PART 5000

AGGREGATE ORGANIC CONSTITUENTS

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5210 Biochemical Oxygen Demand (BOD) ....James C. Young
5220 Chemical Oxygen Demand (COD) ....Clarence G. Johnson
5310 Total Organic Carbon (TOC) ....Edward W.D. Huffman
5320 Dissolved Organic Halogen ....James M. Symons
5520 Oil and Grease ....John Gute
5560 Organic and Volatile Acids ....Theadore C. Heesen
5710 Formation of Trihalomethanes and Other Disinfection By-Products ....Leown A. Moore
5910 UV-Absorbing Organic Constituents ....James P. Malley, Jr.

Summary of Major Changes since 1998

A new solid-phase, partition-gravimetric method appears in Oil and Grease (5520), and the 80% *n*-hexane and 20% methyl-*tert*-butyl ether solvent mixture was dropped from the liquid-liquid partition-gravimetric method. Organic and Volatile Acids (5560) contains a new gas chromatographic method.

Biochemical Oxygen Demand (5210) was significantly revised to clarify specific procedural steps and QA/QC practices. New quality control checks also were added. Updated quality control information was added to Total Organic Carbon (5310), Dissolved Organic Halogen (5320), and UV-Absorbing Organic Constituents (5910).

5010 INTRODUCTION

5010 A. General Discussion

Analyses for organic matter in water and wastewater can be classified into two general types of measurements: those that quantify an aggregate amount of organic matter comprising organic constituents with a common characteristic and those that quantify individual organic compounds. The latter can be found in Part 6000. The former, described here in Part 5000, have been grouped into four categories: oxygen-demanding substances, organically bound elements, classes of compounds, and formation potentials.

Methods for total organic carbon and chemical oxygen demand are used to assess the total amount of organics present. Gross fractions of the organic matter can be identified analytically, as in the measurement of BOD, which is an index of the biodegradable organics present, oil and grease, which represents material extractable from a sample by a nonpolar solvent, or dissolved organic halide (DOX), which measures organically bound halogens. Trihalomethane formation potential is an aggregate measure of the total concentration of trihalomethanes formed upon chlorination of a water sample.

Analyses of organics are made to assess the concentration and general composition of organic matter in raw water supplies, wastewaters, treated effluents, and receiving waters; and to determine the efficiency of treatment processes.

5010 B. Sample Collection and Preservation

The sampling, field treatment, preservation, and storage of samples taken for organic matter analysis are covered in detail in the individual introductions to the methods. If possible, analyze samples immediately because preservatives often interfere with the tests. Otherwise, store at a low temperature (4°C) immediately after collection to preserve most samples. Use chemical preservatives only when they are shown not to interfere with the examinations to be made (see Section 1060). Never use preservatives for samples to be analyzed for BOD. When preservatives are used, add them to the sample bottle initially so that all portions are preserved as soon as collected. No single method of preservation is entirely satisfactory; choose the preservative with due regard to the determinations that are to be made. All methods of preservation may be inadequate when applied to samples containing significant amounts of suspended matter.

5020 QUALITY ASSURANCE/QUALITY CONTROL

Part 1000 contains important information relevant to analyses included in Part 5000. Give particular attention to Sections 1020B (Quality Control), 1060 (Collection and Preservation of Samples), 1080 (Reagent Water), and 1090 (Laboratory Occupational Health and Safety), all of which are critical for many of the Part 5000 methods.

Take special precautions when analyses are performed by independent laboratories. Reliable use of independent laboratories deserves the same quality assurance procedures observed for in-house analyses: replicate samples, samples with known additions, and blanks.

Preparation of samples with known additions may not be feasible for certain analyses. In such cases, consider using a mixture, in varying ratios, of several samples. Use the reported concentrations in the samples and the proportions in which they were mixed to calculate the expected concentration in the mixture. Examine laboratory performance using externally prepared standards and check samples (see Section 1020B).

Reagent water (Section 1080) should give satisfactory results for most of the analyses in Part 5000, but additional purification steps may be needed for certain methods, such as dissolved organic halogen (DOX) and disinfection by-product formation potential (DBPFP).

5210 BIOCHEMICAL OXYGEN DEMAND (BOD)*

5210 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 BOD_5, 5210B), oxygen consumed after 60 to 90 d of incubation (ultimate BOD or UBOD, 5210C), and continuous oxygen uptake (respirometric method, 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:BOD_5 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, and the inclusion of ammonia in the dilution water contributes an external source of nitrogenous demand. The interference from nitrogenous demand can now be prevented by an inhibitory chemical.[1] If an inhibiting chemical is not used, the oxygen demand measured is the sum of carbonaceous and nitrogenous demands.

Measurements that include nitrogenous demand generally are not useful for assessing the oxygen demand associated with organic material. Nitrogenous demand can be estimated directly from ammonia nitrogen (Section 4500-NH_3); and carbonaceous demand can be estimated by subtracting the theoretical equivalent of the nitrite and nitrate produced in 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 5210B.5*e*) is recommended for samples of secondary effluent, for samples seeded with secondary effluent, and for samples of polluted waters.

3. Reference

1. YOUNG, J.C. 1973. Chemical methods for nitrification control. *J. Water Pollut. Control Fed.* 45:637.

* Approved by Standard Methods Committee, 2001.
Joint Task Group: James C. Young (chair), George T. Bowman, Sabry M. Kamhawy, Terry G. Mills, Marlene Patillo, Ray C. Whittemore.

5210 B. 5-Day BOD Test

1. General Discussion

The method consists of filling with diluted and seeded sample, to overflowing, an airtight bottle of 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.

For sampling and storage procedures, see ¶ 4*a* below.

2. Apparatus

a. Incubation bottles: Use glass bottles having 60 mL or greater capacity (300-mL bottles having a ground-glass stopper and a flared mouth are preferred). Clean bottles with a detergent, rinse thoroughly, and drain before use.

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. Use reagent grade or better for all chemicals and use distilled or equivalent water, preferably sterilized, for making all solutions.

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 and 1.7 g NH_4Cl 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 to 1 L.

c. Calcium chloride solution: Dissolve 27.5 g $CaCl_2$ in distilled water and dilute to 1 L.

d. Ferric chloride solution: Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to 1 L.

e. Acid and alkali solutions, 1*N*, 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:

1) 2-chloro-6-(trichloromethyl) pyridine—Use pure TCMP or commercial preparations*.

2) Allylthiourea (ATU) solution—Dissolve 2.0 g allylthiourea ($C_4H_8N_2S$) in about 500 mL water and dilute to 1 L. Store at 4°C. The solution is stable for not more than 2 weeks.

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 unless solution is maintained in a sterile condition. Store all glucose-glutamic acid mixtures at 4°C or lower. Commercial preparations may be used but concentrations may vary.

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. Source water for preparing BOD dilution water: Use demineralized, distilled, tap, or natural water for making sample dilutions (see ¶ 4*c*).

4. Preparatory Procedures

a. Sampling and storage: Samples for BOD analysis may degrade significantly during storage between collection and analysis, resulting in low BOD values.

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.

b. Sample preparation and pretreatment:

1) All samples — Check pH; if it is not between 6.0 and 8.0, adjust sample temperature to 20 ± 3°C, then adjust pH to 7.0 to 7.2 using a solution of sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH) of such strength that the quantity of reagent does not dilute the sample by more than 0.5%. Exceptions may be justified with natural waters when the BOD is to be measured at in-situ pH values. 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 residual chlorine is present, dechlorinate sample. In some samples chlorine will dissipate within 1 to 2 h of standing in the light. This dissipation 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 1 + 1 acetic acid or 1 + 50 H_2SO_4, 10 mL potassium iodide (KI) solution (10 g /100 mL) per 1000 mL sample and titrating with Na_2SO_3 solution to the starch-iodine end point for residual. Add to neutralized sample the proportional volume of Na_2SO_3 solution determined by the above test, mix, and after 10 to 20 min check sample for residual chlorine. (NOTE: Excess Na_2SO_3 exerts an oxygen demand and reacts slowly with certain organic chloramine compounds that may be present in chlorinated samples.) Do not test chlorinated/dechlorinated samples without seeding.

3) Samples containing other toxic substances—Certain industrial wastes, for example, plating wastes, contain toxic metals. Such samples often require special study and treatment.

* Nitrification Inhibitor Formula 2533 (2% TCMP on sodium sulfate), Hach Co., Loveland, CO, or equivalent.

4) Samples supersaturated with DO—Samples containing DO concentration above saturation 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 by bringing sample to about 20 ± 3°C in partially filled bottle while agitating by vigorous shaking or by aerating with clean, filtered compressed air.

5) Samples containing hydrogen peroxide—Hydrogen peroxide remaining in samples from some industrial bleaching processes such as those used at paper mills and textile plants can cause supersaturated oxygen levels in samples collected for BOD testing. Mix such samples vigorously in open containers for sufficient time to allow the hydrogen peroxide to dissipate before setting up BOD tests. Check adequacy of peroxide removal by observing dissolved oxygen concentrations over time during mixing or by using peroxide-specific test strips. Mixing times can vary from 1 to 2 h depending on the amount of hydrogen peroxide present. The peroxide reaction can be considered complete when the DO no longer increases during a 30-min period without mixing.

c. Selection and storage of source water for BOD sample dilution: Obtain water from suitable source—distilled, tap, or receiving water. Make sure the water is free of heavy metals, specifically copper, and toxic substances, such as chlorine, that can interfere with BOD measurements. Protect source water quality by using clean glassware, tubing, and bottles. Deionized water often contains sufficient amounts of organics and microorganisms to cause failure of the dilution water quality control check (¶ 6*c*). Source water may be stored before use as long as the prepared dilution water (¶ 5*a*) meets quality control criteria in the dilution water blank (¶ 6*c*). Such storage may improve the quality of some source waters but may allow biological growth to cause deterioration in others. Storage of prepared dilution water (¶ 5*g*) for more than 24 h after adding nutrients, minerals, and buffer is not recommended unless dilution water blanks consistently meet quality control limits. Discard stored source water if the dilution water blank shows more than 0.20 mg/L DO depletion in 5 d (see ¶ 6*c*).

d. Preparation of seed suspension: It is necessary to have present in each BOD bottle a population of microorganisms capable of oxidizing the biodegradable organic matter in the sample. Domestic wastewater, unchlorinated or otherwise undisinfected effluents from biological wastewater treatment plants, and surface waters receiving wastewater discharges usually contain satisfactory microbial populations. Some samples (for example, some untreated industrial wastes, disinfected wastes, high-temperature wastes, wastes having pH values less than 6 or greater than 8, or wastes stored more than 6 h after collection) do not contain a sufficient microbial population. Seed such samples by adding a population of suitable microorganisms. The preferred seed is obtained from a biological treatment system processing the waste. In this case, use supernatant from settled domestic wastewater, effluent from primary clarifiers, diluted mixed liquor from an aeration basin, undisinfected effluent, or receiving water from below the point of discharge. When effluent or mixed liquor from a biological treatment process is used as a seed source, inhibition of nitrification is recommended. Do not use seed from effluents that have been disinfected by chlorine or other means. Commercial seed sources may be used but are more likely to be unadapted to the wastewater constituents. Do not filter seed sources; filtering removes the seed microorganisms.

When acclimated seed sources are not available, develop an acclimated seed in the laboratory by continuously aerating a sample of settled domestic wastewater and adding small daily increments of sample from the waste in question. Use a soil suspension, 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 acclimation.

5. Testing Procedure

a. Preparation of dilution water: Transfer desired working volume of source water (¶ 4*c*) to a suitably sized bottle (glass is preferred). Check to ensure that the dissolved oxygen concentration is at least 7.5 mg/L before using water for BOD tests. If not, add DO by shaking bottle or by aerating with organic-free filtered air. Alternatively, store the water in cotton-plugged bottles long enough for the DO concentration to approach saturation. Add 1 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ solution/L to prepared source water (¶ 4*c*). Mix thoroughly and bring temperature to 20 ± 3°C. Prepare dilution water immediately before use unless dilution water blanks (¶ 6*c*) show that the water is acceptable after longer storage times. If the dilution water blanks show a DO depletion greater than 0.20 mg/L, obtain a satisfactory water by improving purification or use water from another source. Do not add oxidizing agents or expose dilution water to ultraviolet light in attempts to bring the dilution blank into range.

b. Sample temperature adjustment: Bring samples to 20 ± 3°C before making dilutions.

c. Preparation of dilutions: Using the dilution water prepared as in ¶ 5*a*, make at least three dilutions of prepared sample estimated to produce a residual DO of at least 1.0 mg/L and a DO uptake of at least 2.0 mg/L after a 5-d incubation. Five dilutions are recommended if experience with a particular sample does not produce at least three bottles having acceptable minimum DO depletions and residual limits (¶ 6*a*). A more rapid analysis, such as COD (Section 5220), may be correlated approximately with BOD and serve as a guide in selecting dilutions. In the absence of prior knowledge, use the following percentages of wastewater when preparing dilutions: 0.01 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. The number of bottles to be prepared for each dilution depends on the DO technique and the number of replicates desired. Prepare dilutions in volumetric containers (Class A glass or equivalent) and then transfer to BOD bottles or prepare directly in BOD bottles. Either dilution method can be combined with any DO measurement technique.

1) Dilutions prepared in volumetric containers—Using a wide-tipped pipet, add desired amount of prepared sample to individual volumetric cylinders or flasks. Mix the sample well immediately before pipetting to avoid loss of solids by settling. For dilutions greater than 1:100 make a primary dilution before making final dilution in the bottle. Fill cylinders or flasks at least two-thirds full of dilution water without entraining air. Add

appropriate amounts of seed suspension (¶ *5d*) and nitrification inhibitor (¶ *5e*). Dilute to final level with dilution water (¶ *5a*). Mix well but avoid entraining air. Siphon mixed dilution into a suitable number of BOD bottles, taking care not to let solids settle in the cylinder or flask during transfer.

2) Dilutions prepared directly in BOD bottles—Using a wide-tip volumetric pipet, add the desired sample volume to individual BOD bottles. Fill each BOD bottle approximately two-thirds full with dilution water. Add appropriate amounts of seed suspension (¶ *5d*) and nitrification inhibitor (¶ *5e*) to the individual 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 (¶s *3a–e*) directly to diluted sample at a rate of 1 mL/ L (0.30 mL/300-mL bottle) or use commercially prepared solutions designed to dose the appropriate bottle size.

d. Addition of seed suspension: If seeding is used, add seed suspensions to the dilution vessels or to individual BOD bottles before final dilution as described in ¶ *5c*. Do not add seed directly to wastewater samples if they contain materials that are toxic before dilution. Generally, 1 to 3 mL of settled raw wastewater or primary effluent or 1 to 2 mL of a 1:10 dilution of mixed liquor/300-mL bottle will provide a suitable amount of microorganisms. Do not filter seed suspension before use. Agitate the seed suspension during transfer to ensure that the same quantity of microorganisms is added to each BOD bottle. Always record the exact volume of seed suspension added to each bottle. The DO uptake attributable to the seed added to each bottle generally 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 (GGA) check results of 198 ± 30.5 mg/L. For example, if 1 mL of seed suspension is required to achieve 198 ± 30.5 mg/L BOD in the glucose-glutamic acid check, then use 1 mL in each BOD bottle receiving the test wastewater.

e. Addition of nitrification inhibitor: Samples that may require nitrification inhibition[1] include, but are not limited to, biologically treated effluents, samples seeded with biologically treated effluents, and river waters. Note the use of nitrogen inhibition and the chemical used when reporting results. (NOTE: TCMP is the preferred nitrification inhibitor but requires handling and transfer in a solid form. Allylthiourea is not always effective in inhibiting nitrification within the 5-d incubation period and concentrations above 2 mg/L may cause increases in carbonaceous BOD measurements. ATU concentrations above 2 mg/L also can adversely affect the azide modification of the iodometric method). Seed all samples to which nitrification inhibitor has been added. The amount of seed should be consistent with that required to achieve GGA test results in the range of 198 ± 30.5 mg/L (¶ *6b*).

1) Nitrification inhibition using 2-chloro-6-(trichloromethyl) pyridine (TCMP)—Add 10 mg TCMP/L to diluted sample or 3 mg TCMP to each 300-mL bottle or sample dilution vessel, or proportional amounts to other sized bottles, after initial sample dilution but before final filling of the bottles with dilution water. Do not add TCMP to BOD bottles before they are at least two-thirds filled with diluted sample. (NOTE: TCMP dissolves slowly and can float on top of the sample if not mixed well). Some commercial TCMP formulations are not 100% TCMP; adjust dosage appropriately.

2) Nitrification inhibition using allylthiourea (ATU)—Add 1 mL ATU solution (¶ *3g*)/L diluted sample or 0.3 mL/300mL test bottle or sample dilution vessel. Do not add ATU to BOD bottles until they are at least two-thirds filled with diluted sample.

f. Sealing of bottles: Complete filling of each bottle by adding enough dilution water that insertion of the stopper leaves no bubbles in the bottle. Mix the sample by turning the bottle manually several times unless a DO probe having a stirrer is used immediately to measure initial DO concentration. 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.

g. Determination of initial DO: 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. Replace any displaced contents with sufficient diluted sample or dilution water to fill the bottle, stopper all bottles tightly, and water seal before beginning incubation. After preparing dilution, measure initial DO within 30 min. If the membrane electrode method is used, take care to eliminate drift in calibration between initial and final DO readings. If the azide modification of the titrimetric iodometric method is used, prepare an extra bottle for initial DO determination for each sample dilution.

h. Sample incubation: Incubate at 20°C ± 1°C the stoppered and sealed BOD bottles containing desired dilutions (¶ *5a*), seed controls (¶ *6d*), dilution water blanks (¶ *6c*), and glucose-glutamic acid checks (¶ *6b*). Exclude light to avoid growth of algae in the bottles during incubation.

i. Determination of final DO: After 5 d ± 6 h of incubation, determine DO in all sample dilutions, and in all blanks and checks as in ¶ *6b–d*, using the azide modification of the titrimetric method or the membrane electrode method.

6. Quality Control Checks

a. Minimum residual DO and minimum DO depletion: Only bottles, including seed controls, giving a minimum DO depletion of 2.0 mg/L and a residual DO of at least 1.0 mg/L after 5 d of incubation are considered to produce valid data, because at least 2.0 mg oxygen/uptake L is required to give a meaningful measure of oxygen uptake and at least 1.0 mg/L must remain throughout the test to ensure that insufficient DO does not affect the rate of oxidation of waste constituents. Exceptions occur for reporting purposes only when the depletions for tests using undiluted samples in all bottles fall below 2.0 mg/L and when the residual DO in all dilutions is less than 1.0 mg/L (see ¶ 7). When using membrane electrodes for measuring DO, make frequent calibration checks to ensure accurate DO readings (see 4500-O.C).

b. Glucose-glutamic acid check: The glucose-glutamic acid check is the primary basis for establishing accuracy and precision of the BOD test and is the principal measure of seed quality and set-up procedure. Together with each batch of samples,

check seed effectiveness and analytical technique by using procedures in ¶ 5 to make BOD measurements on an equal weight mixture of glucose and glutamic acid as follows: Add sufficient amounts of standard glucose-glutamic acid solution (¶ 3*h*) to give 3.0 mg glucose/L and 3.0 mg glutamic acid /L in each of three test bottles (20 mL GGA solution/L seeded dilution water, or 6.0 mL/300-mL bottle). Commercial solutions may contain other glucose-glutamic acid concentrations; adjust doses accordingly. Add nitrification inhibitor if seed is obtained from a source that is nitrifying. Evaluate data as described in ¶ 8, Precision and Bias. The resulting average BOD for the three bottles, after correction for dilution and seeding, must fall into the range of 198 ± 30.5 mg/L. If the average value falls outside this range, evaluate the cause and make appropriate corrections. Consistently high values can indicate the use of too much seed suspension, contaminated dilution water, or the occurrence of nitrification; consistently low values can indicate poor seed quality or quantity or the presence of a toxic material. If low values persist, prepare a new mixture of glucose and glutamic acid and check the sources of dilution water and source of seed.

c. Dilution water quality check: With each batch of samples incubate one or more bottles of dilution water that contains nutrient, mineral, and buffer solutions but no seed or nitrification inhibitor. This dilution water blank serves as a check on quality of unseeded dilution water and cleanliness of incubation bottles. Determine initial and final DO as in ¶s 5*e* and *g*. The DO uptake in 5 d must not be more than 0.20 mg/L and preferably not more than 0.10 mg/L, before making seed corrections. If the dilution water blank exceeds 0.20 mg/L, discard all data for tests using this dilution water or clearly identify such samples in data records.

d. Seed control: Determine BOD of the seed suspension as for any other sample. This is the *seed control.* Ideally, make three dilutions of seed such that the smallest quantity gives at least 2.0 mg/L DO depletion and the largest quantity results in at least 1.0 mg/L DO residual after 5 d of incubation. Determine the DO uptake per milliliter of seed added to each bottle using either the slope method or the ratio method. For the slope method, plot DO depletion in milligrams per liter versus milliliters of seed for all seed control bottles having a 2.0 mg/L depletion and 1.0 minimum residual DO. The plot 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 0.20 mg/L (see ¶ 6*c*). For the ratio method, divide the DO depletion by the volume of seed in milliliters for each seed control bottle having a 2.0 mg/L depletion and greater than 1.0 mg/L minimum residual DO and average the results. Seed dilutions showing widely varying depletions per milliliter of seed (± 30%) suggest the presence of toxic substances or large particulates in the seed suspension. In this case, check or change the seed source.

7. Data Analysis and Reporting

a. Calculations:

1) For each test bottle having 2.0 mg/L minimum DO depletion and at least 1.0 mg/L residual DO, calculate BOD as follows:

$$\text{BOD}_5\text{, mg/L} = \frac{(D_1 - D_2) - (S)V_s}{P}$$

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,
S = oxygen uptake of seed, Δ DO/mL seed suspension added per bottle (¶ 6*d*) ($S = 0$ if samples are not seeded),
V_s = volume of seed in the respective test bottle, mL, and
P = decimal volumetric fraction of sample used; $1/P$ = dilution factor.

