TOC and BOD, AOC, or COD for a specific source water then TOC can be used to estimate the accompanying BOD, AOC, or COD. This relationship must be established independently for each set of matrix conditions, such as various points in a treatment process. Unlike BOD or COD, TOC is independent of the oxidation state of the organic matter and does not measure other organically bound elements, such as nitrogen and hydrogen, and inorganics that can contribute to the oxygen demand measured by BOD and COD. TOC measurement does not replace BOD, AOC, and COD testing.

Measurement of TOC is of vital importance to the operation of water treatment and waste treatment plants. Drinking water TOCs range from less than 100 µg/L to more than 25,000 µg/L. Wastewater may contain very high levels of organic compounds (TOC >100 mg/L). Some of these applications may include waters with substantial ionic impurities as well as organic matter.

In many applications, the presence of organic contaminants may degrade ion-exchange capacity, serve as a nutrient source for undesired biological growth, or be otherwise detrimental to the process for which the water is to be utilized. For drinking waters in particular, organic compounds may react with disinfectants to produce potentially toxic and carcinogenic compounds.

To determine the quantity of organically bound carbon, the organic molecules must be broken down and converted to a single molecular form that can be measured quantitatively. TOC methods utilize high temperature, catalysts, and oxygen, or lower temperatures (<100°C) with ultraviolet irradiation, chemical oxidants, or combinations of these oxidants to convert organic carbon to carbon dioxide (CO₂). The CO₂ may be purged from the sample, dried, and transferred with a carrier gas to a nondispersive infrared analyzer or coulometric titrator. Alternatively, it may be separated from the sample liquid phase by a membrane selective to CO₂ into a high-purity water in which corresponding increase in conductivity is related to the CO₂ passing the membrane.

2. Fractions of Total Carbon

The methods and instruments used in measuring TOC analyze fractions of total carbon (TC) and measure TOC by two or more determinations. These fractions of total carbon are defined as: inorganic carbon—the carbonate, bicarbonate, and dissolved CO₂; total organic carbon (TOC)—all carbon atoms covalently bonded in organic molecules; dissolved organic carbon (DOC)—the fraction of TOC that passes through a 0.45-µm-pore-diam filter; suspended organic carbon—also referred to as particulate organic carbon, the fraction of TOC retained by a 0.45-µm filter; purgeable organic carbon—also referred to as volatile organic carbon, the fraction of TOC removed from an aqueous solution by gas stripping under specified conditions; and nonpurgeable organic carbon—the fraction of TOC not removed by gas stripping.

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In most water samples, the inorganic carbon fraction is many times greater than the TOC fraction. Eliminating or compensating for inorganic carbon interferences requires determinations of both TC and inorganic carbon to measure TOC. Inorganic carbon interference can be eliminated by acidifying samples to pH 2 or less to convert inorganic carbon species to CO₂. Subsequent purging of the sample with a purified gas or vacuum degassing removes the CO₂ by volatilization. Sample purging also removes purgeable organic carbon so that the organic carbon measurement made after eliminating inorganic carbon interferences is actually a nonpurgeable organic carbon determination: determine purgeable organic carbon to measure TOC. In many surface and ground waters the purgeable organic carbon contribution to TOC is negligible. Therefore, in practice, the nonpurgeable organic carbon determination is substituted for TOC.

Alternatively, inorganic carbon interference may be compensated for by separately measuring total carbon (TC) and inorganic carbon. The difference between TC and inorganic carbon is TOC.

The purgeable fraction of TOC is a function of the specific conditions and equipment employed. Sample temperature and salinity, gas-flow rate, type of gas diffuser, purging-vessel dimensions, volume purged, and purging time affect the division of TOC into purgeable and nonpurgeable fractions. When separately measuring purgeable organic carbon and nonpurgeable organic carbon on the same sample, use identical conditions for purging during the purgeable organic carbon measurement as in purging to prepare the nonpurgeable organic carbon portion for analysis. Consider the conditions of purging when comparing purgeable organic carbon or nonpurgeable organic carbon data from different laboratories or different instruments.

3. Selection of Method

The high-temperature combustion method (B) is suitable for samples with higher levels of TOC that would require dilution for the various persulfate methods (Method C or Method D). Generally, it also will determine organic carbon from compounds that are chemically refractory and not determined by Method C or Method D. High-temperature combustion may be desirable for samples containing high levels of suspended organic carbon, which may not be efficiently oxidized by persulfate and/or UV methods. Interlaboratory studies have shown biases on the order of 1 mg/L using older high-temperature instruments. With newer instruments, detection limits as low as 10 µg/L have been reported. Some high-temperature combustion instruments are not designed for levels below 1 mg/L. The high-temperature methods accumulate nonvolatile residues in the analyzer, whereas, in Method C, residuals are drained from the analyzer. Method C generally provides better sensitivity for lower-level (<1 mg/L) samples. Persulfate and/or UV oxidation are useful for TOC as low as 10 µg/L. Because the range of sensitivity of the methods overlaps, other factors may dictate method choice in the range of 1 mg/L to 50 mg/L. A method may be chosen on the basis of desired precision, ease of use, cost, etc. Method D generally is equivalent to Method C, but the equipment for Method D is no longer manufactured.

To qualify a particular instrument for use, demonstrate that the single-user precision and bias given in each method can be reproduced. Also, preferably demonstrate the overall precision by

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conducting in-house studies with more than one operator.

Evaluate the selected method to ensure that data quality objectives are attained. Evaluate method detection limit in a matrix as similar as possible to the unknowns as described in Section 1030. Be aware that instrument blanks are handled in a variety of ways in TOC analyzers and that the true magnitude of the blank may not be readily apparent to the analyst. Some instruments “zero out” much of the blank by adjusting the zero on the detector. Others enter blank values in units such as mv responses rather than absolute concentrations, whereas other instruments accumulate the total blank in the system during a blank run. Carefully observe the variability of low-level measurements and check it any time reagents or instrument operations are changed. The following methods note that when a water blank is run there is a contribution to the observed blank value from the level of carbon in the blank water.

The methods show expected single-operator and multiple-laboratory precision. These equations are based on referenced interlaboratory studies that in some cases were performed on older equipment. The range of testing is important to observe because the error and bias generally will be some significant fraction of the low standard. Consult references to determine type of equipment and conditions of the interlaboratory study. Determine the performance of the instrument being used by analyzing waters with matrices similar to those of unknowns, using the procedures outlined in Section 1040B.

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