Standard Methods for the Examination of Water and Wastewater

2130 TURBIDITY

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 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. 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

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 ±30° 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 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 μm; 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, $(NH_2)_2 \cdot H_2SO_4$, 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, $(CH_2)6N_4$, 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 ± 3°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, and items supplied by instrument manufacturers, 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 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 non representative 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 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.

2340 HARDNESS

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 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.

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 increased indefinitely because of the danger of precipitating calcium carbonate, $CaCO_3$, or magnesium hydroxide, $Mg(OH)_2$, 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 2*b*3)], 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 1*N* hydrochloric acid (HCl), neutralize to pH 7 with 1*N* 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 CaCO3 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:

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 ($MgSO_4 \cdot 7H_2O$) or 644 mg magnesium chloride ($MgCl_2 \cdot 6H_2O$) 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 0.1*N* 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 ($Na_2S \cdot 9H_2O$) or 3.7 g $Na_2S \cdot 5H_2O$ 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 3*b* 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 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,* 0.01*M*:** 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 (2*e*) as described in 3*b* 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 CO2. Cool, add a few drops of methyl red indicator, and adjust to the intermediate orange color by adding 3*N* NH_4OH or 1 + 1 HCl, as required. Transfer quantitatively and dilute to 1000 mL with distilled water; 1mL = 1.00 mg $CaCO_3$.

***f. Sodium hydroxide,* NaOH, 0.1*N*.**

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 (2*b* et seq.) or that the indicator has deteriorated.

Add 1 to 2 drops indicator solution or an appropriate amount of dry-powder indicator formulation [(2*c*3)]. 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 3*c* below.

c. Low-hardness sample: For ion-exchanger effluent or other softened water and for natural wat ers 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

$$\text{Hardness (EDTA) as mg } CaCO_3/L = \frac{A \times B \times 1000}{\text{mL sample}}$$

where:
A = mL titration for sample and
B = mg $CaCO_3$ equivalent to 1.00 mL EDTA titrant.

2540 SOLIDS

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.

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.3*b*).

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.

Residues dried at 180 ± 2°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.

2540 B. Total Solids Dried at 103–105°C

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.
i. Graduated cylinder.
j. Low-form beaker.

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 200mg. 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 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.

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 rolongs 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 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 1L. 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 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.

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 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.2*a* - h is required, and in addition:

a. Glass-fiber filter disks 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.

c. Suction flask, of sufficient capacity for sample size selected.

d. Drying oven, for operation at 180 ± 2°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 ± 2°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 ± 2°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 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.

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

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 to1 L with distilled water.

***b. Standard silver nitrate titrant,* 0.0141*M* (0.0141*N*):** Dissolve 2.395 g $AgNO_3$ in distilled water and dilute to 1000 mL. Standardize against NaCl by the procedure described in 4*b* below; 1.00 mL = 500 μg Cl^-. Store in a brown bottle.

***c. Standard sodium chloride,* 0.0141*M* (0.0141*N*):** 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, 1*N*.

4) *Sulfuric acid,* H_2SO_4, 1*N*.

5) *Hydrogen peroxide,* H_2O_2, 30%.

4. Procedure

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 $Al(OH)_3$ 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

$$\text{mg } Cl^-/L = \frac{(A - B) \times N \times 35\,450}{\text{mL sample}}$$

where:

A = mL titration for sample,

B = mL titration for blank, and

N = normality of $AgNO_3$.

mg NaCl/L = (mg Cl^-/L) × 1.65

2320 ALKALINITY

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 constitutents. 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 ($CaCO_3$)/L.1

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 $CaCO_3$/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.

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 (CO_2) at that stage. CO_2 concentration depends, in turn, on the total carbonate species originally present and any losses that may have occurred during titration. The pH values: are suggested as the equivalence points for the corresponding

alkalinity concentrations as milligrams $CaCO_3$ 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 $CaCO_3$/L only if it has been determined by the low-alkalinity method of 4*d*.

Construct a titration curve for standardization of reagents. 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 (H_2SO_4) 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* H_2SO_4 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 Na_2CO_3 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* Na_2CO_3 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 Na_2CO_3 weighed into 1-L flask,
B = mL Na_2CO_3 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 $CaCO_3$.

***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 15.00 mL 0.05*N* Na_2CO_3 according to the procedure of 3*b*; 1 mL = 1.00 mg $CaCO_3$.