2) If DO depletion is less than 2.0 mg/L and sample concentration is 100% (no dilution except for seed, nutrient, mineral, and buffer solutions), actual seed-corrected, DO depletion may be reported as the BOD even if it is less than 2.0 mg/L.

3) When all dilutions result in a residual DO < 1.0, select the bottle having the lowest DO concentration (greatest dilution) and report:

$$\text{BOD, mg/L} > \frac{(D_1 - D_2) - (S)V_s}{P}$$

In the above 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 in ¶ 6*c*. 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.

b. Reporting: Average the test results for all qualified bottles within each dilution series. Report the result as BOD_5 if nitrification is not inhibited. Report results as $CBOD_5$ if nitrification is inhibited. Samples showing large differences between the computed BOD for different dilutions, for example, greater than 30%, may indicate the presence of a toxic substance or analytical problems. When the effect becomes repetitive, investigate to identify the cause. Identify results in the test reports when any of the following quality control parameters is not met:

- Dilution water blank exceeds 0.20 mg/L (¶ 6*c*),
- Glucose-glutamic acid check falls outside acceptable limits (¶ 6*b*),
- Test replicates show more than 30% difference between high and low values,
- Seed control samples do not meet the above criteria in all dilutions (¶ 6*d*), or
- Minimum DO is less than 1.0 mg/L [¶ 7*a*3)].

8. Precision and Bias

There is no measurement for establishing bias of the BOD procedure. The glucose-glutamic acid check prescribed in ¶ 6*b* is intended to be a reference point for evaluation of dilution water quality, seed effectiveness, and analytical technique. Single-laboratory tests using a 300-mg/L mixed glucose-glutamic acid solution provided the following results:

Number of months:	14
Number of triplicates:	421
Average monthly recovery:	204 mg/L
Average monthly standard deviation:	10.4 mg/L

In a series of interlaboratory studies,[2] each involving 2 to 112 laboratories (and as many analysts and seed sources), 5-d BOD measurements were made on synthetic water samples containing a 1:1 mixture of glucose and glutamic acid in the total concentration range of 3.3 to 231 mg/L. The regression equations for mean value, *X*, and standard deviation, *S*, from these studies were:

$X = 0.658$ (added concentration, mg/L) $+ 0.280$ mg/L

$S = 0.100$ (added concentration, mg/L) $+ 0.547$ mg/L

For the 300-mg/L mixed primary standard, the average 5-d BOD would be 198 mg/L with a standard deviation of 30.5 mg/L. When nitrification inhibitors are used, GGA test results falling outside the 198 ± 30.5 control limit quite often indicate use of incorrect amounts of seed. Adjust amount of seed added to the GGA test to achieve results falling within this range.

a. Control limits: Because of many factors affecting BOD tests in multilaboratory studies and the resulting extreme variability in test results, one standard deviation, as determined by interlaboratory tests, is recommended as a control limit for individual laboratories. Alternatively, each laboratory may establish its control limits by performing a minimum of 25 glucose-glutamic acid checks (¶ 6*b*) over a period of several weeks or months and calculating the mean and standard deviation. Use the mean ±3 standard deviations as the control limit for future glucose-glutamic acid checks. Compare calculated control limits to the single-laboratory tests presented above and to interlaboratory results. If the glucose-glutamic acid test results are outside the range of 198 ± 30.5, re-evaluate the control limits and investigate source of the problem. If measured BOD for a glucose-glutamic acid check is outside the accepted control limit range, reject tests made with that seed and dilution water or identify such tests clearly in all data records and reports.

b. Working range and detection limit: The working range is equal to the difference between the maximum initial DO (7 to 9 mg/L) and minimum DO residual of 1 mg/L corrected for seed, and multiplied by the dilution factor.

Detection limits are established by the minimum DO depletion and minimum DO residuals as follows:

• The lower detection limit for unseeded samples that require dilution ($S = 0$; $P < 1.0$) is 2 mg/L multiplied by the dilution factor as established by the requirement for a minimum DO depletion of 2 mg/L.

• The lower limit for seeded samples that require dilution ($S > 0$; $P < 1.0$) is approximately 1 mg/L as established by the minimum depletion of 2.0 mg/L minus the maximum seed correction, which should be less than about 1 mg/L.

• The lower limit for unseeded samples that require no dilution ($S = 0$; $P = 1.0$) is equal to the detection limit of the DO measurement method (~0.1 mg/L).

• The lower detection limit for seeded samples that require no dilution ($S > 0$; $P = 1.0$) is 0 mg/L, as established by the difference between the sample DO depletion and the seed correction.

9. References

1. YOUNG, J.C. 1973. Chemical methods for nitrification control. *J. Water Pollut. Control Fed.* 45:637.
2. U.S. ENVIRONMENTAL PROTECTION AGENCY, OFFICE OF RESEARCH AND DEVELOPMENT. 1986. Method-by-Method Statistics from Water Pollution (WP) Laboratory Performance Evaluation Studies. Quality Assurance Branch, Environmental Monitoring and Support Lab., Cincinnati, Ohio.

10. Bibliography

THERIAULT, E.J., P.D. MCNAMEE & C.T. BUTTERFIELD. 1931. Selection of dilution water for use in oxygen demand tests. *Pub. Health Rep.* 46:1084.

LEA, W.L. & M.S. NICHOLS. 1937. Influence of phosphorus and nitrogen on biochemical oxygen demand. *Sewage Works J.* 9:34.

RUCHHOFT, C.C. 1941. Report on the cooperative study of dilution waters made for the Standard Methods Committee of the Federation of Sewage Works Associations. *Sewage Works J.* 13:669.

MOHLMAN, F.W., E. HURWITZ, G.R. BARNETT & H.K. RAMER. 1950. Experience with modified methods for BOD. *Sewage Ind. Wastes* 22:31.

YOUNG, J.C., G.N. MCDERMOTT & D. JENKINS . 1981. Alterations in the BOD procedure for the 15th edition of Standard Methods for the Examination of Water and Wastewater. *J. Water Pollut. Control Fed.* 53:1253.

5210 C. Ultimate BOD Test

1. General Discussion

The ultimate BOD test is an extension of the 5-d dilution BOD test as described in 5210B but with a number of specific test requirements and differences in application. The user should be familar with the 5210B procedure before conducting tests for UBOD.

a. Principle: The method consists of placing a single sample dilution in full, airtight bottles and incubating under specified conditions for an extended period depending on wastewater, effluent, river, or estuary quality.[1] Dissolved oxygen (DO) is measured (with probes) initially and intermittently during the test. From the DO versus time series, UBOD is calculated by an appropriate statistical technique. For improved accuracy, run tests in triplicate.

Bottle size and incubation time are flexible to accommodate individual sample characteristics and laboratory limitations. Incubation temperature, however, is 20°C. Most effluents and some naturally occurring surface waters contain materials with oxygen demands exceeding the DO available in air-saturated water. Therefore, it is necessary either to dilute the sample or to monitor DO frequently to ensure that low DO or anaerobic conditions do not occur. When DO concentrations approach 2 mg/L, the sample should be reaerated.

Because bacterial growth requires nutrients such as nitrogen, phosphorus, and trace metals, the necessary amounts may be added to the dilution water together with buffer to ensure that pH remains in a range suitable for bacterial growth and seed to provide an adequate bacterial population. However, if the result is being used to estimate the rate of oxidation of naturally occurring surface waters, addition of nutrients and seed probably accelerates the decay rate and produces misleading results. If only UBOD is desired, it may be advantageous to add supplemental nutrients that accelerate decay and reduce the test duration. When nutrients are used, they also should be used in the dilution water blank. Because of the wide range of water and wastewater characteristics and varied applications of UBOD data, no specific nutrient or buffer formulations are included.

The extent of oxidation of nitrogenous compounds during the prescribed incubation period depends on the presence of microorganisms capable of carrying out this oxidation. Such organisms may not be present in wastewaters in sufficient numbers to oxidize significant quantities of reduced nitrogen. This situation may be reversed in naturally occurring surface waters. Erratic results may be obtained when a nitrification inhibitor is used;[2] therefore, the specified method precludes use of a nitrogen inhibitor unless prior experimental evidence on the particular sample suggests that it is acceptable.* Monitor NO_2^--N and NO_3^--N to compute the oxygen equivalency of the nitrification reaction. When these values are subtracted from the DO vs. time series, the carbonaceous BOD time series can be constructed.[3]

b. Sampling and storage: See Section 5210B.4*a*.

2. Apparatus

a. Incubation bottles: Glass bottles with ground-glass stoppers,† 2-L (or larger) capacity. Glass serum bottles of 4- to 10-L capacity are available. Alternatively use nonground-glass bottles with nonbiodegradable plastic caps as a plug insert. Do not reuse the plugs because discoloration occurs with continued use. Replace plugs every 7 to 14 d. Do not use rubber stoppers that may exert an oxygen demand. Clean bottles with a detergent and wash with dilute HCl (3*N*) to remove surface films and precipitated inorganic salts; rinse thoroughly with DI water before use. Cover top of bottles with paper after rinsing to prevent dust from collecting. To prevent drawing air into the sample bottle during incubation, use a water seal. If the bottle does not have a flared mouth, construct a water seal by making a watertight dam around the stopper (or plug) and fill with water from the reservoir as necessary. Cover dam with clean aluminum foil to retard evaporation. If a 2-L BOD bottle is used, fill reservoir with sample and cover with a polyethylene cap before incubation.

Place a clean magnetic stirring bar in each bottle to mix contents before making DO measurement or taking a subsample. Do not remove the magnets until the test is complete.

Alternatively use a series of 300-mL BOD bottles as described in 5210B, if larger bottles are not available or incubation space is limited.

b. Reservoir bottle: 4-L or larger glass bottle. Close with screw plastic cap or non-rubber plug.

c. Incubator or water bath, thermostatically controlled at 20 ± 1°C. Exclude all light to prevent the possibility of photosynthetic production of DO.

d. Oxygen-sensitive membrane electrode: See Section 4500-O.G.2.

3. Procedure

a. River water samples: Preferably fill large BOD bottle (>2 L, or alternatively 6 or more 300-mL BOD bottles) with sample at 20°C. Add no nutrients, seed, or nitrification inhibitor if in-bottle decay rates will be used to estimate in-stream rates. Do not dilute sample unless it is known by pretesting or by experience to have a high ultimate BOD (>20 mg/L).

Measure DO in each bottle, stopper, and make an airtight seal. Incubate at 20°C in the dark.

Measure DO in each bottle at intervals of at least 2 to 5 d over a period of 30 to 60 d (minimum of 6 to 8 readings) or longer under special circumstances. To avoid oxygen depletion in samples containing NH_3-N, measure DO more frequently until nitrification has taken place. If DO falls to about 2 mg/L, reaerate as directed below. Replace sample lost by the cap and DO probe displacement by adding 1 to 2 mL sample from the reservoir bottle.

When DO approaches 2 mg/L, reaerate. Pour a small amount of sample into a clean vessel and reaerate the remainder directly in the bottle by vigorous shaking or bubbling with purified air (medical grade). Refill bottle from the storage reservoir and measure DO. This concentration becomes the initial DO for the next measurement. If using 300-mL BOD bottles, pour all of the sample from the several bottles used into a clean vessel, reaerate, and refill the small bottles.

Analyze for nitrate plus nitrite nitrogen (NO_3^--N + NO_2^--N) (see Sections 4500-NO_2^- and 4500-NO_3^-) on Days 0, 5, 10, 15, 20, and 30. Alternatively, determine NO_2^--N and NO_3^--N each time DO is determined, thereby producing corresponding BOD and nitrogen determinations. If the ultimate demand occurs at a time greater than 30 d, make additional analyses at 30-d intervals. Remove 10 to 20 mL from the bottle for these analyses. Refill bottle as necessary from the reservoir bottle. Preserve NO_2^--N + NO_3^--N subsample with H_2SO_4 to pH <2 and refrigerate. If the purpose of the UBOD test is to assess the UBOD and not to provide data for rate calculations, measure nitrate nitrogen concentration only at Day 0 and on the last day of the test (kinetic rate estimates are not useful when the nitrification reaction is not followed).

Calculate oxygen consumption during each time interval and make appropriate corrections for nitrogenous oxygen demand. Correct by using 3.43 × the NH_3-N to NO_2^--N conversion plus 1.14 × the NO_2^--N to NO_3^--N conversion to reflect the stoichiometry of the oxidation of NH_4^+ to NO_2^- or NO_3^-.

When using a dilution water blank, subtract DO uptake of the blank from the total DO consumed. High-quality reagent water without nutrients typically will consume a maximum of 1 mg DO/L in a 30- to 90-d period. If DO uptake of the dilution water is greater than 0.5 mg/L for a 20-d period, or 1 mg/L for a 90-d period, report the magnitude of the correction and try to obtain higher-quality dilution water for use with subsequent UBOD tests.

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* Some analysts have reported satisfactory results with 2-chloro-6-(trichloromethyl) pyridine (Nitrification Inhibitor, Formula 2533, Hach Co., Loveland, CO, or equivalent).

† Wheaton 2-L BOD bottle No. 227580, 1000 North Tenth St., Millville, NJ, or equivalent.

TABLE 5210:I. UBOD RESULTS FOR WASTEWATER SAMPLE

Day	(1) Average DO* mg/L	(2) Average Blank DO† mg/L	(3) Accumulated DO Consumed by Sample‡ mg/L	(4) Average NO_3-N mg/L	(5) NBOD mg/L§	(6) CBOD mg/L‖
0	8.1	—	0	0.0	0	0
3	5.6	—	2.5	—	0	2.5
5	3.5/8.0	—	4.6	0.0	0	4.6
7	6.2	—	6.4	—	0.23	6.2
10	3.2/8.2	—	9.4	0.10	0.46	8.9
15	4.3	—	13.3	—	0.58	12.7
18	2.7/8.1	—	14.9	0.15	0.69	14.2
20	6.6	—	16.4	—	0.80	15.6
25	5.4	—	17.6	0.20	0.92	16.7
30	2.6/8.2	—	20.4	—	0.92	19.5
40	5.3	—	23.3	0.20	0.92	22.4
50	3.1/8.0	—	25.5	—	0.92	24.6
60	4.5	—	29.0	—	0.92	28.1
70	3.3/8.1	—	30.2	—	0.92	29.3
90	5.4	—	32.9	0.20	0.92	32.0

* Two readings indicate concentrations before and after reaeration.
† None was used.
‡ Column (1)–blank correction (none needed in the example).
§ Column (4) × 4.57 (linear interpolation between values).
‖ [Column (3)–Column (5)] × dilution factor.

Ultimate CBOD = 34.5 mg/L; CBOD decay rate = 0.03/d (calculated with first-order equation from 5210C.4).

When the weekly DO consumption drops below 1 to 2% of the total accumulative consumption, calculate the ultimate BOD using a nonlinear regression method.

b. Wastewater treatment plant samples: Use high-quality reagent water (see Section 1080) for dilution water. Add no nitrification inhibitors if decay rates are desired. If seed and nutrients are necessary, add the same amounts of each to the dilution water blank. Use minimal sample dilution. As a rule of thumb, the ultimate BOD of the diluted sample should be in the range of 20 to 30 mg/L. Dilution to this level probably will require two or three sample reaerations during the incubation period to avoid having dissolved oxygen concentrations fall below 2 mg/L.

Use 2-L or larger BOD bottles (alternatively, multiple 300-mL BOD bottles) for each dilution. Add desired volume of sample to each bottle and fill with dilution water.

Fill a BOD bottle with dilution water to serve as a dilution water blank. Treat blank the same as all samples. Follow procedure given in ¶ 3*a* and incubate for at least as long as UBOD test.

4. Calculations

An example of results obtained for a wastewater sample, undiluted, without seed and nutrients, is given in Table 5210:I.

UBOD can be estimated by using a first-order model described as follows:

$$BOD_t = UBOD\,(1 - e^{-kt})$$

where:

BOD_t = oxygen uptake measured at time t, mg/L, and
k = first-order oxygen uptake rate.

The data in Table 5210:I were analyzed with a nonlinear regression technique applied to the above first-order model.[4] However, a first-order kinetic model may not always be the best choice. Significantly better statistical fits usually are obtained with alternative kinetic models including sum of two first-order and logistic function models.[1,3–8]

5. Precision and Bias

The precision of the ultimate BOD test was assessed with a series of replicate tests in a single laboratory. Interlaboratory studies have not been conducted.

Reference	Replicate No.	UBOD mg/L	Precision Summary*
2	1	154	μ = 151 mg/L
	2	154	
	3	145	CV = 3.5%
5	1	10.3	
	2	11.1	
	3	9.6	μ = 10.0 mg/L
	4	9.9	
	5	9.8	CV = 5.8%
	6	9.6	
6	1	12.8	μ = 12.4 mg/L
	2	12.6	
	3	12.6	CV = 4.4%
	4	11.6	

* μ = mean,
CV = coefficient of variation.

Bias was assessed by determining the BOD of a known concentration of glucose (150 mg/L) and glutamic acid (150 mg/L). This solution has a UBOD of 321 mg/L to 308 mg/L, depending on extent of nitrification. The results of the study conducted in triplicate were:[1]

Estimated* UBOD *mg/L*	Theoretical BOD *mg/L*	Percent Difference
276	308/321	−10/−14
310	308/321	+1/−3
303	308/321	−2/−6

* By statistical model.

6. References

1. MARTONE, C.H. 1976. Studies Related to the Determination of Biodegradability and Long Term BOD. M.S. thesis, Dep. Civil Engineering, Tufts Univ., Medford, Mass.
2. NATIONAL COUNCIL OF THE PAPER INDUSTRY FOR AIR AND STREAM IMPROVEMENT, INC. 1986. A Review of the Separation of Carbonaceous and Nitrogenous BOD in Long-Term BOD Measurements. Tech. Bull. No. 461, New York, N.Y.
3. NATIONAL COUNCIL OF THE PAPER INDUSTRY FOR AIR AND STREAM IMPROVEMENT, INC. 1982. A Review of Ultimate BOD and Its Kinetic Formulation for Pulp and Paper Mill Effluents. Tech. Bull. No. 382, New York, N.Y.
4. BARNWELL, T. 1980. Least Squares Estimates of BOD Parameters. *J. Environ. Eng. Div., Proc. Amer. Soc. Civil Eng.* 107(EE6):1197.
5. NATIONAL COUNCIL OF THE PAPER INDUSTRY FOR AIR AND STREAM IMPROVEMENT, INC. 1982. A Proposal to Examine the Effect of Mixing on Long Term BOD Test. NE82-01, New York, N.Y.
6. NATIONAL COUNCIL OF THE PAPER INDUSTRY FOR AIR AND STREAM IMPROVEMENT, INC. 1982. A Study of the Selection, Calibration, and Verification of Mathematical Water Quality Models. Tech. Bull. No. 367, New York, N.Y.
7. NATIONAL COUNCIL OF THE PAPER INDUSTRY FOR AIR AND STREAM IMPROVEMENT, INC. 1987. User's Manual for Parameter Estimation for First Order Ultimate BOD Decay, BODFO. Tech. Bull. No. 529, New York, N.Y.
8. CHU, W.S. & E.W. STRECKER. 1972. Parameter Identification In Water System Model. Dep. Civil Engineering, Univ. Washington, Seattle.

5210 D. Respirometric Method

1. General Discussion

a. Principle: Respirometric methods provide direct measurement of the oxygen consumed by microorganisms from an air or oxygen-enriched environment in a closed vessel under conditions of constant temperature and agitation.

b. Uses: Respirometry measures oxygen uptake more or less continuously over time. Respirometric methods are useful for assessing: biodegradation of specific chemicals; treatability of organic industrial wastes; the effect of known amounts of toxic compounds on the oxygen-uptake reaction of a test wastewater or organic chemical; the concentration at which a pollutant or a wastewater measurably inhibits biological degradation; the effect of various treatments such as disinfection, nutrient addition, and pH adjustment on oxidation rates; the oxygen requirement for essentially complete oxidation of biologically oxidizable matter; the need for using adapted seed in other biochemical oxygen-uptake measurements, such as the dilution BOD test; and stability of sludges.