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: 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,* 0.1*N*:** Dissolve 25 g Na2S2O3 · 5H2O and dilute to 1000 mL with distilled water.

4. Procedure

a. Color change: Select sample size and normality of titrant according to criteria of 1*e*. 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) 0.1*M* Na2S2O3 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.

b. Potentiometric titration curve: Follow the procedure for determining acidity (Section 2310B.4*c*), 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.

***Potentiometric titration curve(*(Section 2310B.4*c*)**

1) Rinse electrodes and titration vessel with distilled water and drain. Select sample size and normality of titrant according to the criteria of 1*e*. 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.

c. Potentiometric titration to preselected pH: Determine the appropriate end-point pH according to 1*b*. Prepare sample and titration assembly (Section 2310B.4*c*) see above. 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 4*c*, above, using a 10-mL microburet and 0.02*N* 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:

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times N \times 50\,000}{\text{mL sample}}$$

where:
A = mL standard acid used and
N = normality of standard acid
or

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times t \times 1000}{\text{mL sample}}$$

where:

t = titer of standard acid, mg $CaCO_3$/mL.

Report pH of end point used as follows: ‘‘The alkalinity to pH _____ = _____ mg $CaCO_3$/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 $CaCO_3$/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 (CO_3^{2-}) 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 (HCO_3^-) alkalinity is present if phenolphthalein alkalinity is less than half the phenolphthalein alkalinity and T is total alkalinity (1*b*):

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 $CaCO_3$.

Accurately measure pH, calculate OH^- concentration as milligrams CaCO3 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:

$$CO_3^{2-} = 2P - 2[OH^-]$$

$$HCO_3^- = T - 2P + [OH^-]$$

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:

$P = 1/2\ [CO_3^{2-}] + [OH^-]$

4500-NH3 NITROGEN (AMMONIA)

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.

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 NH_3-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 NH_3-N/L.

The manual phenate method is applicable to both fresh water and seawater and is linear to 0.6 mg NH_3-N/L. Distill into sulfuric acid (H_2SO_4) absorbent for the phentate method when interferences are present.

The automated phenate method is applicable over the range of 0.02 to 2.0 mg NH_3-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.3*d* (As below)

Dechlorinating reagent: Dissolve 3.5 g sodium thiosulfate ($Na_2S_2O_3 \cdot 5H2O$) 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.

3. Storage of Samples

Most reliable results are obtained on fresh samples. If samples are to be analyzed within 24 h 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.

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 orfiltration. 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 (≥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-NH_3.D.3*d*.(as below)

Stock ammonium chloride solution: Dissolve 3.819 g anhydrous NH_4Cl (dried at 100°C) in water, and dilute to 1000 mL; 1.00 mL = 1.00 mg N = 1.22 mg NH_3.

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 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.

4500-NO2 ⁻ NITROGEN (NITRITE)

A. Introduction

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 μg NO_2^--N/L (See B.1*a*). 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-NO3⁻.I).

4500-NO2 ⁻ B. Colorimetric Method

1. General Discussion

a. Principle: Nitrite ($NO2^-$) 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 μg NO_2^--N/L. Photometric measurements can be made in the range 5 to 50 μg 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 μg 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 $NO2^-$, free chlorine, and nitrogen trichloride (NCl_3) will coexist. NCl3 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 NH3. 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:

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.2*b*) indicates the presence of permanganate.

2) Add 1 mL conc H_2SO_4 and 0.2 mL MnSO4 solution (36.4 g $MnSO_4 \cdot H_2O$/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,* 0.025*M* (0.05*N*):** Dissolve 3.350 g $Na_2C_2O_4$, primary standard grade, in water and dilute to 1000 mL.

***d. Ferrous ammonium sulfate,* 0.05*M* (0.05*N*):** Dissolve 19.607 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ plus 20 mL conc H_2SO_4 in water and dilute to 1000 mL. Standardize as in Section 5220B.3*d*.

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 0.01*M* (0.05*N*) KMnO4 solution (see h below), discharge permanganate color with a known quantity of standard reductant such as 0.025*M* $Na_2C_2O_4$ or 0.05*M* $Fe(NH_4)_2(SO_4)_2$, 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 0.01*M* (0.05*N*) $KMnO_4$, 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 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 0.025*M* $Na_2C_2O_4$. Titrate excess $Na_2C_2O_4$ with 0.01*M* (0.05*N*) $KMnO_4$ 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 0.05*M* ferrous ammonium sulfate solution is substituted for $Na_2C_2O_4$, 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 0.01*M* (0.05*N*) $KMnO_4$ 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,* 0.01*M* (0.05*N*):** 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 $Na_2C_2O_4$ into 400-mL beakers. To each beaker, in turn, add 100 mL distilled water and stir to dissolve. Add 10 mL 1 + 1 H_2SO_4 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 H_2SO_4.

$$\text{Normality of } KMnO_4 = \frac{\text{g } Na_2C_2O_4}{(A - B) \times 0.335\ 05}$$

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-μ m-pore-diam membrane filter.

b. Color development: If sample pH is not between 5 and 9, adjust to that range with 1*N* HCl or NH_4OH 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 NO_2^--N concentrations:

Light Path Length *cm*	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 NO_2^--N concentration.
Compute sample concentration directly from curve.

4500-NO3 – NITROGEN (NITRATE)

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 or capillary ion electrophoresis. 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.

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 2d at 4°C; disinfected samples are stable much longer without acid preservation. For longer storage of unchlorinated samples, preserve with 2 mL conc H_2SO_4/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-NO3– 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 monitoringNO_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 1*N* 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.

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 mL = 100 μg NO_3^--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 mL = 10.0 μg NO_3^--N. Preserve with 2 mL $CHCl_3$/L. This solution is stable for 6 months.

d. Hydrochloric acid solution, HCl, 1*N*.

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 NO_3^--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 NO_3^--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.

4500-P PHOSPHORUS

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.

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. 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.

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 1to 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 and capillary ion electrophoresis are useful for determination of orthophosphate in undigested samples.

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 HgCl2 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 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.

B. Sample Preparation

For information on selection of digestion method (3 through 5 below), see 4500-P.A.3*a*.

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 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, 6*N*.

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.

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,* $HClO_4 \cdot 2H_2O$, purchased as 70 to 72% $HClO_4$, reagent-grade.
3) *Sodium hydroxide,* NaOH, 6*N*.
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 HClO4 fuming or a glass fume eradicator 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 6*N* 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 (seeMethod C, D, or E) through digestion step. Do not use orthophosphate standards without 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, 1*N*.

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 1*N* 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.

3) *Ammonium persulfate,* $(NH_4)2S_2O_8$, solid, or potassium persulfate, $K_2S_2O_8$, solid.

4) *Sodium hydroxide,* NaOH, 1*N*.

c. Procedure: Use 50 mL or a suitable portion of thoroughly mixed sample. Add 0.05 mL (1drop) phenolphthalein indicator solution. If a red color develops, add H2SO4 solution dropwise to just discharge the color. Then add 1 mL H_2SO_4 solution and either 0.4 g solid $(NH_4)2S_2O_8$ or 0.5 g solid $K_2S_2O_8$.

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.

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 1000mg/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^{-}, SiO44–, 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 μ 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.

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 0.5*N* is recommended.

c. Activated carbon.Remove fine particles by rinsing with distilled water.

d. Vanadate-molybdate reagent:

1) *Solution A:* Dissolve 25 g ammonium molybdate, $(NH_4)6Mo_7O_{24} \cdot 4H_2O$, 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 μg PO_4^{3-}-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 2*a* 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 4*c*. 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

$$\text{mg P/L} = \frac{\text{mg P(in 50 mL final volume)} \times 1000}{\text{mL sample}}$$

4500-S^{2-} SULFIDE

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 H_2S in clean water is between 0.025 and 0.25 μg/L. Gaseous H_2S 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 H_2S. It attacks metals directly and indirectly has caused serious corrosion of concrete sewers because it is oxidized biologically to H_2SO_4 on the pipe wall. Dissolved H_2S 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 H_2S and HS^-, as well as acid-soluble metallic sulfides present in suspended matter. The S^{2-} 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 H_2S.

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 2*N* zinc acetate soution 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 6*N* 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.3*a* 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 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 methlylene 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 ($Na_2S \cdot 9H_2O$) 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 $Na_2S \cdot 9H_2O$ 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 $Na_2S \cdot 9H_2O$ in weighing bottle by difference, then multiply the weight by 0.133 to determine the amount of S^{2-}. 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 $Na_2S \cdot 9H_2O$ diluted to a final volume of 500 mL will give a stock solution of which 1.00 mL = 1.00 mg S2–). Alternatively, purchase 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.