Respirometric data typically will be used comparatively, that is, in a direct comparison between oxygen uptakes from two test samples or from a test sample and a control. Because of inherent differences among uses, among seed cultures, among applications of results, and among instruments, a single procedure for respirometric tests applicable to all cases cannot be defined. Therefore, only basic recommendations and guidelines for overall test setup and procedure are given. Follow manufacturer's instructions for operating details for specific commercial instruments.

c. Types of respirometers: Four principal types of commercial respirometers are available. Manometric respirometers relate oxygen uptake to the change in pressure caused by oxygen consumption while maintaining a constant volume. Volumetric respirometers measure oxygen uptake in incremental changes in gas volume while maintaining a constant pressure at the time of reading. Electrolytic respirometers monitor the amount of oxygen produced by electrolysis of water to maintain a constant oxygen pressure within the reaction vessel. Direct-input respirometers deliver oxygen to the sample from a pure oxygen supply through metering on demand as detected by minute pressure differences. Most respirometers have been instrumented to permit data collection and processing by computer. Reaction-vessel contents are mixed by using a magnetic or mechanical stirring device or by bubbling the gaseous phase within the reaction vessel through the liquid phase. All respirometers remove carbon dioxide produced during biological growth by suspending a concentrated adsorbent (granular or solution) within the closed reaction chamber or by recirculating the gas phase through an external scrubber.

d. Interferences: Evolution of gases other than CO_2 may introduce errors in pressure or volume measurements; this is uncommon in the presence of dissolved oxygen. Incomplete CO_2 absorption will introduce errors if appropriate amounts and concentrations of alkaline absorbent are not used. Temperature fluctuations or inadequate mixing will introduce error. Fluctuations in barometric pressure can cause errors with some respirometers. Become familiar with the limits of the instrument to be used.

e. Minimum detectable concentration: Most commercial respirometers can detect oxygen demand in increments as small as 0.1 mg but test precision depends on the total amount of oxygen consumed at the time of reading, the precision of pressure or volume measurement, and the effect of temperature and barometric pressure changes. Upper limits of oxygen uptake rate are

determined by the ability to transfer oxygen into the solution from the gas phase, which typically is related to mixing intensity. Transfer limits typically range from less than 10 mg O_2/L/h for low-intensity mixing to above 100 mg O_2/L/h for high-intensity mixing.

f. Relationship to dilution BOD: Variations in waste composition, substrate concentration, mixing, and oxygen concentrations from one wastewater source to another generally preclude use of a general relationship between oxygen uptake by respirometers and the 5-d, 20°C, BOD (see 5210B, above). Reasonably accurate correlations may be possible for a specific wastewater. The incubation period for respirometric measurements need not be 5 d because equally valid correlations can be made between the 5-d dilution BOD and respirometric oxygen uptake at any time after 2 d.[1,2] The point of common dilution and respirometric BOD seems to occur at about 2 to 3 d incubation for municipal wastewaters. Correlations between respirometric measurements and 5-d BOD for industrial wastes and specific chemicals are less certain. Respirometric measurements also can provide an indication of the ultimate biochemical oxygen demand (UBOD) (see Section 5210C). In many cases, it is reasonable to consider that the 28- to 30-d oxygen uptake is essentially equal to the UBOD.[3]

More commonly, respirometers are used as a diagnostic tool. The continuous readout of oxygen consumption in respirometric measurements indicates lag, toxicity, or any abnormalities in the biodegradation reaction. The change in the normal shape of an oxygen-uptake curve in the first few hours may help to identify the effect of toxic or unusual wastes entering a treatment plant in time to make operating corrections.

g. Relationship to other test methods and protocols: This method supports most of the protocols and guidelines established by the European Organization for Economic Co-operation and Development[3] (OECD) that require measurement of oxygen uptake.

h. Sampling and storage:

1) Grab samples—If analysis is begun within 2 h of sample collection, cold storage is unnecessary. Otherwise, keep sample at or below 4°C from the time of collection. Begin analysis within 6 h of collection; when this is not possible, store at or below 4°C and report length and temperature of storage. Never start analysis more than 24 h after grab sample collection.

2) Composite samples—Keep samples at or below 4°C during compositing. Limit compositing period to 24 h. Use the same criteria as for storage of grab samples, starting the measurement of holding time from the end of the compositing period. State storage time and conditions with results.

2. Apparatus

a. Respirometer system: Use commercial apparatus and check manufacturer's instructions for specific system requirements, reaction vessel type and volume, and instrument operating characteristics.

b. Incubator or water bath: Use a constant-temperature room, incubator chamber, or water bath to control temperature to ±1°C. Exclude all light to prevent oxygen formation by algae in the sample. Use red, actinic-coated bottles for analysis outside of a darkened incubator.

3. Reagents

Formulations of reagent solutions are given for 1-L volumes, but smaller or larger volumes may be prepared according to need. Discard any reagent showing signs of biological growth or chemical precipitation. Stock solutions can be sterilized by autoclaving to provide longer shelf life.

a. Distilled water: Use only high-quality water distilled from a block tin or all-glass still (see Section 1080). Deionized water may be used but often contains high bacterial counts. The water must contain less than 0.01 mg heavy metals/L and be free of chlorine, chloramines, caustic alkalinity, organic material, or acids. Make all reagents with this water. When other waters are required for special-purpose testing, state clearly their source and quality characteristics.

b. Phosphate buffer solution, 1.5N: Dissolve 207 g sodium dihydrogen phosphate, $NaH_2PO_4 \cdot H_2O$, in water. Neutralize to pH 7.2 with 6*N* KOH (¶ 3*g* below) and dilute to 1 L.

c. Ammonium chloride solution, 0.71N: Dissolve 38.2 g ammonium chloride, NH_4Cl, in water. Neutralize to pH 7.0 with KOH. Dilute to 1.0 L; 1 mL = 10 mg N.

d. Calcium chloride solution, 0.25N: Dissolve 27.7 g $CaCl_2$ in water and dilute to 1 L; 1 mL = 10 mg Ca.

e. Magnesium sulfate solution, 0.41N: Dissolve 101 g $MgSO_4 \cdot 7H_2O$ in water and dilute to 1 L; 1 mL = 10 mg Mg.

f. Ferric chloride solution, 0.018N: Dissolve 4.84 g $FeCl_3 \cdot 6H_2O$ in water and dilute to 1 L; 1 mL = 1.0 mg Fe.

g. Potassium hydroxide solution, 6N: Dissolve 336 g KOH in about 700 mL water and dilute to 1 L. CAUTION: *Add KOH to water slowly and use constant mixing to prevent excessive heat buildup.* Alternately, use commercial solutions containing 30 to 50% KOH by weight.

h. Acid solutions, 1N: Add 28 mL conc H_2SO_4 or 83 mL conc HCl to about 700 mL water. Dilute to 1 L.

i. Alkali solution, 1N: Add 40 g NaOH to 700 mL water. Dilute to 1 L.

j. Nitrification inhibitor: Reagent-grade 2-chloro-6-(trichloromethyl) pyridine (TCMP) or equivalent.[3]*

k. Glucose-glutamic acid solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 h. Add 15.0 g glucose and 15.0 g glutamic acid to distilled water and dilute to 1 L. Neutralize to pH 7.0 using 6*N* potassium hydroxide (¶ 3*g*). This solution may be stored for up to 1 week at 4°C.

l. Electrolyte solution (for electrolytic respirometers): Use manufacturer's recommended solution.

m. Sodium sulfite solution, 0.025N: Dissolve 1.575 g Na_2SO_3 in about 800 mL water. Dilute to 1 L. This solution is not stable; prepare daily or as needed.

n. Trace element solution: Dissolve 40 mg $MnSO_4 \cdot 4H_2O$, 57 mg H_3BO_3, 43 mg $ZnSO_4 \cdot 7H_2O$, 35 mg $(NH_4)_6 Mo_7O_{24}$, and 100 mg Fe-chelate ($FeCl_3$-EDTA) in about 800 mL water. Dilute to 1 L. Sterilize at 120°C and 200 kPa (2 atm) pressure for 20 min.

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* Formula 2533, Hach Chemical Co., Loveland, CO, or equivalent. NOTE: Some commercial formulations are not pure TCMP. Check with supplier to verify compound purity and adjust dosages accordingly.

o. Yeast extract solution:[3] Add 15 mg laboratory- or pharmaceutical-grade brewer's yeast extract to 100 mL water. Make this solution fresh immediately before each test in which it is used.

p. Nutrient solution:[3] Add 2.5 mL phosphate buffer solution (3*b*), 0.65 mL ammonium chloride solution (3*c*), 1.0 mL calcium chloride solution (3*d*), 0.22 mL magnesium sulfate solution (3*e*), 0.1 mL ferric chloride solution (3*f*), 1 mL trace element solution (3*n*), and 1 mL yeast extract solution (3*o*) to about 900 mL water. Dilute to 1 L. This nutrient solution and those of ¶s *n* and *o* above are specifically formulated for use with the OECD method.[3] (NOTE: A 10:1 concentrated nutrient solution can be made and diluted accordingly.)

4. Procedure

a. Instrument operation: Follow respirometer manufacturer's instructions for assembly, testing, calibration, and operation of the instrument. NOTE: The manufacturer's stated maximum and minimum limits of measurement are not always the same as the instrument output limits. Make sure that test conditions are within the limits of measurement.

b. Sample volume: Sample volume or concentration of organic chemicals to be added to test vessels is a function of expected oxygen uptake characteristics and oxygen transfer capability of the instrument. Small volumes or low concentrations may be required for high-strength wastes. Large volumes may be required for low-strength wastes to improve accuracy.

c. Data recording interval: Set instrument to give data readings at suitable intervals. Intervals of 15 min to 6 h typically are used.

d. Sample preparation:

1) Homogenization—If sample contains large settleable or floatable solids, homogenize it with a blender and transfer representative test portions while all solids are in suspension. If there is a concern for changing sample characteristics, skip this step.

2) pH adjustment—Neutralize samples to pH 7.0 with H_2SO_4 or NaOH of such strength (¶s 3*h* and *i*) that reagent quantity does not dilute the sample more than 0.5%.

3) Dechlorination—Avoid analyzing samples containing residual chlorine by collecting the samples ahead of chlorination processes. If residual chlorine is present, aerate as described in ¶ 5) below or let stand in light for 1 to 2 h. If a chlorine residual persists, add Na_2SO_3 solution. Determine required volume of Na_2SO_3 solution by adding 10 mL 1 + 1 acetic acid or 1 + 50 H_2SO_4 and 10 mL potassium iodide solution (10 g/100 mL) to a portion of the sample. Titrate with 0.025*N* Na_2SO_3 solution to the starch-iodine end point (see Section 4500-Cl.B). Add to the neutralized sample a proportional volume of Na_2SO_3 solution determined above, mix, and after 10 to 20 min check for residual chlorine. Re-seed the sample (see ¶ 4*h* below).

4) Samples containing toxic substances—Certain industrial wastes contain toxic metals or organic compounds. These often require special study and treatment.[3]

5) Initial oxygen concentration—If samples contain dissolved oxygen concentrations above or below the desired concentration, agitate or aerate with clean and filtered compressed air for about 1 h immediately before testing. Minimum and maximum actual DO concentrations will vary with test objectives. In some cases, pure oxygen may be added to respirometer vessels to increase oxygen levels above ambient.

6) Temperature adjustment—Bring samples and dilution water to desired test temperature (±1°C) before making dilutions or transferring to test vessels.

e. Sample dilution: Use distilled water or water from other appropriate sources free of organic matter. In some cases, receiving stream water may be used for dilution. Add desired sample volume to test vessels using a wide-tip volumetric pipet or other suitable volumetric glassware. Add dilution water to bring sample to about 80% of desired final volume. Add appropriate amounts of nutrients, minerals, buffer, nitrification inhibitor if desired, and seed culture as described in ¶s 4*f* and *h* below. Dilute sample to desired final volume. The number of test vessels to prepare for each dilution depends on test objectives and number of replicates desired.

f. Nutrients, minerals, and buffer: Add sufficient ammonia nitrogen to provide a COD:N:P ratio of 100:5:1 or a TOC:N:P ratio of 30:5:1. Add 2 mL each of calcium, magnesium, ferric chloride, and trace mineral solutions to each liter of diluted sample unless sufficient amounts of these minerals are present in the original sample. Phosphorus requirements will be met by the phosphate buffer if it is used (1 mL/50 mg/L COD or ultimate BOD of diluted sample usually is sufficient to maintain pH between 6.8 and 7.2). Be cautious in adding phosphate buffer to samples containing metal salts because metal phosphates may precipitate and show less toxic or beneficial effect than when phosphate is not present. For OECD-compatible tests, substitute the nutrient, mineral, and buffer amounts listed in ¶ 3*p* for the above nutrient/mineral/buffer quantities.

g. Nitrification inhibition: If nitrification inhibition is desired, add 10 mg 2-chloro-6-(trichloromethyl) pyridine (TCMP)/L sample in the test vessel. Samples that may nitrify readily include biologically treated effluents, samples seeded with biologically treated effluents, and river waters.[4]

h. Seeding: See 5210B.4*d* for seed preparation. Use sufficient amounts of seed culture to prevent major lags in the oxygen uptake reaction but not so much that the oxygen uptake of the seed exceeds about 10% of the oxygen uptake of the seeded sample.

Determine the oxygen uptake of the seeding material as for any other sample. This is the seed control. Typically, the seed volume in the seed control should be 10 times the volume used in seeded samples.

i. Incubation: Incubate samples at 20°C or other suitable temperature ±1.0°C. Take care that the stirring device does not increase the temperature of the sample.

5. Calculations

To convert instrument readings to oxygen uptake, refer to manufacturer's procedures.

Correct oxygen uptake for seed and dilution by the following equation:

$$C = [A - B(S_A/S_B)](1000/N_A)$$

where:

C = corrected oxygen uptake of sample, mg/L,
A = measured oxygen uptake in seeded sample, mg,

B = measured oxygen uptake in seed control, mg,
S_A = volume of seed in Sample A, mL,
S_B = volume of seed in Sample B, mL, and
N_A = volume of undiluted sample in Sample A, mL.

6. Quality Control

Periodically use the following procedure to check distilled water quality, instrument quality, instrument function, and analytical technique by making oxygen uptake measurements using a mixture of glucose and glutamic acid as a standard check solution.

Adjust water for sample formulation to test temperature and saturate with DO by aerating with clean, organic-free filtered air. Protect water quality by using clean glassware, tubing, and bottles.

Prepare a *test solution* by adding 10 mL glucose-glutamic acid solution (3*k*); 6 mL phosphate buffer (3*b*); 2 mL each of ammonium chloride (3*c*), magnesium sulfate (3*e*), calcium chloride (3*d*), ferric chloride (3*f*), and trace element solution (3*n*) to approximately 800 mL water. Add 10 mg nitrification inhibitor (TCMP)/L. Add sufficient seed from a suitable source as described in ¶ 4*h* to give a lag time less than 6 h (usually 25 mL supernatant from settled primary effluent/L test solution is sufficient). Dilute to 1 L. Adjust temperature to 20 ± 1°C.

Prepare a *seed blank* by diluting 500 mL or more of the seed solution to 800 mL with distilled water. Add the same amount of buffer, nutrients, and TCMP as in the test solution, and dilute to 1 L. Adjust temperature to 20 ± 1°C.

Place test solution and seed blank solution in separate reaction vessels of respirometer and incubate for 5 d at 20°C. Run at least three replicates of each. The seed-corrected oxygen uptake after 5 d incubation should be 260 ± 30 mg/L. If the value of the check is outside this range, repeat the test using a fresh seed culture and seek the cause of the problem.

7. Precision and Bias

a. Precision: No standard is available to check the accuracy of respirometric oxygen uptake measurements. To obtain laboratory precision data, use a glucose-glutamic acid mixture (¶ 6 above) having a known theoretical maximum oxygen uptake value. Tests with this and similar organic compound mixtures have shown that the standard deviation, expressed as the coefficient of variation, C_v, is approximately 5% for samples having total oxygen uptakes of 50 to 100 mg/L and 3% for more concentrated samples.[1,2] Individual instruments have different readability limits that can affect precision. The minimum response or sensitivity of most commercial respirometers ranges from 0.05 to 1 mg oxygen. Check manufacturer's specifications for sensitivity of the instrument at hand.

b. Control limits: To establish laboratory control limits, perform a minimum of 25 glucose-glutamic acid checks over a period of several weeks or months and calculate mean and standard deviation. If measured oxygen uptake in 5 d at 20°C is outside the 260 ± 30 mg/L range, re-evaluate procedure to identify source of error. For other samples, use the mean ±3 standard deviations as the control limit.

c. Working range and detection limits: The working range and detection limits are established by the limits of each commercial instrument. Refer to manufacturer's specifications.

8. References

1. YOUNG, J.C. & E.R. BAUMANN. 1976. The electrolytic respirometer—Factors affecting oxygen uptake measurements. *Water Res.* 10:1031.
2. YOUNG, J.C. & E.R. BAUMANN. 1976. The electrolytic respirometer—Use in water pollution control plant laboratories. *Water Res.* 10:1141.
3. ORGANIZATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT. 1981. Method 5.2, Annex V, Part C *in* OECD Guidelines for Testing of Chemicals. OECD, Paris, France.
4. YOUNG, J.C. 1973. Chemical methods for nitrification control. *J. Water Poll. Control Fed.* 45:637.

9. Bibliography

HEUKELEKIAN, H. 1947. Use of direct method of oxygen utilization in waste treatment studies. *Sew. Works J.* 19:375.

CALDWELL, D.H. & W.F. LANGELIER. 1948. Manometric measurement of the biochemical oxygen demand of sewage. *Sew. Works J.* 20:202.

GELLMAN, I. & H. HEUKELEKIAN. 1951. Studies of biochemical oxidation by direct methods. *Sew. Ind. Wastes.* 23:1267.

JENKINS, D. 1960. The use of manometric methods in the study of sewage and trade wastes. *In* Waste Treatment, p. 99. Pergamon Press, Oxford, U.K.

MONTGOMERY, H.A.C. 1967. The determination of biochemical oxygen demand by respirometric methods. *Water Res.* 1:631.

CEDENA, F., A. DROHOBYCZAR, M.I. BEACH & D. BARNES. 1988. A novel approach to simplified respirometric oxygen demand determinations. *Proc. 43rd Ind. Waste Conf.*, Purdue Univ., Lafayette, Ind.

5220 CHEMICAL OXYGEN DEMAND (COD)*

5220 A. Introduction

Chemical oxygen demand (COD) is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence. Because of its unique chemical properties, the dichromate ion ($Cr_2O_7^{2-}$) is the specified oxidant in Methods 5220B, C, and D; 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 5220C and D 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 5220B.4*a*, C.4, or D.4. Use procedure 5220B.4*b* to determine, with lesser accuracy, COD values from 5 to 50 mg O_2/L.

2. Interferences and Limitations

Oxidation of most organic compounds is 95 to 100% of the theoretical value. Pyridine and related compounds resist oxidation and volatile organic compounds will react in proportion to their contact with the oxidant. Straight-chain aliphatic compounds are oxidized more effectively in the presence of a silver sulfate catalyst.

The most common interferent is the chloride ion. Chloride reacts with silver ion to precipitate silver chloride, and thus inhibits the catalytic activity of silver. Bromide, iodide, and any other reagent that inactivates the silver ion can interfere similarly. Such interferences are negative in that they tend to restrict the oxidizing action of the dichromate ion itself. However, under the rigorous digestion procedures for COD analyses, chloride, bromide, or iodide can react with dichromate to produce the elemental form of the halogen and the chromic ion. Results then are in error on the high side. The difficulties caused by the presence of the chloride can be overcome largely, though not completely, by complexing with mercuric sulfate ($HgSO_4$) before the refluxing procedure. Although 1 g $HgSO_4$ is specified for 50 mL sample, a lesser amount may be used where sample chloride concentration is known to be less than 2000 mg/L, as long as 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.[1,2]

Halide interferences may be removed by precipitation with silver ion and filtration before digestion. This approach may introduce substantial errors due to the occlusion and carrydown of COD matter from 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 O_2/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 to COD is negligible, $HgSO_4$ can be omitted. Smaller sample sizes (see 5220C and D) reduce the waste. Recovery of the waste material may be feasible if allowed by regulatory authority.[3]

3. Sampling and Storage

Preferably collect samples in glass bottles. Test unstable samples without delay. If delay before analysis is unavoidable, preserve sample by acidification to pH ≤ 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 prelim-

* Approved by Standard Methods Committee, 1997.
Joint Task Group: 20th Edition–Clarence G. Johnson (chair), Donald G. Miller, John T. Pivinski.

inary dilutions for wastes containing a high COD to reduce the error inherent in measuring small sample volumes.

4. References

1. BURNS, E.R. & C. MARSHALL. 1965. Correction for chloride interference in the chemical oxygen demand test. *J. Water Pollut. Control Fed.* 37:1716.
2. BAUMANN, F.I. 1974. Dichromate reflux chemical oxygen demand: A proposed method for chloride correction in highly saline waters. *Anal. Chem.* 46:1336.
3. HOLM, T.R. 1996. Treatment of Spent Chemical Oxygen Demand Solutions for Safe Disposal. Illinois State Water Survey, Champaign.

5220 B. Open Reflux Method

1. General Discussion

a. Principle: Most types of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfate to determine the amount of $K_2Cr_2O_7$ consumed and the oxidizable matter is calculated in terms of oxygen equivalent. Keep ratios of reagent weights, volumes, and strengths constant when sample volumes other than 50 mL are used. The standard 2-h reflux time may be reduced if it has been shown that a shorter period yields the same results. Some samples with very low COD or with highly heterogeneous solids content may need to be analyzed in replicate to yield the most reliable data. Results are further enhanced by reacting a maximum quantity of dichromate, provided that some residual dichromate remains.

2. Apparatus

a. Reflux apparatus, consisting of 500- or 250-mL erlenmeyer flasks with ground-glass 24/40 neck and 300-mm jacket Liebig, West, or equivalent condenser with 24/40 ground-glass joint, and a hot plate having sufficient power to produce at least 1.4 W/cm^2 of heating surface, or equivalent.

b. Blender.

c. Pipets, Class A and wide-bore.

3. Reagents

a. Standard potassium dichromate solution, 0.04167*M:* Dissolve 12.259 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, in distilled water and dilute to 1000 mL. This reagent undergoes a six-electron reduction reaction; the equivalent concentration is 6 × 0.04167*M* or 0.2500*N*.

b. Sulfuric acid reagent: Add Ag_2SO_4, reagent or technical grade, crystals or powder, to conc H_2SO_4 at the rate of 5.5 g Ag_2SO_4/kg H_2SO_4. Let stand 1 to 2 d to dissolve. Mix.

c. Ferroin indicator solution: Dissolve 1.485 g 1,10-phenanthroline monohydrate and 695 mg $FeSO_4 \cdot 7H_2O$ in distilled water and dilute to 100 mL. This indicator solution may be purchased already prepared.*

* GFS Chemicals, Inc., Columbus, OH, or equivalent.

d. Standard ferrous ammonium sulfate (FAS) titrant, approximately 0.25*M:* Dissolve 98 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 this solution daily against standard $K_2Cr_2O_7$ solution as follows:

Dilute 25.00 mL standard $K_2Cr_2O_7$ to about 100 mL. Add 30 mL conc H_2SO_4 and cool. Titrate with FAS titrant using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator.