4500-S2– F. Iodometric Method

1. Reagents

***a. Hydrochloric acid,* HCl, 6*N*.**

***b. Standard iodine solution,* 0.0250*N*:** 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 0.0250*N* $Na_2S_2O_3$, using starch solution as indicator.

***c. Standard sodium thiosulfate solution,* 0.0250*N*:** See Section 4500-O.C.2*e*.

d. Starch solution: See Section 4500-O.C.2*d*.

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 6*N* 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 2*a* above.

3. Calculation

One milliliter 0.0250*N* 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,

C = mL $Na_2S_2O_3$ solution, and

D = normality of $Na_2S_2O_3$ solution.

4500-SO_4^{2-} SULFATE

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 and capillary ion electrophoresis (CIE) 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 CIE 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-SO_4- E. Turbidimetric Method

1. General Discussion

a. Principle: Sulfate ion (SO_4^{2-}) is precipitated in an acetic acid medium with barium 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 4*d* 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 420nm 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, $MgCl_2 \cdot 6H_2O$, 5 g sodium acetate, $CH_3COONa \cdot 3H_2O$, 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 $MgCl_2 \cdot 6H_2O$, 5 g $CH_3COONa \cdot 3H_2O$, 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.

d. Standard sulfate solution: Prepare a standard sulfate solution as described in 1) or 2) below; 1.00 mL = 100 μg SO_4^{2-}.

1) Dilute 10.4 mL standard 0.0200*N* H_2SO_4 titrant specified in Alkalinity, Section 2320B.3*c*, 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 BaSO4 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.

4500-CN^- CYANIDE

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 obtained as CN^- are classed as simple and complex cyanides.

Simple cyanides are represented by the formula $A(CN)_x$, 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 (p*K*a ~ 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, $Zn(CN)_2$], 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 AyM(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 $M(CN)_x^{y-}$. 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; 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, as the pH of most natural waters is substantially lower than the p*K*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. Analytical distinction between HCN and other cyanide species in solutions of complex cyanides is possible.

The degree of dissociation of the various metallocyanide 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. 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.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 toxic HCN, on exposure of dilute solutions to direct sunlight. 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:

$$NaCN + Cl_2 \rightarrow CNCl + NaCl \quad (1)$$

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. At an alkaline pH, CNCl hydrolyzes to the cyanate ion (CNO^-), which has onlylimited toxicity.

There is no known natural reduction reaction that may convert CNO^- to CN^-.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.

$$CNCl + 2NaOH \rightarrow NaCNO + NaCl + H_2O \quad (2)$$

CNO^- can be oxidized further with chlorine at a nearly neutral pH to CO_2 and N_2:

$$2NaCNO + 4NaOH + 3Cl_2 \rightarrow 6NaCl + 2CO_2 + N_2 + 2H_2O \quad (3)$$

CNO^- also will be converted on acidification to NH_4^+:

$$2NaCNO + H_2SO_4 + 4H_2O \rightarrow (NH_4)_2SO_4 + 2NaHCO_3 \quad (4)$$

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 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).Molecular ammonia and metal-ammonia complexes are toxic to aquatic life.

Thiocyanate (SCN^-) is not very toxic to aquatic life. However, upon chlorination, toxic CNCl is formed, as discussed above. 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. 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 pH ≤ 2.

3. Selection of Method

a. Total cyanide after distillation: After removal of interfering substances, the metal cyanideis converted to HCN gas, which is distilled and absorbed in sodium hydroxide (NaOH) solution. Because of the catalytic decomposition of cyanide in the presence of cobalt at high temperature in a strong acid solution, 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 μ g/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

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 μg/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, $(NH_4)_2CO_3$, 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. Measure NH_3 by either:

1) The selective electrode method, using the NH_3 gas electrode (Section 4500-NH3.D); or

2) The colorimetric method, using the phenate method for NH_3 (Section 4500-NH3.F orSection 4500-NH_3.G).

f. Thiocyanate: Use the colorimetric determination with ferric nitrate as a color-producing compound.

4500-CN^- E. Colorimetric Method

1. General Discussion

a. Principle: CN^- in the alkaline distillate from preliminary treatment is converted to CNCl by reaction with chloramine-T at pH <8 without hydrolyzing to CNO^-.1 (CAUTION—*CNCl is atoxic 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. 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 (3*b*) calculate volume required (approximately 10 mL) to prepare 1 L of a 10 μg CN^-/mL solution. Dilute with the NaOH dilution solution. Dilute 10 mL of the 10 μg CN^-/mL solution to 100 mL with the NaOH dilution solution; 1.0 mL = 1.0 μg 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, $NaC_2H_3O_2 \cdot 3H_2O$, 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 μg CN^- into 50-mL volumetric flasks (0.02 to 0.2 μg CN^-/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 μg CN^-/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.

$$CN^-, \text{ mg/L} = (ma_1 + b) \times \frac{50}{X} \times \frac{250}{Y}$$

where:

X = absorption solution, mL,

Y = original sample, mL, and

$a1$ = absorbance of sample solution.

5210 BIOCHEMICAL OXYGEN DEMAND (BOD)

A. Introduction

1. General Discussion

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. 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 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.4*e*6) 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 ($CBOD_5$) when inhibiting the nitrogenous oxygen demand. When nitrification is not inhibited, report results as BOD_5.

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).

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 ± 3°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 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 ±1°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 $Na_2HPO_4 \cdot 7H_2O$, 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 $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to1 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 $FeCl_3 \cdot 6H_2O$ 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.

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

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 ¶ 4*d*. Test dilution water as described in 4*h* so that water of assured quality always is on hand.

Before use bring dilution water temperature to 20 ± 3°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 (4*h*). 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 4*d-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 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 (4*h*). 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 (H2SO4) 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 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 Na2SO3 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 ± 1°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 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 (3*a* through *e*) directly to individual BOD bottles at a rate of 1mL/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 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 4*g* 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°C ± 1°C BOD bottles containing desired dilutions, seed controls, dilution water blanks, and glucose-glutamic acid checks. Water-seal bottles asdescribed in 4 *f*.
j. Determination of final DO: After 5 d incubation determine DO in sample dilutions,blanks, and checks as in 4*g*.

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:

$$BOD_5,\ mg/L = \frac{D_1 - D_2}{P}$$

When dilution water is seeded:

$$BOD_5,\ mg/L = \frac{D_1 - D_2}{P}$$

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 (4*d*),
*B*2 = DO of seed control after incubation mg/L (4*d*), 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 = (volume of seed in diluted sample)/(volume of seed in seed control)

Report results as $CBOD_5$ 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.

5220 CHEMICAL OXYGEN DEMAND (COD)

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 ($Cr_2O_7^{2-}$) 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.

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.4*a*, Section 5220C.4, or Section 5220D.4. Use procedure Section 5220B.4*b* to determine, with lesser accuracy, COD values from 5 to 50 mg O2/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 a 10:1 weight ratio of $HgSO_4$: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. 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 heterogenous 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 O2/mg NO_2^--N. Because concentrations of NO_2^- in waters rarely exceed 1 or 2 mg NO_2^--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 NO_2^--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 toCOD 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. 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 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.

5220 C. Closed Reflux, Titrimetric 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.

***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

0.10*M* 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.

b. Block heater or similar device to operate at 150 ± 2°C, with holes to accommodate digestion vessels. Use of culture tubes probably requires the caps to be outside the vessel to 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 mLdistilled water 4.903 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, 167mL 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.3*b*.

***c. Ferroin indicator solution*:** See Section 5220B.3*c*. Dilute this reagent by a factor of 5 (1 +4).

d. Standard ferrous ammonium sulfate titrant (FAS), approximately 0.10*M*: 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 ferroin indicator and titrate with FAS titrant.

Molarity of FAS solution

$$= \frac{\text{Volume } 0.01667M \text{ } K_2Cr_2O_7 \text{ solution titrated, mL}}{\text{Volume FAS used in titration, mL}} \times 0.1000$$

***e. Sulfamic acid*:** See Section 5220B.3 *f*.

***f. Potassium hydrogen phthalate standard*:** See Section 5220B.3*g*.

4. Procedure

Wash culture tubes and caps with 20% H_2SO_4 before first use to prevent contamination.

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 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 0.10*M* 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 × 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 ±5% of their average unless the condition of the sample dictates otherwise.