Molarity of FAS solution

$$= \frac{\text{Volume } 0.04167M\ K_2Cr_2O_7 \text{ solution titrated, mL}}{\text{Volume FAS used in titration, mL}} \times 0.2500$$

e. Mercuric sulfate, $HgSO_4$, crystals or powder.

f. Sulfamic acid: Required only if the interference of nitrites is to be eliminated (see 5220A.2 above).

g. Potassium hydrogen phthalate (KHP) standard, $HOOCC_6H_4COOK$: Lightly crush and then dry KHP to constant weight at 110°C. Dissolve 425 mg in distilled water and dilute to 1000 mL. KHP has a theoretical COD[1] of 1.176 mg O_2/mg and this solution has a theoretical COD of 500 μg O_2/ mL. This solution is stable when refrigerated, but not indefinitely. Be alert to development of visible biological growth. If practical, prepare and transfer solution under sterile conditions. Weekly preparation usually is satisfactory.

4. Procedure

a. Treatment of samples with COD of >50 mg O_2/L: Blend sample if necessary and pipet 50.00 mL into a 500-mL refluxing flask. For samples with a COD of >900 mg O_2/L, use a smaller portion diluted to 50.00 mL. Add 1 g $HgSO_4$, several glass beads, and very slowly add 5.0 mL sulfuric acid reagent, with mixing to dissolve $HgSO_4$. Cool while mixing to avoid possible loss of volatile materials. Add 25.00 mL 0.04167*M* $K_2Cr_2O_7$ solution and mix. Attach flask to condenser and turn on cooling water. Add remaining sulfuric acid reagent (70 mL) through open end of condenser. Continue swirling and mixing while adding sulfuric acid reagent. CAUTION: *Mix reflux mixture thoroughly before applying heat to prevent local heating of flask bottom and a possible blowout of flask contents.*

Cover open end of condenser with a small beaker to prevent foreign material from entering refluxing mixture and reflux for 2 h. Cool and wash down condenser with distilled water. Disconnect reflux condenser and dilute mixture to about twice its volume with

distilled water. Cool to room temperature and titrate excess $K_2Cr_2O_7$ with FAS, using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator. Although the quantity of ferroin indicator is not critical, use the same volume for all titrations. Take as the end point of the titration the first sharp color change from blue-green to reddish brown that persists for 1 min or longer. Duplicate determinations should agree within 5% of their average. Samples with suspended solids or components that are slow to oxidize may require additional determinations. The blue-green may reappear. In the same manner, reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of sample.

b. Alternate procedure for low-COD samples: Follow procedure of ¶ 4*a*, with two exceptions: (*i*) use standard 0.004167*M* $K_2Cr_2O_7$, and (*ii*) titrate with standardized 0.025*M* FAS. Exercise extreme care with this procedure because even a trace of organic matter on the glassware or from the atmosphere may cause gross errors. If a further increase in sensitivity is required, concentrate a larger volume of sample before digesting under reflux as follows: Add all reagents to a sample larger than 50 mL and reduce total volume to 150 mL by boiling in the refluxing flask open to the atmosphere without the condenser attached. Compute amount of $HgSO_4$ to be added (before concentration) on the basis of a weight ratio of 10:1, $HgSO_4:Cl^-$, using the amount of Cl^- present in the original volume of sample. Carry a blank reagent through the same procedure. This technique has the advantage of concentrating the sample without significant losses of easily digested volatile materials. Hard-to-digest volatile materials such as volatile acids are lost, but an improvement is gained over ordinary evaporative concentration methods. Duplicate determinations are not expected to be as precise as in 5220B.4*a*.

c. Determination of standard solution: Evaluate the technique and quality of reagents by conducting the test on a standard potassium hydrogen phthalate solution.

5. Calculation

$$\text{COD as mg } O_2/L = \frac{(A - B) \times M \times 8000}{\text{mL sample}}$$

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.

6. Precision and Bias

A set of synthetic samples containing potassium hydrogen phthalate and NaCl was tested by 74 laboratories. At a COD of 200 mg O_2/L in the absence of chloride, the standard deviation was ±13 mg/L (coefficient of variation, 6.5%). At COD of 160 mg O_2/L and 100 mg Cl^-/L, the standard deviation was ±14 mg/L (coefficient of variation, 10.8%).

7. Reference

1. PITWELL, L.R. 1983. Standard COD. *Chem. Brit.* 19:907.

8. Bibliography

MOORE, W.A., R.C. KRONER & C.C. RUCHHOFT. 1949. Dichromate reflux method for determination of oxygen consumed. *Anal. Chem.* 21:953.
MEDALIA, A.I. 1951. Test for traces of organic matter in water. *Anal. Chem.* 23:1318.
MOORE, W.A., F.J. LUDZACK & C.C. RUCHHOFT. 1951. Determination of oxygen-consumed values of organic wastes. *Anal. Chem.* 23:1297.
DOBBS, R.A. & R.T. WILLIAMS. 1963. Elimination of chloride interference in the chemical oxygen demand test. *Anal. Chem.* 35:1064.

5220 C. Closed Reflux, Titrimetric Method

1. General Discussion

a. Principle: See 5220B.1*a*.

b. Interferences and limitations: See 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*

* Hach Co., Bioscience, Inc., or equivalent.

the possibility of leaking samples generating a corrosive and possibly explosive atmosphere. Also, culture tube caps may not withstand the 150°C temperature in an oven.

c. Microburet.

d. Ampule sealer: Use only a mechanical sealer to insure strong, consistent seals.

3. Reagents

a. Standard potassium dichromate digestion solution, 0.01667M: Add to about 500 mL distilled water 4.903 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, 167 mL conc H_2SO_4, and 33.3 g $HgSO_4$. Dissolve, cool to room temperature, and dilute to 1000 mL.

b. Sulfuric acid reagent: See Section 5220B.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

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

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. Refer to Table 5220:I for proper sample and reagent volumes. Make volumetric measurements as accurate as practical; use Class A volumetric ware. The most critical volumes are of the sample and digestion solution. Use a microburet for titrations. Measure H_2SO_4 to ±0.1 mL. The use of hand-held pipettors with non-wetting (polyethylene) pipet tips is practical and adequate. Place sample in culture tube or ampule and add digestion solution. Carefully run sulfuric acid reagent down inside of vessel so an acid layer is formed under the sample-digestion solution layer. Tightly cap tubes or seal ampules, and invert each several times to mix completely. CAUTION: *Wear face shield and protect hands from heat produced when contents of vessels are mixed. Mix 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.

TABLE 5220:I. SAMPLE AND REAGENT QUANTITIES FOR VARIOUS DIGESTION VESSELS

Digestion Vessel	Sample *mL*	Digestion Solution *mL*	Sulfuric Acid Reagent *mL*	Total Final Volume *mL*
Culture tubes:				
16 × 100 mm	2.50	1.50	3.5	7.5
20 × 150 mm	5.00	3.00	7.0	15.0
25 × 150 mm	10.00	6.00	14.0	30.0
Standard 10-mL ampules	2.50	1.50	3.5	7.5

5. Calculation

$$\text{COD as mg } O_2/L = \frac{(A-B) \times M \times 8000}{\text{mL sample}}$$

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.

6. Precision and Bias

Sixty synthetic samples containing potassium hydrogen phthalate and NaCl were tested by six laboratories. At an average COD of 195 mg O_2/L in the absence of chloride, the standard deviation was ±11 mg O_2/L (coefficient of variation, 5.6%). At an average COD of 208 mg O_2/L and 100 mg Cl^-/L, the standard deviation was ±10 mg O_2/L (coefficient of variation, 4.8%).

5220 D. Closed Reflux, Colorimetric Method

1. General Discussion

a. Principle: See Section 5220B.1*a*. When a sample is digested, the dichromate ion oxidizes COD material in the sample. This results in the change of chromium from the hexavalent (VI) state to the trivalent (III) state. Both of these chromium species are colored and absorb in the visible region of the spectrum. The dichromate ion ($Cr_2O_7^{2-}$) absorbs strongly in the 400-nm region, where the chromic ion (Cr^{3+}) absorption is much less. The chromic ion absorbs strongly in the 600-nm region, where the dichromate has nearly zero absorption. In 9*M* sulfuric acid solution, the approximate molar extinction coefficients for these chromium species are as follows: Cr^{3+} − 50 L/mole cm at 604 nm; $Cr_2O_7^{2-}$ − 380 L/mole cm at 444 nm; Cr^{3+} − 25 L/mole cm at 426 nm. The Cr^{3+} ion has a minimum in the region of 400 nm. Thus a working absorption maximum is at 420 nm.

For COD values between 100 and 900 mg/L, increase in Cr^{3+} in the 600-nm region is determined. Higher values can be obtained by sample dilution. COD values of 90 mg/L or less can be determined by following the decrease in $Cr_2O_7^{2-}$ at 420 nm. The corresponding generation of Cr^{3+} gives a small absorption increase at 420 nm, but this is compensated for in the calibration procedure.

b. Interferences and limitations: See Section 5220C.1*b*.

For this procedure to be applicable, all visible light-absorbing interferents must be absent or be compensated for. This includes insoluble suspended matter as well as colored components. If either type of interference occurs, the test is not necessarily lost because COD can be determined titrimetrically as in 5220C.

2. Apparatus

a. See Section 5220C.2. Ensure that reaction vessels are of optical quality. Other types of absorption cells with varying path lengths may be used. Use the extinction coefficients of the ions of interest for this approach.

b. Spectrophotometer, for use at 600 nm and/or 420 nm with access opening adapter for ampule or 16-, 20-, or 25-mm tubes. Verify that the instrument operates in the region of 420 nm and 600 nm. Values slightly different from these may be found, depending on the spectral bandpass of the instrument.

3. Reagents

a. Digestion solution, high range: Add to about 500 mL distilled water 10.216 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, 167 mL conc H_2SO_4, and 33.3 g $HgSO_4$. Dissolve, cool to room temperature, and dilute to 1000 mL.

b. Digestion solution, low range: Prepare as in 3*a*, but use only 1.022 g potassium dichromate.

c. Sulfuric acid reagent: See Section 5220B.3*b*.

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

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

4. Procedure

a. Treatment of samples: Measure suitable volume of sample and reagents into tube or ampule as indicated in Table 5220:I. Prepare, digest, and cool samples, blank, and one or more standards as directed in Section 5220C.4. *Note the safety precautions.* It is critical that the volume of each component be known and that the total volume be the same for each reaction vessel. If volumetric control is difficult, transfer digested sample, dilute to a known volume, and read. Premixed reagents in digestion tubes are available commercially.

b. Measurement of dichromate reduction: Cool sample to room temperature slowly to avoid precipitate formation. Once samples are cooled, vent, if necessary, to relieve any pressure generated during digestion. Mix contents of reaction vessels to combine condensed water and dislodge insoluble matter. Let suspended matter settle and ensure that optical path is clear. Measure absorption of each sample blank and standard at selected wavelength (420 nm or 600 nm). At 600 nm, use an undigested blank as reference solution. Analyze a digested blank to confirm good analytical reagents and to determine the blank COD; subtract blank COD from sample COD. Alternately, use digested blank as the reference solution once it is established that the blank has a low COD.

At 420 nm, use reagent water as a reference solution. Measure all samples, blanks, and standards against this solution. The absorption measurement of an undigested blank containing dichromate, with reagent water replacing sample, will give initial dichromate absorption. Any digested sample, blank, or standard that has a COD value will give lower absorbance because of the decrease in dichromate ion. Analyze a digested blank with reagent water replacing sample to ensure reagent quality and to determine the reagents' contribution to the decrease in absorbance during a given digestion. The difference between absorbances of a given digested sample and the digested blank is a measure of the sample COD. When standards are run, plot differences of digested blank absorbance and digested standard absorbance versus COD values for each standard.

c. Preparation of calibration curve: Prepare at least five standards from potassium hydrogen phthalate solution with COD equivalents to cover each concentration range. Make up to volume with reagent water; use same reagent volumes, tube, or ampule size, and digestion procedure as for samples. Prepare calibration curve for each new lot of tubes or ampules or when standards prepared in ¶ 4*a* differ by ≥5% from calibration curve. Curves should be linear. However, some nonlinearity may occur, depending on instrument used and overall accuracy needed.

5. Calculation

If samples, standards, and blanks are run under same conditions of volume and optical path length, calculate COD as follows:

$$\text{COD as mg } O_2/L = \frac{\text{mg } O_2 \text{ in final volume} \times 1000}{\text{mL sample}}$$

Preferably analyze samples in duplicate because of small sample size. Samples that are inhomogeneous may require multiple determinations for accurate analysis. These should not differ from their average by more than ±5% for the high-level COD test unless the condition of the sample dictates otherwise.

In the low-level procedure, results below 25 mg/L may tend to be qualitative rather than quantitative.

6. Precision and Bias

Forty-eight synthetic samples containing potassium hydrogen phthalate and NaCl were tested by five laboratories. At an average COD of 193 mg O_2/L in the absence of chloride, the standard deviation was ±17 mg O_2/L (coefficient of variation 8.7%). At an average COD of 212 mg O_2/L and 100 mg Cl^-/L, the standard deviation was ±20 mg O_2/L (coefficient of variation, 9.6%). Additional QA/QC data for both high- and low-level procedures may be found elsewhere.[1]

7. Reference

1. AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1995. Standard test methods for chemical oxygen demand (dichromate oxygen demand) of water. D1252-95, ASTM Annual Book of Standards. American Soc. Testing & Materials, Philadelphia, Pa.

8. Bibliography

JIRKA, A.M. & M.J. CARTER. 1975. Micro semi-automated analysis of surface and wastewaters for chemical oxygen demand. *Anal. Chem.* 47:1397.

HIMEBAUGH, R.R. & M.J. SMITH. 1979. Semi-micro tube method for chemical oxygen demand. *Anal. Chem.* 51:1085.

5310 TOTAL ORGANIC CARBON (TOC)*

5310 A. Introduction

1. General Discussion

The organic carbon in water and wastewater is composed of a variety of organic compounds in various oxidation states. Some of these carbon compounds can be oxidized further by biological or chemical processes, and the biochemical oxygen demand (BOD), assimilable organic carbon (AOC), and chemical oxygen demand (COD) methods may be used to characterize these fractions. Total organic carbon (TOC) is a more convenient and direct expression of total organic content than either BOD, AOC, or COD, but does not provide the same kind of information. If a repeatable empirical relationship is established between TOC and BOD, AOC, or COD for a specific source water then TOC can be used to estimate the accompanying BOD, AOC, or COD. This relationship must be established independently for each set of matrix conditions, such as various points in a treatment process. Unlike BOD or COD, TOC is independent of the oxidation state of the organic matter and does not measure other organically bound elements, such as nitrogen and hydrogen, and inorganics that can contribute to the oxygen demand measured by BOD and COD. TOC measurement does not replace BOD, AOC, and COD testing.

Measurement of TOC is of vital importance to the operation of water treatment and waste treatment plants. Drinking water TOCs range from less than 100 μg/L to more than 25,000 μg/L. Wastewater may contain very high levels of organic compounds (TOC >100 mg/L). Some of these applications may include waters with substantial ionic impurities as well as organic matter.

In many applications, the presence of organic contaminants may degrade ion-exchange capacity, serve as a nutrient source for undesired biological growth, or be otherwise detrimental to the process for which the water is to be utilized. For drinking waters in particular, organic compounds may react with disinfectants to produce potentially toxic and carcinogenic compounds.

To determine the quantity of organically bound carbon, the organic molecules must be broken down and converted to a single molecular form that can be measured quantitatively. TOC methods utilize high temperature, catalysts, and oxygen, or lower temperatures (<100°C) with ultraviolet irradiation, chemical oxidants, or combinations of these oxidants to convert organic carbon to carbon dioxide (CO_2). The CO_2 may be purged from the sample, dried, and transferred with a carrier gas to a nondispersive infrared analyzer or coulometric titrator. Alternatively, it may be separated from the sample liquid phase by a membrane selective to CO_2 into a high-purity water in which corresponding increase in conductivity is related to the CO_2 passing the membrane.

2. Fractions of Total Carbon

The methods and instruments used in measuring TOC analyze fractions of total carbon (TC) and measure TOC by two or more determinations. These fractions of total carbon are defined as: inorganic carbon—the carbonate, bicarbonate, and dissolved CO_2; total organic carbon (TOC)—all carbon atoms covalently bonded in organic molecules; dissolved organic carbon (DOC)—the fraction of TOC that passes through a 0.45-μm-pore-diam filter; suspended organic carbon—also referred to as particulate organic carbon, the fraction of TOC retained by a 0.45-μm filter; purgeable organic carbon—also referred to as volatile organic carbon, the fraction of TOC removed from an aqueous solution by gas stripping under specified conditions; and nonpurgeable organic carbon—the fraction of TOC not removed by gas stripping.

In most water samples, the inorganic carbon fraction is many times greater than the TOC fraction. Eliminating or compensating for inorganic carbon interferences requires determinations of both TC and inorganic carbon to measure TOC. Inorganic carbon interference can be eliminated by acidifying samples to pH 2 or less to convert inorganic carbon species to CO_2. Subsequent

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purging of the sample with a purified gas or vacuum degassing removes the CO_2 by volatilization. Sample purging also removes purgeable organic carbon so that the organic carbon measurement made after eliminating inorganic carbon interferences is actually a nonpurgeable organic carbon determination: determine purgeable organic carbon to measure TOC. In many surface and ground waters the purgeable organic carbon contribution to TOC is negligible. Therefore, in practice, the nonpurgeable organic carbon determination is substituted for TOC.

Alternatively, inorganic carbon interference may be compensated for by separately measuring total carbon (TC) and inorganic carbon. The difference between TC and inorganic carbon is TOC.

The purgeable fraction of TOC is a function of the specific conditions and equipment employed. Sample temperature and salinity, gas-flow rate, type of gas diffuser, purging-vessel dimensions, volume purged, and purging time affect the division of TOC into purgeable and nonpurgeable fractions. When separately measuring purgeable organic carbon and nonpurgeable organic carbon on the same sample, use identical conditions for purging during the purgeable organic carbon measurement as in purging to prepare the nonpurgeable organic carbon portion for analysis. Consider the conditions of purging when comparing purgeable organic carbon or nonpurgeable organic carbon data from different laboratories or different instruments.

3. Selection of Method

The high-temperature combustion method (B) is suitable for samples with higher levels of TOC that would require dilution for the various persulfate methods (Method C or Method D). Generally, it also will determine organic carbon from compounds that are chemically refractory and not determined by Method C or Method D. High-temperature combustion may be desirable for samples containing high levels of suspended organic carbon, which may not be efficiently oxidized by persulfate and/or UV methods. Interlaboratory studies have shown biases on the order of 1 mg/L using older high-temperature instruments. With newer instruments, detection limits as low as 10 μg/L have been reported. Some high-temperature combustion instruments are not designed for levels below 1 mg/L. The high-temperature methods accumulate nonvolatile residues in the analyzer, whereas, in Method C, residuals are drained from the analyzer. Method C generally provides better sensitivity for lower-level (<1 mg/L) samples. Persulfate and/or UV oxidation are useful for TOC as low as 10 μg/L. Because the range of sensitivity of the methods overlaps, other factors may dictate method choice in the range of 1 mg/L to 50 mg/L. A method may be chosen on the basis of desired precision, ease of use, cost, etc. Method D generally is equivalent to Method C, but the equipment for Method D is no longer manufactured.

To qualify a particular instrument for use, demonstrate that the single-user precision and bias given in each method can be reproduced. Also, preferably demonstrate the overall precision by conducting in-house studies with more than one operator.

Evaluate the selected method to ensure that data quality objectives are attained. Evaluate method detection level in a matrix as similar as possible to the unknowns as described in Section 1030. Be aware that instrument blanks are handled in a variety of ways in TOC analyzers and that the true magnitude of the blank may not be readily apparent to the analyst. Some instruments "zero out" much of the blank by adjusting the zero on the detector. Others enter blank values in units such as mv responses rather than absolute concentrations, whereas other instruments accumulate the total blank in the system during a blank run. Carefully observe the variability of low-level measurements and check it any time reagents or instrument operations are changed. The following methods note that when a water blank is run there is a contribution to the observed blank value from the level of carbon in the blank water.

The methods show expected single-operator and multiple-laboratory precision. These equations are based on referenced interlaboratory studies that in some cases were performed on older equipment. The range of testing is important to observe because the error and bias generally will be some significant fraction of the low standard. Consult references to determine type of equipment and conditions of the interlaboratory study. Determine the performance of the instrument being used by analyzing waters with matrices similar to those of unknowns, using the procedures outlined in Section 1040B.

4. Bibliography

FORD, D.L. 1968. Total organic carbon as a wastewater parameter. *Pub. Works* 99:89.

FREDERICKS, A.D. 1968. Concentration of organic carbon in the Gulf of Mexico: Off. Naval Res. Rep. 68–27T.

WILLIAMS, P.M. 1969. The determination of dissolved organic carbon in seawater: A comparison of two methods. *Limnol. Oceanogr.* 14: 297.

CROLL, B.T. 1972. Determination of organic carbon in water. *Chem. Ind.* (London) 110:386.

SHARP, J. 1973. Total organic carbon in seawater—comparison of measurements using persulfate oxidation and high temperature combustion. *Mar. Chem.* 1:211.

BORTLUZ, J. 1976. Instrumental TOC analysis. *Vom Wasser* 46:35.

VAN STEENDEREN, R.A. 1976. Parameters which influence the organic carbon determination in water. *Water SA* 2:156.

TAKAHASHI, Y. 1979. Analysis Techniques for Organic Carbon and Organic Halogen. Proc. EPA/NATO-CCMS Conf. on Adsorption Techniques. Reston, Va.

STANDING COMMITTEE OF ANALYSIS, DEPARTMENT OF THE ENVIRONMENT, NATIONAL WATER COUNCIL. 1980. The instrumental determination of total organic carbon, total oxygen demand and related determinants. 1979. Her Majesty's Stationery Off., London.