5520 OIL AND GREASE

A. Introduction

In the determination of oil and grease, an absolute quantity of a specific substance is not measured. Rather, groups of substances with similar physical characteristics are determined quantitatively on the basis of their common solubility in an organic extracting solvent. ‘‘Oil and grease’’ is defined as any material recovered as a substance soluble in the solvent. It includes other material extracted by the solvent from an acidified sample (such as sulfur compounds, certain organic dyes, and chlorophyll) and not volatilized during the test. The 12th edition of *Standard Methods* prescribed the use of petroleum ether as the solvent for natural and treated waters and *n*-hexane for polluted waters. The 13th edition added trichlorotrifluoroethane as an optional solvent for all sample types. In the 14th through the 17th editions, only trichlorotrifluoroethane was specified. However, because of environmental problems associated with chlorofluorocarbons, an alternative solvent (80% *n*-hexane and 20% methyl-*tert*-butyl ether) was included for gravimetric methods in the 19th edition. In the 20th edition, trichlorotrifluoroethane has been dropped from all gravimetric procedures (retained for 5520C, an infrared method), and replaced by *n*-hexane. Solvent-recovery techniques are included and solvent recycling is strongly recommended.

It is important to understand that, unlike some constituents that represent distinct chemical elements, ions, compounds, or groups of compounds, oils and greases are defined by the method used for their determination. In a detailed study involving many complex organic matrices, it was shown that either *n*-hexane or 80/ 20 *n*-hexane/methyl-*tert*-butyl ether gave results that were not statistically different from results obtained with trichlorotrifluoroethane. Although 5520B allows either solvent system for extraction of wastewaters, note that for certain regulatory purposes U.S. EPA currently recommends only *n*-hexane.The methods presented here are suitable for biological lipids and mineral hydrocarbons.

They also may be suitable for most industrial wastewaters or treated effluents containing these materials, although sample complexity may result in either low or high results because of lack of analytical specificity. The method is not applicable to measurement of low-boiling fractions that volatilize at temperatures below 85°C.

1. Significance

Certain constituents measured by the oil and grease analysis may influence wastewater treatment systems. If present in excessive amounts, they may interfere with aerobic and anaerobic biological processes and lead to decreased wastewater treatment efficiency. When discharged in wastewater or treated effluents, they may cause surface films and shoreline deposits leading to environmental degradation.

A knowledge of the quantity of oil and grease present is helpful in proper design and operation of wastewater treatment systems and also may call attention to certain treatment difficulties.
In the absence of specially modified industrial products, oil and grease is composed primarily of fatty matter from animal and vegetable sources and from hydrocarbons of petroleum origin.
The portion of oil and grease from each of these two major sources can be determined with Method 5520F. A knowledge of the relative composition of a sample minimizes the difficulty in determining the major source of the material and simplifies the correction of oil and grease problems in wastewater treatment plant operation and stream pollution abatement.

2. Selection of Method

For liquid samples, three methods are presented: the partition-gravimetric method (B), the partition-infrared method (C), and the Soxhlet method (D). Method C is designed for samples that might contain volatile hydrocarbons that otherwise would be lost in the solvent-removal operations of the gravimetric procedure. Method D is the method of choice when relatively polar, heavy petroleum fractions are present, or when the levels of nonvolatile greases may challenge the solubility limit of the solvent. For low levels of oil and grease (<10 mg/L), Method C is the method of choice because gravimetric methods do not provide the needed precision.
Method E is a modification of the Soxhlet method and is suitable for sludges and similar materials. Method F can be used in conjunction with Methods B, C, D, or E to obtain a hydrocarbon measurement in addition to, or instead of, the oil and grease measurement. This method makes use of silica gel to separate hydrocarbons from the total oil and grease on the basis of polarity.

3. Sample Collection, Preservation, and Storage

Collect a representative grab sample in a wide-mouth glass bottle that has been washed with soap, rinsed with water, and finally rinsed with solvent to remove any residues that might interfere with the analysis. As an alternative to solvent rinsing, cap bottle with aluminum foil and bake at 200 to 250°C for at least 1 h. Use PTFE-lined caps for sample bottles; clean liners as above, but limit temperature to 110 to 200°C. Collect a separate sample for an oil and grease determination. Do not overfill the sample container and do not subdivide the sample in the laboratory. Collect replicate samples for replicate analyses or known-addition QA checks.

Collect replicates either in rapid succession, in parallel, or in one large container with mechanical stirring (in the latter case, siphon individual

portions). Typically, collect wastewater samples of approximately 1 L. If sample concentration is expected to be greater than 1000 mg extractable material/L, collect proportionately smaller volumes. If analysis is to be delayed for more than 2 h, acidify to pH 2 or lower with either 1:1 HCl or 1:1 H_2SO_4 and refrigerate. When information is required about average grease concentration over an extended period, examine individual portions collected at prescribed time intervals to eliminate losses of grease on sampling equipment during collection of a composite sample.