WERSHAW, R.L., M.J. FISHMAN, R.R. GRABBE & L.E. LOWE, eds. 1983. Methods for the Determination of Organic Substances in Water and Fluvial Sediments. U.S. Geological Survey Techniques of Water Resources Investigations, Book 5. Laboratory Analysis. Chapter A3.

KAPLAN, L.A. 1992. Comparison of high-temperature and persulfate oxidation methods for determination of dissolved organic carbon in freshwaters. *Limnol. Oceanogr.* 37:1119.

BENNER, R. & J.I. HEDGES. 1993. A test of the accuracy of freshwater DOC measurements by high-temperature catalytic oxidation and UV-promoted persulfate oxidation. *Mar. Chem.* 41:75.

WANGERSKY, P.J. 1993. Dissolved organic carbon methods: A critical review. *Mar. Chem.* 41:61.

HUTTE, R., R. GODEC & K. O'NEILL. 1994. Transferring NASA-sponsored advances in total organic carbon measurement to industrial applications. *Microcontamination* 12(4):21.

KAPLAN, L.A. 1994. A field and laboratory procedure to collect, process, and preserve freshwater samples for dissolved organic carbon analysis. *Limnol. Oceanogr.* 39:1470.

5310 B. High-Temperature Combustion Method

1. General Discussion

The high-temperature combustion method has been used for a wide variety of samples, but its utility is dependent on particle size reduction because it uses small-orifice syringes.

a. Principle: The sample is homogenized and diluted as necessary and a microportion is injected into a heated reaction chamber packed with an oxidative catalyst such as cobalt oxide, platinum group metals, or barium chromate. The water is vaporized and the organic carbon is oxidized to CO_2 and H_2O. The CO_2 from oxidation of organic and inorganic carbon is transported in the carrier-gas streams and is measured by means of a nondispersive infrared analyzer, or titrated coulometrically.

Because total carbon is measured, inorganic carbon must be removed by acidification and sparging or measured separately and TOC obtained by difference.

Measure inorganic carbon by injecting the sample into a reaction chamber where it is acidified. Under acidic conditions, all inorganic carbon is converted to CO_2, which is transferred to the detector and measured. Under these conditions organic carbon is not oxidized and only inorganic carbon is measured.

Alternatively, convert inorganic carbonates to CO_2 with acid and remove the CO_2 by purging before sample injection. The sample contains only the nonpurgeable organic carbon fraction of total carbon: a purgeable organic carbon determination also is necessary to measure TOC.

b. Interference: Removal of carbonate and bicarbonate by acidification and purging with purified gas results in the loss of volatile organic substances. The volatiles also can be lost during sample blending, particularly if the temperature is allowed to rise. Another important loss can occur if large carbon-containing particles fail to enter the needle used for injection. Filtration, although necessary to eliminate particulate organic matter when only DOC is to be determined, can result in loss or gain of DOC, depending on the physical properties of the carbon-containing compounds and the adsorption or desorption of carbonaceous material on the filter. Check filters for their contribution to DOC by analyzing a filtered blank. Note that any contact with organic material may contaminate a sample. Avoid contaminated glassware, plastic containers, and rubber tubing. Analyze sample treatment, system, and reagent blanks.

Combustion temperatures above 950°C are required to decompose some carbonates. Systems that use lower temperatures must destroy carbonates by acidification. Elemental carbon may not be oxidized at lower temperatures but generally it is not present in water samples nor is it formed during combustion of dilute samples. The advantage of using lower temperatures (680°C) is that fusion of dissolved salts is minimized, resulting in lower blank values. Gases evolved from combustion, such as water, halide compounds, and nitrogen oxides, may interfere with the detection system. Consult manufacturers' recommendations regarding proper selection of scrubber materials and check for any matrix interferences.

The major limitation to high-temperature techniques is the magnitude and variability of the blank. Instrument manufacturers have developed new catalysts and procedures that yield lower blanks, resulting in lower detection levels.

c. Minimum detectable concentration: 1 mg C/L or less, depending on the instrument used. This can be achieved with most high-temperature combustion analyzers although instrument performance varies. The minimum detectable concentration may be reduced by concentrating the sample, or by increasing the portion taken for analysis.

d. Sampling and storage: If possible, rinse bottles with sample before filling and carry field blanks through sampling procedure to check for any contamination that may occur. Collect and store samples in glass bottles protected from sunlight and seal with TFE-backed septa. Before use, wash bottles with acid, seal with aluminum foil, and bake at 400°C for at least 1 h. Wash uncleaned TFE septa with detergent, rinse repeatedly with organic-free water, wrap in aluminum foil, and bake at 100°C for 1 h. Check performance of new or cleaned septa by running appropriate blanks. Preferably use thick silicone rubber-backed TFE septa with open ring caps to produce a positive seal. Less rigorous cleaning may be acceptable if the concentration range is relatively high. Check bottle blanks with each set of sample bottles to determine effectiveness or necessity of cleaning. Preserve samples that cannot be examined immediately by holding at 4°C with minimal exposure to light and atmosphere. Acidification with phosphoric or sulfuric acid to a pH ≤ 2 at the time of collection is especially desirable for unstable samples, and may be used on all samples: acid preservation, however, invalidates any inorganic carbon determination on the samples.

2. Apparatus

a. Total organic carbon analyzer, using combustion techniques.

b. Sampling, injection, and sample preparation accessories, as prescribed by instrument manufacturer.

c. Sample blender or homogenizer.

d. Magnetic stirrer and TFE-coated stirring bars.

e. Filtering apparatus and 0.45-μm-pore-diam filters. Preferably use HPLC syringe filters with no detectable TOC blank. Glass fiber or silver membrane filters also can be used. Rinse filters before use and monitor filter blanks.

3. Reagents

a. Reagent water: Prepare reagents, blanks, and standard solutions from reagent water with a TOC value less than 2 × the MDL (see Sections 1030 and 1080).

b. Acid: Phosphoric acid, H_3PO_4. Alternatively use sulfuric acid, H_2SO_4.

c. Organic carbon stock solution: Dissolve 2.1254 g anhydrous primary-standard-grade potassium biphthalate, $C_8H_5KO_4$, in carbon-free water and dilute to 1000 mL; 1.00 mL = 1.00 mg carbon. Prepare laboratory control standards using any other appropriate organic-carbon-containing compound of adequate purity, stability, and water solubility. Preserve by acidifying with H_3PO_4 or H_2SO_4 to pH ≤ 2, and store at 4°C.

d. Inorganic carbon stock solution: Dissolve 4.4122 g anhydrous sodium carbonate, Na_2CO_3, in water, add 3.497 g anhydrous sodium bicarbonate, $NaHCO_3$, and dilute to 1000 mL; 1.00 mL = 1.00 mg carbon. Alternatively, use any other inorganic

carbonate compound of adequate purity, stability, and water solubility. Keep tightly stoppered. Do not acidify.

e. Carrier gas: Purified oxygen or air, CO_2-free and containing less than 1 ppm hydrocarbon (as methane).

f. Purging gas: Any gas free of CO_2 and hydrocarbons.

4. Procedure

a. Instrument operation: Follow manufacturer's instructions for analyzer assembly, testing, calibration, and operation. Adjust to optimum combustion temperature before using instrument; monitor temperature to insure stability.

b. Sample treatment: If a sample contains gross solids or insoluble matter, homogenize until satisfactory replication is obtained. Analyze a homogenizing blank consisting of reagent water carried through the homogenizing treatment.

If inorganic carbon must be removed before analysis, transfer a representative portion (10 to 15 mL) to a 30-mL beaker, add acid to reduce pH to 2 or less, and purge with gas for 10 min. Inorganic carbon also may be removed by stirring the acidified sample in a beaker while directing a stream of purified gas into the beaker. Because volatile organic carbon will be lost during purging of the acidified solution, report organic carbon as total nonpurgeable organic carbon. Check efficiency of inorganic carbon removal for each sample matrix by splitting a sample into two portions and adding to one portion an inorganic carbon level similar to that of the sample. The TOC values should agree; if they do not, adjust sample container, sample volume, pH, purge gas flow rate, and purge time to obtain complete removal of inorganic carbon.

If the available instrument provides for a separate determination of inorganic carbon (carbonate, bicarbonate, free CO_2) and total carbon, omit decarbonation and determine TOC by difference between TC and inorganic carbon.

If dissolved organic carbon is to be determined, filter sample through 0.45-μm-pore-diam filter; analyze a filtering blank.

c. Sample injection: Withdraw a portion of prepared sample using a syringe fitted with a blunt-tipped needle. Select sample volume according to manufacturer's direction. Stir samples containing particulates with a magnetic stirrer. Select needle size consistent with sample particulate size. Other sample injection techniques, such as sample loops, may be used. Inject samples and standards into analyzer according to manufacturer's directions and record response. Repeat injection until consecutive measurements are obtained that are reproducible to within ± 10%.

d. Preparation of standard curve: Prepare standard organic and inorganic carbon series by diluting stock solutions to cover the expected range in samples within the linear range of the instrument. Dilute samples higher than the linear range of the instrument in reagent water. Inject and record peak height or area of these standards and a dilution water blank. Plot carbon concentration in milligrams per liter against corrected peak height or area on rectangular coordinate paper. This is unnecessary for instruments provided with a digital readout of concentration.

With most TOC analyzers, it is not possible to determine separate blanks for reagent water, reagents, and the entire system. In addition, some TOC analyzers produce a variable and erratic blank that cannot be corrected reliably. In many laboratories, reagent water is the major contributor to the blank value. Correcting only the instrument response of standards (which contain reagent water + reagents + system blank) creates a positive error, while also correcting samples (which contain only reagents and system blank contributions) for the reagent water blank creates a negative error. Minimize errors by using reagent water and reagents low in carbon.

Inject samples and procedural blanks (consisting of reagent water taken through any pre-analysis steps—values are typically higher than those for reagent water) and determine sample organic carbon concentrations directly from the readout or measurements by comparing corrected instrument response to the calibration curve. Instruments with coulometric detectors do not require calibration curves. Regularly analyze laboratory control samples to confirm performance of the instrument (see Quality Control, below). These detectors accumulate the system blank; therefore, monitor system blank regularly.

5. Calculations

Calculate corrected instrument response of standards and samples by subtracting the reagent-water blank instrument response from that of the standard and sample. Prepare a standard curve of corrected instrument response vs. TOC concentration. Subtract procedural blank from each sample instrument response and compare to standard curve to determine carbon content. Apply appropriate dilution factor when necessary. Subtract inorganic carbon from total carbon when TOC is determined by difference.

NOTE: The reagent water blank may include an instrument contribution not dependent on reagent-water carbon, and a true response due to reagent-water carbon. When reagent-water carbon is a significant fraction of reagent-water blank, a negative error no larger than reagent-water blank is introduced in the sample values. If TOC analyzer design permits isolation of each of the contributions to the total blank, apply appropriate blank corrections to instrument response of standards (reagent blank, water blank, system blank) and sample (reagent blank and system blank).

6. Quality Control

Determine instrument detection limit according to Section 1030.

After every tenth analysis, analyze a blank and a laboratory control sample prepared from a source of material other than the calibration standards, at a level similar to the analytical samples. Preferably prepare the laboratory control sample in a matrix similar to that of the samples. Alternatively, periodically make known additions to samples to ensure recovery from unknown matrices.

7. Precision

The difficulty of sampling particulate matter on unfiltered samples limits the precision of the method to approximately 5 to 10%.

Interlaboratory studies of high-temperature combustion methods have been conducted in the range above 2 mg/L.[1] The resulting equation for single-operator precision on matrix water is:

$$S_o = 0.027x + 0.29$$

Overall precision is:

$$S_t = 0.044x + 1.49$$

where:

S_o = single-operator precision,
S_t = overall precision, and
x = TOC concentration, mg/L.

8. Reference

1. AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1994. Standard Test Method for Total and Organic Carbon in Water by High Temperature Oxidation and by Coulometric Detection. D4129–88. Annual Book of ASTM Standards. American Soc. Testing & Materials, Philadelphia, Pa.

9. Bibliography

KATZ, J., S. ABRAHAM & N. BAKER. 1954. Analytical procedure using a combined combustion-diffusion vessel: Improved method for combustion of organic compounds in aqueous solution. *Anal. Chem.* 26:1503.

VAN HALL, C.E., J. SAFRANKO & V. A. STENGER. 1963. Rapid combustion method for the determination of organic substances in aqueous solutions. *Anal. Chem.* 35:315.

SCHAFFER, R.B. et al. 1965. Application of a carbon analyzer in waste treatment. *J. Water Pollut. Control Fed.* 37:1545.

VAN HALL, C.E., D. BARTH & V. A. STENGER. 1965. Elimination of carbonates from aqueous solutions prior to organic carbon determinations. *Anal. Chem.* 37:769.

BUSCH, A.W. 1966. Energy, total carbon and oxygen demand. *Water Resour. Res.* 2:59.

BLACKMORE, R.H. & D. VOSHEL. 1967. Rapid determination of total organic carbon (TOC) in sewage. *Water Sewage Works* 114:398.

WILLIAMS, R.T. 1967. Water-pollution instrumentation—Analyzer looks for organic carbon. *Instrum. Technol.* 14:63.

AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1994. Standard Test Method for Total Organic Carbon in Water. D2579-93. Annual Book of ASTM Standards. American Soc. Testing & Materials, Philadelphia, Pa.

5310 C. Persulfate-Ultraviolet or Heated-Persulfate Oxidation Method

1. General Discussion

Many instruments utilizing persulfate oxidation of organic carbon are available. They depend either on heat or ultraviolet irradiation activation of the reagents. These oxidation methods provide rapid and precise measurement of trace levels of organic carbon in water.

a. Principle: Organic carbon is oxidized to carbon dioxide, CO_2, by persulfate in the presence of heat or ultraviolet light. The CO_2 produced may be purged from the sample, dried, and transferred with a carrier gas to a nondispersive infrared (NDIR) analyzer, or be coulometrically titrated, or be separated from the liquid stream by a membrane that allows the specific passage of CO_2 to high-purity water where a change in conductivity is measured and related to the CO_2 passing the membrane.

Some instruments utilize an ultraviolet lamp submerged in a continuously gas-purged reactor that is filled with a constant-feed persulfate solution. The samples are introduced serially into the reactor by an autosampler or they are injected manually. The CO_2 produced is sparged continuously from the solution and is carried in the gas stream to an infrared analyzer that is specifically tuned to the absorptive wavelength of CO_2. The instrument's microprocessor calculates the area of the peaks produced by the analyzer, compares them to the peak area of the calibration standard stored in its memory, and prints out a calculated organic carbon value in milligrams per liter.

Other UV-persulfate instruments use continuous-flow injection of the sample into the instrument. Removal of inorganic carbon by vacuum degassing is provided optionally. The sample is acidified and persulfate added. Sample flow is split; one channel passes to a delay coil while the other passes through the UV reactor. The CO_2 from each stream is separated from the sample stream by membranes selectively permeable to CO_2 that allow the CO_2 to pass into high-purity water where change in conductivity is measured. CO_2 from the non-UV-irradiated stream represents inorganic carbon. CO_2 from the irradiated stream represents TC. The instrument automatically converts the detector signals to unit of concentration (mg/L or μg/L). The TOC is calculated as the difference between the TC and inorganic carbon channels.

Heated-persulfate instruments utilize a digestion vessel heated to 95 to 100°C. Samples are added by direct injection, loop injection, line injection, or autosampler. After inorganic carbon is removed by acidification and sparging, a measured amount of persulfate solution is added to the sample. After an oxidation period, the resulting CO_2 is sparged from the solution and carried to an infrared analyzer specifically tuned to the absorptive wavelength of CO_2. The instrument's microprocessor converts the detector signal to organic carbon concentrations in mg/L based on stored calibration data.

b. Interferences: See Section 5310B.1. Insufficient acidification will result in incomplete release of CO_2.

The intensity of the ultraviolet light reaching the sample matrix may be reduced by highly turbid samples or with aging of the ultraviolet source, resulting in sluggish or incomplete oxidation. Large organic particles or very large or complex organic molecules such as tannins, lignins, and humic acid may be oxidized slowly because persulfate oxidation is rate-limited. However, oxidation of many large biological molecules such as proteins and monoclonal antibodies proceeds rapidly. Because the efficiency of conversion of organic carbon to CO_2 may be affected by many factors, check efficiency of oxidation with selected model compounds representative of the compounds of interest in a matrix representative of the sample.

Some instruments give low results for certain difficult-to-oxidize compounds under certain conditions. The following compounds are difficult to oxidize, are sufficiently soluble in water, and can be mixed and measured accurately at trace levels: urea, nicotinic acid, pyridine, *n*-butanol, acetic acid, leucine, acetonitrile, octoxynol-9, tartaric acid, 1,10-phenanthroline, l-glutonic acid, 2-propanol, and sodium dodecylbenzenesulfonate. Use these compounds as matrix additions to evaluate oxidation efficiency.

Persulfate oxidation of organic molecules is slowed in samples containing significant concentrations of chloride by the preferential oxidation of chloride; at concentrations above 0.05% chloride, ox-

idation of organic matter may be inhibited. To remove this interference add mercuric nitrate* to the persulfate solution in UV-persulfate instruments, or extend reaction time and/or increase amount of persulfate solution in heated-persulfate instruments.

With any organic carbon measurement, contamination during sample handling and treatment is a likely source of interference. This is especially true of trace analysis. Take extreme care in sampling, handling, and analysis of samples below 1 mg TOC/L.

c. Minimum detectable concentration: Concentration of 0.01 mg TOC/L can be measured by some instruments if scrupulous attention is given to minimizing sample contamination and method background. See Section 1030 for procedures to evaluate the MDL for a specific instrument. Use the high-temperature combustion method (B) for high concentrations of TOC or dilute the sample, ensuring that the dilution process does not contaminate the sample.

d. Sampling and storage: See Section 5310B.1*d*.

2. Apparatus

a. Total organic carbon analyzer utilizing persulfate oxidation principle.

b. Sampling and injection accessories, as specified by the instrument manufacturer.

3. Reagents

a. Reagents listed in Section 5310B.3.

b. Persulfate solution: Different instrument manufacturers recommend different forms and concentrations of peroxydisulfate. Typical preparations are as follows:

1) *Sodium peroxydisulfate,* 10%: Dissolve 100 g reagent in water; bring volume to 1 L.

2) *Ammonium peroxydisulfate,* 15%: Dissolve 150 g reagent in water; bring volume to 1 L.

3) *Potassium peroxydisulfate,* 2%: Dissolve 20 g reagent in water; bring volume to 1 L.

Check blank values from reagents and, if values are high, purify reagent or use a higher-purity source.

4. Procedure

a. Instrument operation: Follow manufacturer's instructions for assembly, testing, calibration, and operation.

b. Sample preparation: If a sample contains gross particulates or insoluble matter, homogenize until a representative portion can be withdrawn through the syringe needle, autosampler tubing, or sample inlet system of continuous on-line monitor.

If dissolved organic carbon is to be determined, filter sample and a reagent water blank through 0.45-μm filter. HPLC syringe filters have been found to pass water without contamination. Glass fiber or silver membrane filters also can be used. Check filter blanks regularly.

To determine nonpurgeable organic carbon, transfer 15 to 30 mL sample to a flask or test tube and acidify to a pH of 2. Purge according to manufacturer's recommendations. In some instruments this is performed internally. Check efficiency of inorganic carbon removal for each sample matrix by splitting a sample into two portions; to one of the portions, add inorganic carbon to a level similar to that of the sample. The TOC values should agree. If the values do not agree, adjust conditions such as sample container, sample volume, pH, purge-gas flow rate, and purge time to obtain complete removal of inorganic carbon.

c. Sample injection: See Section 5310B.4*c*.

d. Standard curve preparation: Prepare an organic carbon standard series over the range of organic carbon concentrations in the samples. Run standards and blanks and record analyzer's response. Determine instrument response for each standard and blank. Unless carbon dioxide is trapped and desorbed, producing consistent peak heights, determinations based on peak height may be inadequate because of differences in the rate of oxidation of standards and samples. Correct instrument response of standards by subtracting reagent water blank and plot organic carbon concentration in milligrams per liter against corrected instrument response. For instruments providing a digital computation of concentration, this is not necessary. Be sure that the instrument's algorithm includes blank correction and linearity of response. Analyze standards having concentrations above and below those determined in the samples, preferably prepared in a similar matrix, to confirm proper instrument operation.

5. Calculation

See Section 5310B.5, or use instrument manufacturer's procedure.

6. Quality Control

See Section 5310B.6.

7. Precision and Bias

Interlaboratory studies of persulfate and/or UV with NDIR detection methods have been conducted in the range of 0.1 mg/L to 4000 mg/L of carbon.[1] The resulting equation for organic carbon, single-operator precision is:

$$S_o = 0.04x + 0.1$$

Overall precision is expressed as:

$$S_t = 0.08x + 0.1$$

where:

S_o = single-operator precision,
S_t = overall precision, and
x = TOC concentration, mg/L.

An interlaboratory study was conducted for the membrane conductivity method,† covering samples with 1 to 25 mg/L organic carbon concentrations. The resulting equation for single-operator precision is:

$$S_o = 0.012x - 0.022$$

*NOTE: If mercuric nitrate is used to complex the chloride, use an appropriate disposal method for the treated waste to prevent mercury contamination.

†Data may be obtained from *Standard Methods* manager, American Water Works Association.

Overall precision is expressed as:

$$S_t = 0.027x + 0.09$$

where terms are defined as above.

8. Reference

1. AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1994. Standard Test Method for Total Carbon in Water by Ultraviolet, or Persulfate Oxidation, or Both, and Infrared Detection. D4839-88. Annual Book of ASTM Standards. American Soc. Testing & Materials, Philadelphia, Pa.

9. Bibliography

BEATTIE, J., C. BRICKER & D. GARVIN. 1961. Photolytic determination of trace amounts of organic material in water. *Anal. Chem.* 33:1890.

ARMSTRONG, F.A.J., P.M. WILLIAMS & J.D.H. STRICKLAND. 1966. Photooxidation of organic matter in sea water by ultraviolet radiation, analytical and other applications. *Nature* 211:481.

BRAMER, H.C., M.J. WALSH & S.C. CARUSO. 1966. Instrument for monitoring trace organic compounds in water. *Water Sewage Works* 113:275.

DOBBS, R.A., R.H. WISE & R.B. DEAN. 1967. Measurement of organic carbon in water using the hydrogen flame ionization detector. *Anal. Chem.* 39P:1255.

MONTGOMERY, H.A.C. & N.S. THOM. 1967. The determination of low concentrations of organic carbon in water. *Analyst* 87:689.

ARMSTRONG, F.A.J. & S. TIBBITS. 1968. Photochemical combustion of organic matter in seawater for nitrogen, phosphorus and carbon determination. *J. Mar. Biol. Assoc. U.K.* 48:143.

JONES, R.H. & A.F. DAGEFORD. 1968. Application of a high sensitivity total organic carbon analyzer. *Proc. Instr. Soc. Amer.* 6:43.

TAKAHASHI, Y., R.T. MOORE & R.J. JOYCE. 1972. Direct determination of organic carbon in water by reductive pyrolysis. *Amer. Lab.* 4:31.

COLLINS, K.J. & P.J. LEB. WILLIAMS. 1977. An automated photochemical method for the determination of dissolved organic carbon in sea and estuarine waters. *Mar. Chem.* 5:123.

GRAVELET-BLONDIN, L.R., H.R. VAN VLIET & P.A. MYNHARDT. 1980. An automated method for the determination of dissolved organic carbon in fresh water. *Water SA* 6:138.

OAKE, R.J. 1981. A Review of Photo-Oxidation for the Determination of Total Organic Carbon in Water. Water Research Center, Technical Rep. (TR 160). Medmenham, England.

VAN STEENDEREN, R.A. & J.S. LIN. 1981. Determination of dissolved organic carbon in water. *Anal. Chem.* 53:2157.

AIKEN, G.R. 1992. Chloride interference in the analysis of dissolved organic carbon by the wet oxidation method. *Environ. Sci. Technol.* 26:2435.

GODEC, R., K. O'NEIL & R. HUTTE. 1992. New Technology for TOC Analysis in Water. *Ultrapure Water* 9(9):17.

5310 D. Wet-Oxidation Method

1. General Discussion

The wet-oxidation method is suitable for the analyses of water, water-suspended sediment mixtures, seawaters, brines, and wastewaters containing at least 0.1 mg nonpurgeable organic carbon/L. The method is not suitable for the determination of volatile organic constituents.

a. Principle: The sample is acidified, purged to remove inorganic carbon, and oxidized with persulfate in an autoclave at temperatures from 116 to 130°C. The resultant carbon dioxide (CO_2) is measured by nondispersive infrared spectrometry.

b. Interferences: See Section 5310B.1 and C.1.

c. Minimum detectable concentrations: High concentrations of reducing agents may interfere. Concentration of 0.10 mg TOC/L can be measured if scrupulous attention is given to minimizing sample contamination and method background. Use the high-temperature combustion method (B) for high concentrations of TOC.

d. Sampling and storage: See Section 5310B.1*d*.

2. Apparatus

a. Ampules, precombusted, 10-mL, glass.
b. Ampule purging and sealing unit.
c. Autoclave.
d. Carbon analyzer.
e. Homogenizer.

3. Reagents

In addition to the reagents specified in Section 5310B.3*a*, *c*, *e*, and *f*, the following reagents are required:

a. Phosphoric acid solution, H_3PO_4, 1.2*N:* Add 83 mL H_3PO_4 (85%) to water and dilute to 1 L with water. Store in a tightly stoppered glass bottle.

b. Potassium persulfate, reagent-grade, granular. Avoid using finely divided forms.

4. Procedure

Follow manufacturer's instructions for instrument assembly, testing calibration, and operation. Add 0.5 mL 1.2*N* H_3PO_4, solution to precombusted ampules.

To analyze for dissolved organic carbon, follow the filtration procedure in Method B. Homogenize sample to produce a uniform suspension. Rinse homogenizer with reagent water after each use. Pipet water sample (10.0 mL maximum) into an ampule. Adjust smaller volumes to 10 mL with reagent water. Prepare one reagent blank (10 mL reagent water plus acid and oxidant) for every 15 to 20 water samples. Prepare standards covering the range of 0.1 to 40 mg C/L by diluting the carbon standard solution. Immediately place filled ampules on purging and seating unit and purge them at rate of 60 mL/min for 6 min with purified oxygen. Add 0.2 g potassium persulfate using a dipper calibrated to deliver 0.2 g to the

ampule. Seal samples according to the manufacturer's instructions. Place sealed samples, blanks, and a set of standards in ampule racks in an autoclave and digest 4 h at temperature between 116 and 130°C.

Set sensitivity range of carbon analyzer by adjusting the zero and span controls in accordance with the manufacturer's instructions. Break combusted ampules in the cutter assembly of the carbon analyzer, sweep CO_2 into the infrared cell with nitrogen gas, and record area of each CO_2 peak. CAUTION: *Because combusted ampules are under positive pressure, handle with care to prevent explosion.*

5. Calculations

Prepare an analytical standard curve by plotting peak area of each standard versus concentration (mg/L) of organic carbon standards. The relationship between peak area and carbon concentration is curvilinear. Define operating curves each day samples are analyzed.

Report nonpurgeable organic carbon concentration as follows: 0.1 mg/L to 0.9 mg/L, one significant figure; 1.0 mg/L and above, two significant figures.

6. Quality Control

See Section 5310B.6.

7. Precision and Bias

Multiple determinations of four different concentrations of aqueous potassium acid phthalate samples at 2.00, 5.00, 10.0, and 40.0 mg C/L resulted in mean values of 2.2, 5.3, 9.9, and 38 mg/L and standard deviations of 0.13, 0.15, 0.11, and 1.4, respectively.

Precision also may be expressed in terms of percent relative standard deviation as follows:

Number of Replicates	Mean *mg/L*	Relative Standard Deviation %
9	2.2	5.9
10	5.3	2.8
10	9.9	1.1
10	38.0	3.7

8. Bibliography

WILLIAMS, P.M. 1969. The determination of dissolved organic carbon in seawater: A comparison of two methods. *Limnol. Oceanogr.* 14:297.

OCEANOGRAPHY INTERNATIONAL CORP. 1970. The total carbon system operating manual. College Station, Tex.

MACKINNON, M.D. 1981. The measurement of organic carbon in seawater. *In* E.K. Duursma & R. Dawson, eds., Marine Organic Chemistry: Evolution, Composition, Interactions and Chemistry of Organic Matter in Seawater. Elsevier Scientific Publishing Co., New York, N.Y.

5320 DISSOLVED ORGANIC HALOGEN*

5320 A. Introduction

Dissolved organic halogen (DOX) is a measurement used to estimate the total quantity of dissolved halogenated organic material in a water sample. This is similar to literature references to "total organic halogen" (TOX), "adsorbable organic halogen" (AOX), and carbon-adsorbable organic halogen (CAOX). The presence of halogenated organic molecules is indicative of disinfection by-products and other synthetic chemical contamination. Halogenated compounds that contribute to a DOX result include, but are not limited to: the trihalomethanes (THMs); organic solvents such as trichloroethene, tetrachloroethene, and other halogenated alkanes and alkenes; chlorinated and brominated pesticides and herbicides; polychlorinated biphenyls (PCBs); chlorinated aromatics such as hexachlorobenzene and 2,4-dichlorophenol; and high-molecular-weight, partially chlorinated aquatic humic substances. Compound-specific methods such as gas chromatography typically are more sensitive than DOX measurements.

The adsorption-pyrolysis-titrimetric method for DOX measures only the total molar amount of dissolved organically bound halogen retained on the activated carbon adsorbent; it yields no information about the structure or nature of the organic compounds to which the halogens are bound or about the individual halogens present. It is sensitive to organic chloride, bromide, and iodide, but does not detect fluorinated organics.

DOX measurement is an inexpensive and useful method for screening large numbers of samples before specific (and often more complex) analyses; for extensive field surveying for pollution by certain classes of synthetic organic compounds in natural waters; for mapping the extent of organohalide contamination in groundwater; for monitoring the breakthrough of some synthetic organic compounds in water treatment processes; and for estimating the level of formation of chlorinated organic by-products after disinfection. When used as a screening tool, a large positive (i.e., above background measurements) DOX test result indicates the need for identifying and quantifying specific substances. In saline or brackish waters the high inorganic halogen concentrations interfere. The possibility of overestimating DOX concentration because of inorganic halide interference always should be considered when interpreting results.

* Approved by Standard Methods Committee, 1997.
Joint Task Group: 20th Edition — James M. Symons (chair), Jarmila Banovic, Karl O. Brinkmann.

5320 B. Adsorption-Pyrolysis-Titrimetric Method

1. General Discussion

a. Principle: The method consists of four processes. First, dissolved organic material is separated from inorganic halides and concentrated from aqueous solution by adsorption onto activated carbon. Second, inorganic halides present on the activated carbon are removed by competitive displacement by nitrate ions. Third, the activated carbon with adsorbed organic material is introduced into a furnace that pyrolyzes organic carbon to carbon dioxide (CO_2) and the bound halogens to hydrogen halide (HX). Fourth, the HX is transported in a carrier gas stream to a microcoulometric titration cell where the amount of halide is quantified by measuring the current produced by silver-ion precipitation of the halides. The microcoulometric detector operates by maintaining a constant silver-ion concentration in a titration cell. An electric potential is applied to a solid silver electrode to produce silver ions in the cell solution. As hydrogen halide from the pyrolysis furnace enters the cell in the carrier gas, it is partitioned into the acetic acid solution where it precipitates as silver halide. The current that is produced is integrated over the period of the pyrolysis. The integrated area under the curve is proportional to the number of moles of halogen recovered. The mass concentration of organic halides is reported as an equivalent concentration of organically bound chloride in micrograms per liter. Because this DOX procedure relies on activated carbon to adsorb organic halides, it also has been referred to as carbon-adsorbable organic halogen (CAOX). Because of the poor adsorption efficiency of some organic compounds containing halogen and the desorption of some halogen-containing compounds during the removal of adsorbed inorganic halogen, this method does not measure total organic halogen.

When a sample is purged with inert gas before activated carbon adsorption, analysis of that sample determines the nonpurgeable dissolved organic halogen (NPDOX) fraction of DOX. The purgeable organic halogen concentration (POX) may be estimated by subtracting the NPDOX value from the DOX value. Alternatively, the POX fraction may be determined directly by purging the sample with carrier gas and introducing that gas stream and the volatilized organics directly into the pyrolysis furnace. Thus, depending on approach, the analysis of POX, DOX, and NPDOX may be determined directly or by difference. Finally, because the POX often is dominated by the THMs, they may be determined as directed in Section 6200 and used to estimate POX. However, this approach is not included here as a standardized procedure.

b. Interferences: The method is applicable only to aqueous samples free of visible particulate matter. Different instruments vary in tolerance of small amounts of suspended matter. Inorganic substances such as chloride, chlorite, chlorate, bromate, bromide, and iodide will adsorb on activated carbon to an extent dependent on their original concentration in the aqueous solution and the volume of sample adsorbed.[1] Positive interference will result if inorganic halides are not removed. Treating the activated carbon with a concentrated aqueous solution of nitrate ion causes competitive desorption from the activated carbon of inorganic halide species and washes inorganic halides from other surfaces. However, if the inorganic halide concentration is greater than 10 000 times[2] the concentration of organic halides, the DOX results may be affected significantly. In general, this procedure may not be applicable to samples with inorganic halide concentrations above 500 mg Cl^-/L, based on activated carbon quality testing results. Therefore, consider both the results of mineral analysis for inorganic halides and the results of the activated carbon quality test (see ¶ 5, below) when interpreting results.

Halogenated organic compounds that are weakly adsorbed on activated carbon are recovered only partially. These include certain alcohols and acids (e.g., chloroethanol), and such compounds as chloroacetic acid, that can be removed from activated carbon by the nitrate ion wash. However, for most halogenated organic molecules, recovery is very good; the activated carbon adsorbable organic halide (CAOX) therefore is a good estimate of true DOX.

Failure to acidify samples with nitric acid or sulfuric acid may result in reduced adsorption efficiency for some halogenated organic compounds and may intensify the inorganic halide interference. However, acidification may result in precipitation loss of humic acids and any DOX associated with that fraction. Further, if the water contains residual chlorine, reduce it before adsorption to eliminate positive interference resulting from continued chlorination reactions with organic compounds adsorbed on the activated carbon surface or with the activated carbon surface itself. The sulfite dechlorinating agent may cause decomposition of a small fraction of the DOX if nitric acid is used; this decomposition is avoided if sulfuric acid is used. Do not add acid in great excess.

Highly volatile components of the POX fraction may be lost during sampling, shipment, sample storage, sample handling, and sample preparation, or during sample adsorption. A laboratory quality-control program to ensure sample integrity from time of sampling until analysis is vital. During sample filtration for the analysis of samples containing undissolved solids, major losses of POX can be expected. Syringe-type filtration systems can minimize losses. Analyze for POX before sample filtration and analyze for NPDOX after filtration; the sum of POX and NPDOX is the total DOX. In preparing samples for DOX analysis, process a blank and a standard solution to determine effect of this procedure on DOX measurement. If an insignificant loss of POX occurs during the removal of particulate matter by filtration, DOX may be measured directly.

Granular activated carbon used to concentrate organic material from the sample can be a major source of variability in the analysis and has a dramatic effect on the minimum detectable concentration. Ideally, activated carbon should have a low halide content, readily release adsorbed inorganic halides on nitrate washing, be homogeneous, and readily adsorb *all* organic halide compounds even in the presence of large excesses of other organic material. An essential element of quality control for DOX requires testing and monitoring of activated carbon (see ¶ 5 below). Nonhomogeneous activated carbon or activated carbon with a high background value affects the method reliability at low concentrations of DOX. A high and/or variable blank value raises the minimum detectable concentration. Random positive bias, in part because of the ease of activated carbon contamination during use, may necessitate analyzing duplicates of each sample. Because activated carbon from different sources may vary widely in the ease of releasing inorganic halides, test for this quality before using activated carbon. Proper quantification also may be affected by the adsorptive capacity of the

activated carbon. If excessive organic loading occurs, some DOX may break through and not be recovered. For this reason, make serial adsorptions of each sample portion and individual analyses.

c. Sampling and storage: Collect and store samples in amber glass bottles with TFE-lined caps. If amber bottles are not available, store samples in the dark. To prepare sample bottles, acid wash, rinse with deionized water, seal with aluminum foil, and bake at 400°C for at least 1 h. If bottle blanks without baking show no detectable DOX, baking may be omitted. Wash septa with detergent, rinse repeatedly in organic-free, deionized water, wrap in aluminum foil, and bake for 1 h at 100°C. Preferably use thick silicone rubber-backed TFE septa and open ring caps to produce a positive seal that prevents loss of POX and contamination. Store sealed sample bottles in a clean environment until use. Completely fill sample bottles but take care not to volatilize any organic halogen compounds. Preserve samples that cannot be analyzed promptly by acidifying with concentrated nitric acid or sulfuric acid to pH 2. Refrigerate samples at 4°C with minimal exposure to light. Reduce any residual chlorine by adding sodium sulfite crystals (minimum: 5 mg/L). Add 4 drops conc H_2SO_4 plus sodium sulfite crystals to bottles shipped to the field. NOTE: Some organic chloramines are not completely dechlorinated by sodium sulfite, particularly at pH > 7. This may affect reported concentrations.[1] Analyze all samples within 14 d.

d. Minimum detectable concentration: For nonsaline waters free of particulate matter, 5 to 10 μg organic Cl^-/L is considered a typical range for detection levels. The minimum detectable concentration may be influenced by the analytical repeatability, equipment used, activated carbon quality, and the analyst. Determine the detection level for each procedure, instrument, and analyst.

2. Apparatus

a. Adsorption assembly, including gas-tight sample reservoir, activated carbon-packed adsorption columns, column housings, and nitrate solution reservoir. In particular, note the following:

1) *Noncombustible insulating material (microcolumn method only):* Form into plugs to hold activated carbon in columns. NOTE: *Do not touch with fingers.*

2) *Activated carbon columns (microcolumn method only):* Pack 40 ± 5 mg activated carbon (¶ 3*m*) into dry glass tubing approximately 2 to 3 mm ID × 6 mm OD × 40 to 50 mm long. NOTE: *Protect these columns from all sources of halogenated organic vapors.* Clean glass tubes before use with a small-diameter pipe cleaner to remove residual carbon, then soak in chromate cleaning solution for 15 min and dry at 400°C. Rinse between steps with deionized water. NOTE: Use prepacked columns with caution, because of occasional reported contamination.

b. Analyzer assembly, including carrier gas source, boat sampler, and pyrolysis furnace, that can oxidatively pyrolyze halogenated organics at a temperature of 800 to 900°C to produce hydrogen halides and deliver them to the titration cell with a minimum overall efficiency of 90% for 2,4,6-trichlorophenol; including a microcoulometric titration system with integrator, digital display, and data system or chart recorder connection; including (optional) purging apparatus.

c. Chart recorder or microprocessor, controlled data system.

d. Batch adsorption equipment: Use instrument manufacturer's purge vessel or similar purging flask, erlenmeyer flasks (100 to 250 mL), and high-speed stirrers.

e. Filtering apparatus and filters: Use 0.45-μm-pore-diam filters, preferably HPLC syringe filters or similar, with no detectable DOX blank. Rinsed glass-fiber filters are satisfactory for sample filtration. Preferably use membrane filters for separating activated carbon from aqueous phase.

3. Reagents and Materials

Use chemicals of ACS reagent grade or other grades if it can be demonstrated that the reagent is of sufficiently high purity to permit its use without lessening accuracy of the determination.

a. Carbon dioxide, argon, or nitrogen, as recommended by the equipment manufacturer, purity 99.99%.

b. Oxygen, purity 99.99%.

c. Aqueous acetic acid, 70 to 85%, as recommended by the equipment manufacturer.

d. Sodium chloride standard, NaCl: Dissolve 0.1648 g NaCl and dilute to 100 mL with reagent water; 1 μL = 1 μg Cl^-.

e. Ammonium chloride standard, NH_4Cl: Dissolve 0.1509 g NH_4Cl and dilute to 100 mL with reagent water; 1 μL = 1 μg Cl^-.

f. Trichlorophenol stock solution: Dissolve 1.856 g trichlorophenol and dilute to 100 mL with methanol; 1 μL = 10 μg Cl^-.

g. Trichlorophenol standard solution: Make a 1:20 dilution of the trichlorophenol stock solution with methanol; 1 μL = 0.5 μg Cl^-.

h. Trichloroacetic acid stock solution: Dilute 199.44 mg trichloroacetic acid in 1000 mL reagent water; 1 mL = 130 μg Cl^-.

i. Trichloroacetic acid standard solution: Dilute 2.0 mL trichloroacetic acid stock solution into 1000 mL with reagent water; 1 mL = 0.260 μg Cl^-.

j. Chloroform standard solution, $CHCl_3$: Dilute 100 mg $CHCl_3$ to 100 mL with methanol; 1 μL = 1 μg $CHCl_3$.

k. Blank standard: Use reagent water. Reagent water preferably is carbon-filtered, deionized water that has been heated and purged.

l. Nitrate wash solution, 0.08*M*: Dilute 8.2 g KNO_3 to 1000 mL with reagent water. Adjust to pH 2 with HNO_3. 1 L = 5000 mg NO_3^-.

m. Activated carbon, 100 to 200 mesh: Ideally use activated carbon having a very low apparent halide background that readily releases adsorbed inorganic halides on nitrate washing, and reliably adsorbs organic halides in the presence of a large excess of other organic compounds.* See ¶ 5 below for preparation and evaluation of activated carbon. CAUTION: *Protect activated carbon from contact with halogenated organic vapors.*

n. Sodium sulfite, Na_2SO_3, crystals.

o. Nitric acid, HNO_3, conc, or *sulfuric acid,* H_2SO_4, conc.

4. Procedure

Use either the microcolumn (4*a*) or batch adsorption (4*b*) method to determine DOX (as CAOX). If present, determine POX separately (¶4*c*). The microcolumn method utilizes small glass columns packed with activated carbon through which the

* Westvaco or Calgon Filtrasorb 400 or equivalent.

sample is passed under positive pressure to adsorb the organic halogen compounds. The batch adsorption method uses a small quantity of activated carbon that is added to the sample. After stirring, activated carbon is removed by filtration, washed with nitrate, and analyzed. The batch adsorption procedure typically is run on samples that have had POX analyzed directly (¶4*c*), yielding NPDOX directly as well.

a. Microcolumn procedure:

1) Apparatus setup—Adjust equipment in accordance with the manufacturer's instructions. Make several injections of NaCl solution directly into the titration cell [¶ 5*c*1)] as a microcoulometer/titration cell check at the start of each day.

2) Sample pretreatment for DOX analysis—If the sample has not been acidified during collection, adjust pH to 2 with HNO_3 or H_2SO_4. If the samples contain undissolved solids, filter through a glass-fiber filter (other means of removing particulate matter may be used, if it can be demonstrated that they do not cause significant interferences). Also filter a blank and standard. Analyze these to determine the contribution of filtration to the organic halogen measurement. Vacuum filtration will cause some loss of volatile organic halogen. Analyze for POX (¶4*c*) before filtration and NPDOX after filtration, unless it is shown that POX losses during filtration are insignificant for a specific water type.