In sampling sludges, take every possible precaution to obtain a representative sample. When analysis cannot be made within 2 h, preserve samples with 1 mL conc HCl/80 g sample and refrigerate. Never preserve samples with CHCl3 or sodium benzoate.

4. Interferences

a. Organic solvents have the ability to dissolve not only oil and grease but also other organic substances. Any filterable solvent-soluble substances (e.g., elemental sulfur, complex aromatic compounds, hydrocarbon derivatives of chlorine, sulfur, and nitrogen, and certain organic dyes) that are extracted and recovered are defined as oil and grease. No known solvent will dissolve selectively only oil and grease. Heavier residuals of petroleum may contain a significant portion of materials that are not solvent-extractable. The method is entirely empirical; duplicate results with a high degree of precision can be obtained only by strict adherence to all details.

b. For Methods 5520B, D, E, and F, solvent removal results in the loss of short-chain hydrocarbons and simple aromatics by volatilization. Significant portions of petroleum distillates from gasoline through No. 2 fuel oil are lost in this process. Adhere strictly to sample drying time, to standardize gradual loss of weight due to volatilization. For Methods 5520B, D, E, and F, during the cooling of the distillation flask and extracted material, a gradual increase in weight may be observed, presumably due to the absorption of water if a desiccator is not used. For Method 5520C use of an infrared detector offers a degree of selectivity to overcome some coextracted interferences (4*a*). For Methods 5520D and E, use exactly the specified rate and time of extraction in the Soxhlet apparatus because of varying solubilities of different greases.

For Method 5520F, the more polar hydrocarbons, such as complex aromatic compounds and hydrocarbon derivatives of chlorine, sulfur, and nitrogen, may be adsorbed by the silica gel.

Extracted compounds other than hydrocarbons and fatty matter also interfere.

c. Alternative techniques may be needed for some samples if intractable emulsions form that cannot be broken by centrifugation. Such samples

may include effluents from pulp/paper processing and zeolite manufacturing. Determine such modifications on a case-by-case basis.

d. Some sample matrices can increase the amount of water partitioned into the organic extraction fluid. When the extraction solvent from this type of sample is dried with sodium sulfate, the drying capacity of the sodium sulfate can be exceeded, thus allowing sodium sulfate to dissolve and pass into the tared flask. After drying, sodium sulfate crystals will be visible in the flask. The sodium sulfate that passes into the flask becomes a positive interference in gravimetric methods. If crystals are observed in the tared flask after drying, redissolve any oil and grease with 30 mL of extraction solvent and drain the solvent through a funnel containing a solvent-rinsed filter paper into a clean, tared flask. Rinse the first flask twice more, combining all solvent in the new flask, and treat as an extracted sample.

e. Silica gel fines may give positive interferences in 5520F if they pass through the filter. Use filters with smaller pores if this occurs with a particular batch of silica gel.

5520 B. Partition-Gravimetric Method

1. General Discussion

Dissolved or emulsified oil and grease is extracted from water by intimate contact with an extracting solvent. Some extractables, especially unsaturated fats and fatty acids, oxidize readily; hence, special precautions regarding temperature and solvent vapor displacement are included to minimize this effect. Organic solvents shaken with some samples may form an emulsion that is very difficult to break. This method includes a means for handling such emulsions. Recovery of solvents is discussed. Solvent recovery can reduce both vapor emissions to the atmosphere and costs.

2. Apparatus

a. Separatory funnel, 2-L, with TFE stopcock.

b. Distilling flask, 125-mL.

c. Liquid funnel, glass.

d. Filter paper, 11-cm diam.

e. Centrifuge, capable of spinning at least four 100-mL glass centrifuge tubes at 2400 rpm or more.

f. Centrifuge tubes, 100-mL, glass.

g. Water bath, capable of maintaining 85°C.

h. Vacuum pump or other source of vacuum.

i. Distilling adapter with drip tip. Setup of distillate recovery apparatus. Alternatively, use commercially available solvent recovery equipment.

j. Ice bath.

k. Waste receptacle, for used solvent.

l. Desiccator.