3) Sample adsorption—Transfer a representative portion of sample to the cleaned sample reservoir with two activated carbon adsorption columns in series attached by the column housings to the reservoir outlet. Seal the reservoir. Adjust to produce a flow rate of about 3 mL/min. When the desired volume has been processed, stop the flow, detach the activated carbon housings and columns, and rinse the sample reservoir twice with reagent-grade water. Vary volume processed to produce optimum quantities of adsorbed DOX on the columns. Suggested volumes are as follows:

Volume Processed *mL*	Instrument Optimum Range *μg Cl⁻*	Conc of DOX in Waters *μg/L*
100	0.5–50	5–50
50	12.5–50	250–1000
25	12.5–50	500–2000

If possible, avoid using volumes greater than 100 mL because the maximum adsorptive capacity of the activated carbon may be exceeded, leading to adsorbate breakthrough and loss of DOX. Larger sample volumes processed lead to an increased quantity of inorganic halide accumulated on the activated carbon and may result in a positive interference. Do not use a sample less than 25 mL to minimize volumetric errors. For samples exceeding 2000 μg DOX/L dilute before adsorption. Protect columns from the atmosphere until DOX is determined.

4) Inorganic halide removal—Attach columns through which sample has been processed in series to the nitrate wash reservoir and pass 2 to 5 mL NO_3^- solution through the columns at a rate of approximately 1 mL/min.

5) DOX determination—After concentrating sample on activated carbon and removing inorganic halogens by nitrate washing, pyrolyze contents of each microcolumn and determine organic halogen content. Remove top glass microcolumn from the column housing, taking care not to contaminate the sample with inorganic halides. Using a clean ejector rod, eject the activated carbon and noncombustible insulating material plugs into the sample boat. Prepare sample boat during the preceding 4 h by heating at 400 to 800°C for at least 4 min in an oxygen-rich atmosphere (i.e., in the pyrolysis furnace). Remove residual ash. Place ejector rod on the plug of the effluent end of the carbon microcolumn and place the influent end of the carbon microcolumn in the quartz boat first. Seal sample inlet tube and let instrument stabilize. After NO_3^- wash avoid contact with inorganic halides. Wear latex gloves while carrying out this procedure. Preferably clean work area frequently with deionized water.

Pyrolyze the activated carbon and determine halide content. Repeat for each microcolumn. Check for excess breakthrough (¶ 5*b*) and repeat analysis as necessary.

6) Replicates—When DOX determination is used strictly as a screening tool, total replication is not necessary. Single-operator precision (% CV) is expected to be less than 15% for tap water and wastewater (Table 5320:I). If system performance is consistently worse as demonstrated by routine QA duplicates, or if quality objectives dictate, run replicates of each sample by repeating steps 3, 4, and 5.

7) Blanks—Analyze one method blank [¶ 5*e*2)] with each set of ten samples. Preferably analyze the method blank before starting the sample set and run a blank after the last set of the day.

8) Preparation and analysis of calibration standard—Run daily calibration standards in accordance with ¶ 5*c*3) for POX analysis or ¶ 5*c*5) for microcolumn-adsorption DOX analysis. Accompany by a suitable blank (¶ 5*e*). Be certain that analytical conditions and procedures (e.g., purging temperature) are the same for the analysis of calibration standards as for the analysis of samples.

b. Batch adsorption procedure:

1) Apparatus setup—Adjust equipment in accordance with the manufacturer's instructions.

2) Sample pretreatment—Adjust sample pH to 2 with conc HNO_3 or H_2SO_4 [see ¶ 4*a*2)].

3) Sample adsorption—Prepare carbon suspension by adding high-quality activated carbon to high-purity, deionized, granular activated carbon (GAC)-treated water to produce a uniform suspension of 10 mg carbon/mL. To an erlenmeyer flask, transfer prepurged sample of optimum size from a purging flask standardized in the same manner as the instrument's purging vessel. Add 20 mg activated carbon (2 mL carbon suspension). Using a high-speed mixer (20 000 rpm), stir for 45 min in an organohalide vapor-free environment. Filter through a membrane filter under vacuum or pressure, and collect filtrate. Remove flask containing filtrate. Wash carbon cake and filter with 10 mL NO_3^- wash solution. Add portions of wash solution serially to keep activated carbon and NO_3^- solution in contact for 15 min. Using clean instruments, transfer carbon cake and membrane filter to pyrolysis unit sample boat. Let instrument stabilize, pyrolyze, and determine the halide content of the first serial filter.

Add 20 mg more activated carbon to filtrate in erlenmeyer flask. Repeat carbon mixing, filtering, and washing procedures. Pyrolyze and determine halide content of second serial filter. If the second value is greater than 10% of the total value (first plus second), perform the NPDOX determination on an additional sample portion.

c. POX procedure (optional) (direct purge): Adjust apparatus [¶ 4*a*1)]. Select sample volume by comparing expected POX value (if known) with optimum instrument range. Using a gastight syringe, inject sample through septum into purge vessel, and purge as recommended by equipment manufacturer. Carefully control gas flow rate, sample temperature, and purging time. The maximum POX that can be determined is:

$$POX_{max}, \mu m/L = \frac{0.5 \times 1000}{mL\ sample \times 35.5}$$

If replicates are analyzed, sampling from replicate sample bottles may minimize variability due to volatilization losses.

5. Quality Control

a. Activated carbon quality: Purchase activated carbon ready for use or prepare activated carbon by milling and sieving high-quality activated carbon. Use only 100- to 200-mesh carbon in the microcolumn method. During preparation, take care not to expose the activated carbon to organic vapors. Use of a clean room is helpful. Prepare only small quantities (a month's supply or less) at one time. Discard the activated carbon if its DOX background concentration has increased significantly from the time of preparation or if the background is greater than 1 μg apparent organic Cl^-/40 mg activated carbon. Uniformity of activated carbon is important; therefore, after sieving small portions, combine and mix thoroughly. Transfer representative portions to clean glass bottles with ground-glass stoppers or with rubber-backed TFE septa and open ring caps. Store bottles in a gas-purged, evacuated, sealed desiccator.

Test each newly prepared batch of activated carbon to ensure adequate quality before use. Use only activated carbon meeting the guidelines outlined below.

1) Check activated carbon particle size by applying deionized water to two 40-mg activated carbon microcolumns. If flow rate is significantly less than 3 mL/min, resieve activated carbon to remove excess fines.

2) Analyze a pair of method blanks, ¶ 5*e*2). Reject carbon if the apparent organic halogen exceeds 1.2 μg/40 mg activated carbon.

If the activated carbon originated from a previously untested batch from a commercial supplier, test it for adsorption efficiency and inorganic halide rejection.

3) Adsorb replicate 100-mL portions of solutions containing 100, 500, and 1000 mg inorganic Cl^-/L deionized water. Wash with nitrate solution and analyze. The apparent organic halogen yield should not increase by more than 0.50 μg over the value determined in 2) above. A greater increase indicates significant interference at that concentration.

b. Serial adsorption: Each aqueous standard and sample is serially adsorbed on activated carbon in both procedures given above. Of the net organic halide, 90% or more should be adsorbed on the first activated carbon portion and the remaining 10% or less on the second. If, upon separate analysis of the two serial activated carbon portions, the second shows more than 10% of the net (after subtracting the method blank), reanalyze sample. Inorganic halogen interference or organic breakthrough are the most common reasons for a high second activated carbon value. Sample dilution before adsorption may improve recovery on the first activated carbon in series, but the minimum detectable concentration will be affected.

c. Standards: The standards used in routine analysis, quality control testing, and isolating specific causes during corrective maintenance include:

1) Sodium chloride standard (¶ 3*d*)—Use to check functioning of the titration cell and microcoulometer by injecting directly into the acetic acid solution of the titration cell. By examining the height and shape of the peak produced on the chart recorder and from the integrated value, problems associated with the cell and coulometer may be isolated. Use this standard at startup each day and after cell cleaning throughout the day. At daily startup consecutive duplicates should be within 3% of the historical mean. Depending on sample loading and number of analyses performed, it may be necessary to clean the titration cell several times per day. After cleaning, cell performance may be very unstable; therefore, inject a single NaCl standard before analyzing an instrument calibration standard [see ¶ 4) below]. Do *not* introduce NaCl standards into the pyrolysis furnace by application to the sample boat.

2) Ammonium chloride standard (¶ 3*e*)—Apply this standard to the sample boat to check for loss of halide in the pyrolysis furnace and entrance of the titration cell. Typically, this may be necessary when injection of a NaCl standard indicates proper titration cell and microcoulometer function but the recovery of the calibration standard is poor: suspect either poor conversion of organic chloride to hydrogen chloride or loss of hydrogen halide after conversion but before partitioning into the cell solution. To isolate the possible loss of hydrogen halides inject NH_4Cl standard directly onto the quartz sample boat. Recovery should be better than 95%, with a single peak of uniform shape produced. Use only a new quartz sample boat free of any residue; an encrusted boat dramatically reduces recovery. Use this standard for corrective maintenance problem isolation but not for routine analyses.

3) Purgeable organic halide calibration standards—For the POX analysis use aqueous chloroform solutions for instrument calibration. Also for POX analysis an aqueous bromoform standard can be used initially to insure acceptable purging conditions. Develop a standard curve over the dynamic range of the microcoulometer and check daily as in ¶ 5*c*5). Recovery of chloroform and bromoform should exceed 90% and 80%, respectively.

4) Instrument calibration standard—Direct injection of trichlorophenol working standard onto the nitrate-washed method blank in concentrations over the working range of the instrument determines linearity and calibration of the analyzer module. After checking for proper microcoulometer function by injecting NaCl standard, pyrolyze duplicate instrument calibration standards and then duplicate method blanks. The net response to the calibration standards should be within 3% of the calibration curve value. If not, check for loss of halide in the pyrolysis furnace using the ammonium chloride standard [¶ 5*c*2)].

5) Nonvolatile organic halide calibration standards—Develop an initial standard curve by analyzing aqueous solutions of 2,4,6-trichlorophenol, trichloroacetic acid (commonly formed during chlorination), or another appropriate halogenated organic compound over the dynamic range of the microcoulometer. This dynamic range typically is from 0.5 to 50 μg chloride, but will

vary between microcoulometers and titration cells. Construct an initial calibration curve using five calibration standards in range of 0.5 to 50 μg organic chloride; recheck calibration curve after changes in an instrument's configuration, such as replacement of a titration cell or major instrument maintenance. Daily, analyze a calibration standard to check proper function of the instrumentation and procedures. Select check standard in the concentration range of samples to be analyzed that day. When sample filtration is used to remove particulate matter, also use this pretreatment with the calibration standard. If DOX recovery is less than 90%, analyze a set of instrument calibration standards [¶ 5c4)].

d. Standard addition recovery: During routine analyses, ideally make standard additions to every tenth sample. Where the compounds constituting the DOX are known, use standards of these compounds. Where the compounds constituting the DOX are wholly or partially unknown, use standards reflecting the relative abundance of the halogens, the molecular size, and the volatility of the halogenated compounds presumed to be present. Recovery of 90% or more of the added amount indicates that the analyses are in control. Do not base acceptance of data on standard addition recoveries.

e. Blanks: High precision and accuracy of the background or blank value is important to the accurate measurement of DOX. Make blank measurements daily. Blanks that may be required are:

1) Reagent water blank—Analyze each batch of organic-free reagent water. The blank should have less than the minimum detectable concentration. Use this blank to insure that the standards, equipment, and procedures are not contributing to the DOX. Once reagent water blank is demonstrated, it can be used to determine method blank and POX blank as described below.

2) Method blank—Analyze activated carbon that has been nitrate-washed. Analyze method blanks daily before sample analysis and after at least each 10 to 14 sample pyrolyses.

3) Purgeable organic halogen blank—Analyze organic-free, pre-purged, reagent water to determine the POX blank.

6. Calculation

Calculate the net organic halide content as chloride (C_4) of each replicate of each sample and standard:

$$C_4 = \frac{C_1 - C_3 + C_2 - C_3}{V}$$

where:

C_1 = organic halide as Cl^- on the first activated carbon column or activated carbon cake, μg,
C_2 = organic halide as Cl^- on the second activated carbon column or activated carbon cake, μg,
C_3 = mean of method blanks on the same day and same instrument, μg X as Cl^-,
C_4 = uncorrected net organic halide as Cl^- of absorbed sample, μg organic halide as Cl^-/L, and
V = volume of sample absorbed, L.

If $C_2 \leq C_3$, then use:

$$C_4 = \frac{C_1 - C_3}{V}$$

If applicable, calculate net purgeable organic halide as Cl^- content (P_3):

$$P_3 = \frac{P_1 - P_2}{V}$$

where:

P_1 = sample purgeable organic halide as Cl^-, μg,
P_2 = blank purgeable organic halide as Cl^-, μg,
P_3 = uncorrected net purgeable organic halide as Cl^-, μg X as Cl^-/L, and
V = volume of sample or standard purged, L.

TABLE 5320:I. INTRALABORATORY, SINGLE-OPERATOR, DISSOLVED ORGANIC HALOGEN (MICROCOLUMN PROCEDURE)—PRECISION AND BIAS DATA

Characteristic of Analysis	Tap Water	Tap Water + 43.5 μg Organic Chloride	Ground Water (50:1)	Wastewater	Waste-water + 1000 μg Organic Chloride
Concentration determined, μg Cl^-/L:					
Replicate 1	38.5	89.0	123.6	186.0	1178.0
Replicate 2	36.7	90.9	124.8	195.0	1183.0
Replicate 3	43.1	88.4	125.2	195.0	1185.5
Replicate 4	35.9	90.1	123.3	204.0	1196.5
Replicate 5	41.1	91.7	125.3	185.0	1183.0
Replicate 6	48.5	93.0	127.0	236.5	1204.0
Replicate 7	52.8	97.0	123.5	204.0	1138.0
Mean, μg Cl^-/L	42.37	91.5	124.7	200.8	1181.1
Standard deviation:					
μg Cl^-/L	±6.29	±3.0	±1.3	±17.47	±21.04
%	15	3	1	9	2
Value of blank + standard addition, μg Cl^-/L	—	85.87	—	—	1200.8
Recovery, %	—	107	—	—	98
Error, %	—	7	—	—	2

Report sample results and percent recovery of the corresponding calibration standards [¶ 5*c*3) or ¶ 5*c*5)]. Also report the calibration standard curve if it is significantly nonlinear.

7. Precision and Bias

Precision and bias depend on specific procedures, equipment, and analyst. Develop and routinely update precision and bias data for each procedure, each instrument configuration, and each analyst. Table 5320:I shows sample calculations of precision expressed as the standard deviation among replicates and bias in the recovery of 2,4,6-trichlorophenol.

The precision and bias (accuracy) of analyses of TOX by this method were determined under the Information Collection Rule.[3] Precision was determined as relative percent difference for duplicate analyses and was calculated only when both analyses in the duplicate pair showed concentrations at, or greater than, the ICR minimum reporting level (MRL). Accuracy was calculated as percent recovery for cases in which the fortified concentration of organic halide from trichlorophenol was at least half the background concentration. Fortifying concentrations ranged from MRL (50 μg Cl^-/L) to 500 μg Cl^-/L. Results were as follows:[4]

Data Quality		Percentile				
Variable	N	10	25	50 (median)	75	90
Precision (RPD)	13911*	0.0	1.2	3.8	7.6	14
Accuracy (% rec.)	1109	82	93	100	108	126

* 6003 samples excluded — both samples less than MRL.

NOTE: For precision study, median sample concentration was 140 μg Cl^-/L; for accuracy study, median background concentration was 75 μg Cl^-/L.

8. References

1. STANBORO, W.D. & M.J. LENKEVICH. 1982. Slowly dechlorinated organic chloramines. *Science* 215:967.
2. SYMONS, J.M. & R. XIA. 1995. Interference of Br^-, BrO_3^-, and ClO_3^- in the DOX determination. *J. Amer. Water Works Assoc.* 87:81.
3. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1996. Monitoring requirements for Public Drinking Water Supplies: Cryptosporidium, Giardia, Viruses, Disinfection Byproducts, Water Treatment Plant Data and Other Information Requirements. Final Rule. *Federal Register.* 61(94):24354.
4. FAIR, P.S., R. K. SORRELL, M. KARAPONDO, B.J. BOHL, C.P. FREBIS & M.A. FEIGE. 2002. Quality of ICR monitoring data. *In* M. J. McGuire, J. McLain & A. Oblensky, eds. Information Collection Rule Data Analysis. American Water Works Assoc. Research Foundation, Denver, Colo.

9. Bibliography

KUHN, W. 1974. Thesis, Univ. Karlsruhe, West Germany.

KUHN, W., F. FUCHS & H. SONTHEIMER. 1977. Untersuchungen zur Bestimmung des organisch gebundenen Chlors mit Hilfe eines neuartigen Anreicherungsverfahrens. *Z. Wasser-Abwasser Forsch.* 10(6):162.

DRESSMAN, R.C., B.A. NAJAR & R. REDZIKOWSKI. 1979. The analysis of organohalides (OX) in water as a group parameter. Proc. 7th Annual Water Quality Technology Conf., Philadelphia, Pa. American Water Works Assoc., Denver, Colo.

TAKAHASHI, Y., et al. 1980. Measurement of total organic halides (TOX) and purgeable organic halides (POX) in water using carbon adsorption and microcoulometric detection. Proc. Symp. on Chemistry and Chemical Analysis of Water and Waste Water Intended for Reuse, Houston, Tex. American Chemical Soc., Washington, D.C.

DRESSMAN, R.C. 1980. Total Organic Halide, Method 450.1—Interim. Drinking Water Research Div., Municipal Environmental Research Lab., U.S. Environmental Protection Agency, Cincinnati, Ohio.

JEKEL, M.R. & P.V. ROBERTS. 1980. Total organic halogen as a parameter for the characterization of reclaimed waters: measurement, occurrence, formation, and removal. *Environ. Sci. Technol.* 14:970.

DRESSMAN, R.C. & A. STEVENS. 1983. Analysis of organohalides in water—An evaluation update. *J. Amer. Water Works Assoc.* 75:431.

RECKHOW, D.A., C. HULL, E. LEHAN, J.M. SYMONS, H-S KIM, Y-M HANG, L. SIMMS & R.C. DRESSMAN. 1990. The determination of total organic halide in water: A comparative study of two instruments. *Internat. J. Environ. Chem.* 38:1.

5510 AQUATIC HUMIC SUBSTANCES*

5510 A. Introduction

1. General Discussion

Aquatic humic substances (AHS) are heterogeneous, yellow to black, organic materials that include most of the naturally occurring dissolved organic matter in water. Aquatic humic substances have been shown to produce trihalomethanes (THMs) on chlorination and to affect the transport and fate of other organic and inorganic species through partition/adsorption, catalytic, and photolytic reactions.

Humic substances, the major fraction of soil organic matter, are mixtures; their chemical composition is poorly understood. They have been classified into three fractions based on water "solubility"†: humin is the fraction not soluble in water at any pH value; humic acid is not soluble under acidic conditions (pH < 2) but becomes soluble at higher pH; and fulvic acid is soluble at all pH conditions.

* Approved by Standard Methods Committee, 2000.

† "Solubility" is here used as a general description of whether or not the material can be uniformly dispersed in an aqueous phase rather than as an expression of equilibrium between a pure solute and its aqueous solution.

AHS have the solubility characteristics of fulvic acids but they should not be referred to as such unless they have been fractionated by precipitation at pH < 2. Avoid using the terms "humic acid" and "tannic acid" to describe AHS because they represent other classifications of natural organic materials.

The heterogeneity of AHS requires an operational definition. Isolation by the methods included herein most likely will be incomplete and compounds that are not AHS may be isolated incidentally. Users of these methods are cautioned in the interpretation of results; the bibliography suggests several sources for more information.

Measurement of AHS begins by separation of the sample into dissolved (containing AHS) and particulate organic carbon fractions. Although there is no distinct size that separates these two groups, 0.45 μm is used as the compromise between acceptable flow rate and rejection of small colloidal materials. Low-pressure liquid chromatography serves to concentrate these materials and to isolate them from interfering substances. AHS are quantified by measuring dissolved organic carbon (DOC), Method 5310.

2. Selection of Method

Concentration/isolation of AHS may be achieved by sorption on the nonpolar polyacrylate macroporous resin (such as XAD-7 or XAD-8‡) (Method 5510C) or by anion-exchange on diethylaminoethyl (DEAE) cellulose (Method 5510B). In a collaborative study with seven laboratories using deionized water fortified with about 10 mg AHS/L (previously isolated with XAD), the DEAE method gave better recoveries. Nevertheless, the XAD method has been used extensively; refer to the discussions of interferences and minimum detectable concentrations to assist in method selection. Both methods require further quality control development.

‡ Rohm and Haas Co., Philadelphia, PA.

3. Bibliography

CHRISTMAN, R. F. & E. T. GJESSING, eds. 1983. Aquatic and Terrestrial Humic Substances. Ann Arbor Science, Ann Arbor, Mich.

MILES, C. J., J. R. TUSCHALL, JR. & P. L. BREZONIK. 1983. Isolation of aquatic humus with diethylaminoethyl cellulose. *Anal. Chem.* 55: 410.

THURMAN, E. M. 1984. Determination of aquatic humic substances in natural waters. *In* E. L. Meyer, ed. Selected Papers in the Hydrologic Sciences. U.S. Geological Survey Water Supply Paper 2262, p. 47.

TUSCHALL, J. R., JR. & G. GEORGE. 1984. Selective Isolation of Dissolved Organic Matter from Aquatic Systems. UILUWRC-84-190, Water Resources Center, Univ. Illinois, Urbana.

AIKEN, G. R., D. M. MCKNIGHT, R. L. WERSHAW & P. L. MACCARTHY, eds. 1985. Humic Substances in Soil, Sediment, and Water. John Wiley & Sons, New York, N.Y.