3. Reagents

***a. Hydrochloric or sulfuric acid,* 1:1:** Mix equal volumes of either acid and reagent water.

b. n-Hexane, boiling point 69°C. The solvent should leave no measurable residue on evaporation; distill if necessary. Do not use any plastic tubing to transfer solvent between containers.

c. Methyl*-tert-*butyl ether (MTBE), boiling point 55°C to 56°C. The solvent should leave no measurable residue on evaporation; distill if necessary. Do not use any plastic tubing to transfer solvent between containers.

d. Sodium sulfate, Na2SO4, anhydrous crystal.

e. Solvent mixture, 80% *n*-hexane/20% MTBE, v/v.

4. Procedure

When a sample is brought into the laboratory, either mark sample bottle at the water meniscus or weigh the bottle, for later determination of sample volume. If sample has not been acidified previously (see Section 5520A.3), acidify with either 1:1 HCl or 1:1 H_2SO_4 to pH 2 or lower (generally, 5 mL is sufficient for 1 L sample). Using liquid funnel, transfer sample to a separatory funnel. Carefully rinse sample bottle with 30 mL extracting solvent (either 100% *n*-hexane, 3*b*, or solvent mixture, 3*e*) and add solvent washings to separatory funnel. Shake vigorously for 2 min. Let layers separate. Drain aqueous layer and small amount of organic layer into original sample container. Drain solvent layer through a funnel containing a filter paper and 10 g Na2SO4, both of which have been solvent-rinsed, into a clean, tared distilling flask. If a clear solvent layer cannot be obtained and an emulsion of more than about 5 mL exists, drain emulsion and solvent layers into a glass centrifuge tube and centrifuge for 5 min at approximately 2400 rpm. Transfer centrifuged material to an appropriate separatory funnel and drain solvent layer through a funnel with a filter paper and 10 g Na_2SO_4, both of which have been prerinsed, into a clean, tared distilling flask. Recombine aqueous layers and any remaining emulsion or solids in separatory funnel. For samples with <5 mL of emulsion, drain only the clear solvent through a funnel with pre-moistened filter paper and 10 g Na_2SO_4. Recombine aqueous layers and any remaining emulsion or solids in separatory funnel. Extract twice more with 30 mL solvent each time, but first rinse sample container with each solvent portion. Repeat centrifugation step if emulsion persists in subsequent extraction steps. Combine extracts in tared distilling flask, and include in flask a final rinsing of filter and Na_2SO_4 with an additional 10 to 20 mL solvent. Distill solvent from flask in a water bath at 85°C for either solvent system. To maximize solvent recovery, fit distillation flask with a distillation adapter equipped with a drip tip and collect solvent in an ice-bath-cooled receiver. When visible

solvent condensation stops, remove flask from water bath. Cover water bath and dry flasks on top of cover, with water bath still at 85°C, for 15 min. Draw air through flask with an applied vacuum for the final 1 min. Cool in desiccator for at least 30 min and weigh. To determine initial sample volume, either fill sample bottle to mark with water and then pour water into a 1-L graduated cylinder, or weigh empty container and cap and calculate the sample volume by difference from the initial weight (assuming a sample density of 1.00).

5. Calculation

If the organic solvent is free of residue, the gain in weight of the tared distilling flask is due to oil and grease. Total gain in weight, *A*, of tared flask, less calculated residue from solvent blank, *B*, is the amount of oil and grease in the sample:

$$\text{mg oil and grease/L} = \frac{(A - B) \times 1000}{\text{mL sample}}$$

3111 METALS BY FLAME ATOMIC ABSORPTION SPECTROMETRY

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 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.

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.

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 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 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. Miscorrection 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.

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.
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, Section 3113B.4*d*2), 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 Boling 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 .

3030 PRELIMINARY TREATMENT OF SAMPLES

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 HNO_3/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 HNO_3 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.

B. Filtration for Dissolved and Suspended Metals

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-μ m-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 0.5*N* HCl or 1*N* HNO_3 (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 HNO_3 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 sytem 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 $HClO_4$).

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. 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. 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. 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 μg/L or less for electrothermal analysis. To address problems with silver halide solubility in HNO_3, digest using method 3030F.3*b*.

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 maybe 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 *mg/L*	Sample Volume* *mL*
<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:

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. 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.

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 HNO3. 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 HNO3 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 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*, 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.

c. Procedure: Soak new polypropylene tubes and caps overnight or for several days in 2*N* HNO_3. Triple rinse with metal-free water, and preferably dry in poly rackets 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 HNO_3 (or 1.0 mL 1 + 1 HNO_3) 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.