5510 B. Diethylaminoethyl (DEAE) Method

1. General Discussion

a. Principle: AHS are concentrated by column chromatography on diethylaminoethyl (DEAE) cellulose and measured as dissolved organic carbon (DOC). AHS are weak organic acids that bind to anion-exchange materials, such as DEAE cellulose, at neutral pH values. The method is based on the assumption that AHS are the major dissolved organic acids present.

b. Interferences: Any carbonaceous nonhumic materials that are concentrated and isolated by the chromatographic method will interfere (false positive response). Substances that have been shown to interfere include fatty acids, phenols, surfactants, proteinaceous materials, and DOC leached from cellulose.

c. Minimum detectable concentration: Estimated limit of detection is 1.1 mg/L using a 50-mL water sample. The detection limit can be decreased by increasing sample volume. The major limitation is blank contamination.

d. Standard substance: Eliminate documentation of false negatives by analyses of a sample of known humic concentration at regular intervals (at least once per batch of samples).

2. Apparatus

a. Membrane filtration apparatus: Use an all-glass filtering device and 0.45-μm silver membrane filters. Consult manufacturer's specifications for filter details. Do not use filters that sorb AHS or are contaminated with detergents and other organic material.

b. Glass column, approximately 1 × 20 cm with silanized glass wool.

c. Dye-impregnated paper or strips for approximate pH measurements.

d. Organic carbon analyzer capable of measuring concentrations as low as 0.1 mg/L (see Section 5310).

*e. Buchner funnel and filter paper.**

3. Reagents

a. Water, DOC-free: Preferably use activated-carbon-filtered, redistilled water.

b. DEAE cellulose, exchange capacity 0.22–1.0 meq/g.† Do not use high-exchange-capacity cellulose, which may decrease recovery of AHS. Take care not to overload low-exchange-capacity cellulose.

c. Hydrochloric acid, HCl, 0.1*N:* Add 8.3 mL conc HCl to 1000 mL water.

d. Hydrochloric acid, HCl, 0.5*N:* Add 41.5 mL conc HCl to 1000 mL water.

e. Sodium hydroxide, NaOH, 0.1*N:* Dissolve 4.0 g NaOH in 1000 mL water.

f. Sodium hydroxide, NaOH, 0.5*N:* Dissolve 20 g NaOH in 1000 mL water.

* Whatman No. 1 or equivalent.

† Whatman pre-swollen microgranular DE 52 or DE 51, or equivalent.

g. DOC standards: See Section 5310.

h. Potassium chloride, KCl, 0.01*N*: Add 0.75 g KCl to 1000 mL water.

i. Phosphoric acid, H_3PO_4, conc.

4. Procedure

a. Sample concentration and preservation: AHS are sensitive to biodegradation and photodegradation. Collect and store samples in organic-free glass containers. Filter at least duplicate portions through a 0.45-μm silver membrane filter as soon after collection as possible. Store samples in the dark at 4°C.

Use care to avoid overloading chromatographic columns and losing AHS. A rough guideline for sample volume selection is as follows:

Sample DOC *mg/L*	Sample Volume *mL*
0–2	250
2–10	50
10–50	25

b. Preparation of DEAE cellulose: Add 70 g DEAE cellulose to 1000 mL 0.5*N* HCl and stir gently for 1 h. Rinse cellulose with water in a Buchner funnel until funnel effluent pH is about 4. Resuspend DEAE in 1000 mL 0.5*N* NaOH and stir for 1 h. Rinse in a Buchner funnel with water until pH is about 6. Remove fines by suspending the treated DEAE in a 1000-mL graduated cylinder filled with water. Let mixture stand undisturbed for 1 h, then decant and discard the supernatant. Repeat removal of fines. Filter remaining DEAE using a Buchner funnel and store in a refrigerated glass container. Avoid prolonged storage, which may lead to microbial contamination.

c. Chromatography: Add 10 mL water to about 1 g DEAE to make a slurry. Carefully pipet enough into a 1- × 20-cm column fitted with a small (0.5-cm) glass-wool plug to make a 1-cm-deep column bed. Avoid getting DEAE on the sides of the column. Carefully place another 0.5-cm glass-wool plug on top of the bed. Rinse column with 50 mL 0.01*N* KCl (adjusted to pH 6 with 0.1*N* HCl or NaOH) just before sample concentration.

Adjust sample to pH 6 and pass it through the column at a flow rate of about 2 mL/min. Rinse with 5 mL water (pH 6). Elute AHS by adding about 3 mL 0.1*N* NaOH to the top of the column. Start collecting column effluent when it appears colored. (This will occur after about 1 mL has passed out of the column). Collect eluate in a graduated, conical test tube until it becomes colorless (about 2 mL). Acidify with conc H_3PO_4 to a pH of 2 or less (about 2 to 3 drops) and remove dissolved carbon dioxide (inorganic carbon) by purging with nitrogen for 10 min. Avoid exposure of alkaline samples to air (i.e. acidify immediately) to minimize contamination with CO_2. Determine volume and DOC of acidified eluate.

Process two portions of water and a second portion of sample by the same procedure. Pack a fresh column of DEAE for each sample and each control (DEAE cannot be reused).

5. Calculation

Calculate the concentration of AHS as:

$$\text{AHS, mg DOC/L} = [(A - B) \times C]/D$$

where:

A = average DOC concentration of the two sample NaOH eluates, mg C/L,

B = average DOC concentration of the two control NaOH eluates, mg C/L,

C = volume of eluate, L, and

D = volume of sample, L.

Multiplication of AHS, mg DOC/L, by 2 converts concentration to AHS, mg/L, if it is assumed that AHS contain 50% carbon. This will be the minimum concentration of AHS because recoveries are less than 100%.

6. Precision and Bias

For seven single-operator analyses, the relative standard deviation of triplicate samples (about 10 mg/L as AHS) ranged from 2.5 to 14.4% with an average of 4.9% ($n = 7$).

For seven single-operator analyses, recoveries ranged from 59.3 to 97.3% with an average of 77.4% and a relative standard deviation of 18.1%.

5510 C. XAD Method

1. General Discussion

a. Principle: AHS are concentrated by column chromatography on XAD resin and measured as dissolved organic carbon (DOC). Acidification of AHS decreases polarity, allowing partition into the nonpolar XAD matrix. The method is based on the assumption that AHS are the major dissolved organic acids present.

b. Interferences: Any carbonaceous nonhumic materials that are concentrated and isolated by the chromatographic method will interfere. This includes fatty acids, phenols, surfactants, proteinaceous materials, and DOC leached from the resin, chromatography pump, or tubing.

c. Minimum detectable concentration: Estimated limit of detection is 1.4 mg/L using a 50-mL water sample. The detection limit can be decreased by increasing sample volume. The major limitation is blank contaminations.

2. Apparatus

See Section 5510B.2*a*, *c*, and *d*. In addition, the following are required:

a. Glass column, 0.2 × 25 cm with silanized glass wool.

b. Pump, with inert internal parts and tubing, capable of flow rates of 0.2 to 1.0 mL/min.*

c. TFE tubing, 0.2 cm ID.

d. Extraction apparatus, Soxhlet.

* Pump parts may be of stainless steel or TFE.

3. Reagents

In addition to reagents *a, c, e, g,* and *i* of Section 5510B:

a. XAD resin,† approximately 250-μm size.

b. Hexane.

c. Methanol.

d. Acetonitrile.

4. Procedure

a. Sample collection and preservation: See Section 5510B.4*a*.

b. Preparation of XAD resin: Clean resin by successive washing with 0.1*N* NaOH for 5 d. Extract resin sequentially in a Soxhlet extractor with hexane, methanol, acetonitrile, and methanol, for 24 h each. Pack clean resin into a 0.2- × 25-cm glass column that has a 2-mm length of glass wool in one end. After filling, cap column with another 2-mm length of glass wool.

Wet dry column with methanol. When the air has been displaced, pump distilled water through the column until the effluent concentration of DOC decreases to 0.5 mg/L (approximately 20 bed volumes).

c. Chromatography: Preclean column with three cycles of 0.1*N* NaOH and 0.1*N* HCl just before pumping sample into column. Leave column saturated with 0.1*N* HCl. Acidify sample to pH 2.0 with concentrated HCl, and pump it onto the column at rate of 1.0 mL/min. Save column effluent for DOC analysis. Significant concentrations of DOC in the effluent can indicate that the column was overloaded and that a smaller sample volume should be used. Colored organic acids adsorb to the top of the column. Back-elute (reverse flow) the column with 0.1*N* NaOH at 0.2 mL/min and collect eluate in a graduated, conical test tube until it becomes colorless (about 2 mL). Acidify with conc H_3PO_4 to a pH of 2 or less (about 2 to 3 drops) and remove dissolved carbon dioxide (inorganic carbon) by purging with nitrogen for 10 min. Avoid exposure of alkaline samples to air (i.e. acidify immediately) to minimize contamination with CO_2. Determine volume and DOC of acidified column effluent.

After eluting and collecting AHS from the column with back-elution using 0.1*N* NaOH, continue rinsing with about 20 bed-volumes of the basic solution. Rinse with water for about 20 bed volumes. Repeat the triplicate acid/base column precleaning procedure described above, then reuse the column to analyze a replicate sample. Process two portions of water by the same procedure to serve as controls.

The XAD column may be reused to analyze subsequent samples and controls if the triplicate acid/base precleaning procedure is repeated immediately before analysis of each replicate. Replace the column if recovery is poor or the resin becomes discolored.

† XAD-8. XAD is a trademark of Rohm and Haas Co., Philadelphia, PA.

5. Calculation

Calculate the concentration of AHS as given in 5510B.5.

6. Precision and Bias

For seven single-operator analyses, the relative standard deviation of triplicate samples (about 10 mg/L as AHS) ranged from 0.9 to 20.7% with an average of 5.4% (n = 7).

For seven single-operator analyses, recoveries ranged from 15.1 to 71.0% with an average of 51.6% and a relative standard deviation of 35.1%.

5520 OIL AND GREASE*

5520 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 was dropped from all gravimetric procedures (retained for 5520C, an infrared method), and replaced with *n*-hexane. Solvent-recovery techniques were included and solvent recycling was strongly recommended. In the methods given below, the 80% *n*-hexane and 20% methyl-*tert*-butyl ether solvent mix has been dropped from 5520B, D, and E, and an alternative to the liquid/liquid extraction procedure using solid-phase adsorbent disks is included.

It is important to understand that, unlike some constituents that represent distinct chemical elements, ions, compounds, or groups

* Approved by Standard Methods Committee, 2001.
Joint Task Group: John Gute (chair), Jennifer R. Calles, Lavern V. Tamoria.

of compounds, oils and greases are defined by the method used for their determination. In detailed studies involving wastewaters and solid matrices, it was shown that *n*-hexane produced results statistically different from results produced by trichlorotrifluoroethane.[1,2] Trichlorotrifluoroethane may at some point be prohibited entirely by regulatory authorities. EPA currently recommends use of *n*-hexane in place of trichlorotrifluoroethane.[3]

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 has two primary components: fatty matter from animal and vegetable sources and hydrocarbons of petroleum origin. The portion of oil and grease from each of these two major components 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, four methods are presented: the liquid/liquid partition-gravimetric method (B), the partition-infrared method (C), the Soxhlet method (D), and the solid-phase, partition-gravimetric method (G). 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 sensitivity. Method G can be used as an alternate to Method B to reduce solvent volumes and matrix problems.

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 G 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 petroleum 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 $CHCl_3$ 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, F, and G, 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, F, and G, 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* above). 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.

f. Analyte recovery may be compromised unless efforts are made to ensure uniform sample passage through the solid-phase extraction disks used in Method 5520G.

5. References

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1993. Preliminary Report of EPA Efforts to Replace Freon for the Determination of Oil and Grease, Revision 1. EPA-821-R-93-001, U.S. Environmental Protection Agency, Washington, D.C.
2. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1995. Report of EPA Efforts to Replace Freon for the Determination of Oil and Grease and Total Petroleum Hydrocarbons: Phase II. EPA-821-R-95-003, U.S. Environmental Protection Agency, Washington, D.C.
3. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1998. Method 1664, Revision A, *n*-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated *n*-Hexane Extractable Material (HEM; Non-polar material) by Extraction and Gravimetry. EPA-821-R-98-002, U.S. Environmental Protection Agency, Washington, D.C.; 40 CFR Part 136, Table IB, Item 41 (July 1, 2000); *Federal Register* 64:93, 26315.

5520 B. Liquid-Liquid, 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 is shown in Figure 5520:1. Alternatively, use commercially available solvent recovery equipment.
j. Ice bath.

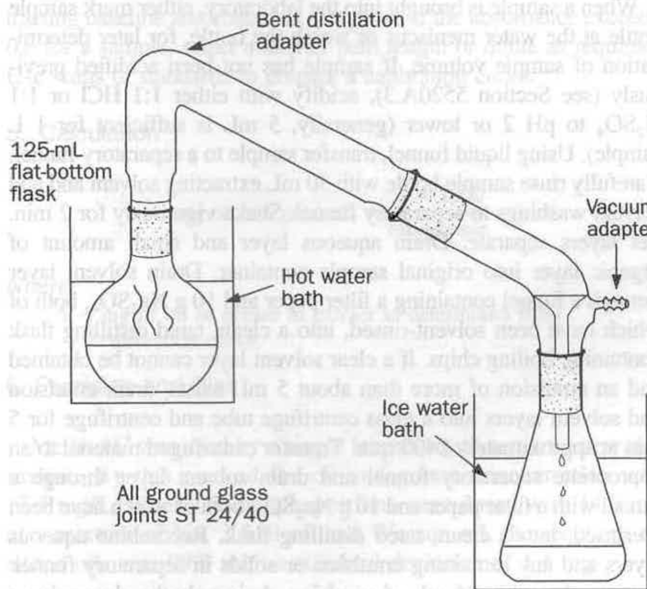

Figure 5520:1. Distillate recovery apparatus.

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.

* Teflon or equivalent.
† Whatman No. 40 or equivalent

b. n-Hexane, 85% minimum purity, 99% minimum saturated C_6 isomers, residue less than 1 mg/L; distill if necessary. Do not use any plastic tubing to transfer solvent between containers.

c. Sodium sulfate, Na_2SO_4, anhydrous crystal. Dry at 200–250°C for 24 h.

d. Acetone, residue less than 1 mg/L.

e. Hexadecane, 98% minimum purity.

f. Stearic acid, 98% minimum purity.

g. Standard mixture, hexadecane/stearic acid 1:1 w/w, in acetone at 2 mg/mL each. Purchase prepared standard mixture, or prepare as follows.[1]

Place 200 ± 2 mg stearic acid and 200 ± 2 mg hexadecane in a 100-mL volumetric flask and fill to mark with acetone. NOTE: The solution may require warming for complete dissolution of stearic acid.

After the hexadecane and stearic acid have dissolved, transfer solution to a 100- to 150-mL vial with TFE-lined cap. Mark solution level on the vial and store in dark at room temperature.

Immediately before use, verify level on vial and bring to volume with acetone, if required. Warm to redissolve all visible precipitate. NOTE: If there is doubt of the concentration, remove 10.0 ± 0.1 mL with a volumetric pipet, place in a tared weighing pan, and evaporate to dryness in a fume hood. The weight must be 40 ± 1 mg. If not, prepare a fresh solution.

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 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 Na_2SO_4, both of which have been solvent-rinsed, into a clean, tared distilling flask containing boiling chips. 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. 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 (Figure 5520:1). When visible solvent condensation stops, replace bent distillation apparatus with vacuum/air adapter connected to vacuum source. Immediately draw air through flask with an applied vacuum for the final 1 min. Remove flask from bath and wipe outside surface to remove moisture. Cool in desiccator until a constant weight is obtained. 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

Calculate oil and grease in sample as follows:

$$\text{mg oil and grease/L} = \frac{W_r}{V_s}$$

where:

W_r = total weight of flask and residue, minus tare weight of flask, mg, and

V_s = initial sample volume, L.

6. Precision and Bias

Method B was tested in a single laboratory study and an interlaboratory method validation study.[1] Combined data from these studies yielded an average recovery of 93% and precision (as relative standard deviation) of 8.7%.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1998. Method 1664, Revision A. *n*-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated *n*-Hexane Extractable Material by Extraction and Gravimetry. EPA-821-R-98-002; 40 CFR Part 136 (July 1, 2000); *Federal Register* 64 (93):26315. U.S. Environmental Protection Agency, Washington, D.C.

8. Bibliography

KIRSCHMAN, H.D. & R. POMEROY. 1949. Determination of oil in oil field waste waters. *Anal. Chem.* 21:793.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1995. Report of the Method 1664 Validation Studies. EPA-821-R-95-036, U.S. Environmental Protection Agency, Washington, D.C.

5520 C. Partition-Infrared Method

1. General Discussion

a. Principle: The use of trichlorotrifluoroethane as extraction solvent allows absorbance of the carbon-hydrogen bond in the infrared to be used to measure oil and grease. Elimination of the evaporation step permits infrared detection of many relatively volatile hydrocarbons. Thus, the lighter petroleum distillates, with the exception of gasoline, may be measured accurately. With adequate instrumentation, as little as 0.2 mg oil and grease/L can be measured.

b. Definitions: A "known oil" is defined as a sample of oil and/or grease that represents the only material of that type used or manufactured in the processes represented by a wastewater. An "unknown oil" is defined as one for which a representative sample of the oil or grease is not available for preparation of a standard.

2. Apparatus

a. Separatory funnel, 2-L, with TFE* stopcock.
b. Volumetric flask, 100-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. Infrared spectrophotometer, double-beam, recording.
h. Cells, near-infrared silica.

3. Reagents

a. Hydrochloric acid, HCl, 1 + 1.

b. Trichlorotrifluoroethane (1,1,2-trichloro-1,2,2-trifluoroethane), boiling point 47°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. Sodium sulfate, Na_2SO_4, anhydrous, crystal. Dry at 200 to 250°C for 24 h.

d. Reference oil: Prepare a mixture, by volume, of 37.5% isooctane, 37.5% hexadecane, and 25.0% benzene. Store in sealed container to prevent evaporation.

4. Procedure

Refer to Section 5520B.4 for sample handling and for method of dealing with sample emulsions. After carefully transferring sample to a separatory funnel, rinse sample bottle with 30 mL trichlorotrifluoroethane and add solvent washings to funnel. Shake vigorously for 2 min. Let layers separate. Drain all but a very small portion of the lower trichlorotrifluoroethane layer through a funnel containing a filter paper and 10 g Na_2SO_4, both of which have been solvent-rinsed, into a clean, 100-mL volumetric flask. If a clear solvent layer cannot be obtained and an emulsion of more than about 5 mL exists, see Section 5520B.4. 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 volumetric flask, and include in flask a final rinsing of filter and Na_2SO_4 with an additional 10 to 20 mL solvent. Adjust final volume to 100 mL with solvent.

Prepare a stock solution of known oil by rapidly transferring about 1 mL (0.5 to 1.0 g) of the oil or grease to a tared 100-mL volumetric flask. Stopper flask and weigh to nearest milligram. Add solvent to dissolve and dilute to mark. If the oil identity is unknown (5520C.1*b*) use the reference oil (5520C.3*d*) as the standard. Using volumetric techniques, prepare a series of standards over the range of interest. Select a pair of matched near-infrared silica cells. A 1-cm-path-length cell is appropriate for a working range of about 4 to 40 mg. Scan standards and samples from 3200 cm^{-1} to 2700 cm^{-1} with solvent in the reference beam and record results on absorbance paper. Measure absorbances of samples and standards by constructing a straight base line over the scan range and measuring absorbance of the peak maximum at 2930 cm^{-1} and subtracting baseline absorbance at that point. If the absorbance exceeds 0.8 for a sample, select a shorter path length or dilute as required. Use scans of standards to prepare a calibration curve.

5. Calculation

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

where:

A = mg of oil or grease in extract as determined from calibration curve.

6. Precision and Bias

Method C was used by a single laboratory to test a wastewater sample. By this method the oil and grease concentration was 17.5 mg/L. When 1-L sample portions were dosed with 14.0 mg of a mixture of No. 2 fuel oil and Wesson oil, the recovery of added oils was 99% with a standard deviation of 1.4 mg.

7. Bibliography

GRUENFELD, M. 1973. Extraction of dispersed oils from water for quantitative analysis by infrared spectrophotometry. *Environ. Sci. Technol.* 7:636.

* Teflon or equivalent.
† Whatman No. 40 or equivalent.

5520 D. Soxhlet Extraction Method

1. General Discussion

Soluble metallic soaps are hydrolyzed by acidification. Any oils and solid or viscous grease present are separated from the liquid samples by filtration. After extraction in a Soxhlet apparatus with solvent, the residue remaining after solvent evaporation is weighed to determine the oil and grease content. Compounds volatilized at or below 103°C will be lost when the filter is dried.

2. Apparatus

a. Extraction apparatus, Soxhlet, with 125-mL extraction flask.
b. Extraction thimble, paper, solvent-extracted.
c. Electric heating mantle.
d. Vacuum pump or other source of vacuum.
e. Vacuum filtration apparatus.
f. Buchner funnel, 12-cm.
g. Filter paper, 11-cm diam.*
h. Muslin cloth disks, 11-cm diam, solvent-extracted.
i. Glass beads or glass wool, solvent-extracted.
j. Water bath, capable of maintaining 85°C.
k. Distilling adapter with drip tip. See 5520B.2*i* and Figure 5520:1.
l. Ice bath.
m. Waste receptacle, for used solvent.
n. Desiccator.

3. Reagents

a. Hydrochloric acid, HCl, 1 + 1.
b. n*-Hexane:* See Section 5520B.3*b*.
c. Diatomaceous-silica filter aid suspension,† 10 g/L distilled water.

4. Procedure

When sample is brought into the laboratory, either mark sample bottle at the meniscus or weigh bottle for later determination of volume. If sample has not been acidified previously (see Section 5520A.3), acidify with 1:1 HCl or 1:1 H_2SO_4 to pH 2 or lower (generally, 5 mL is sufficient).

* Whatman No. 40 or equivalent.
† Hyflo Super-Cel, Manville Corp., or equivalent.

Prepare filter consisting of a muslin cloth disk overlaid with filter paper. Wet paper and muslin and press down edges of paper. Using vacuum, pass 100 mL filter aid suspension through prepared filter and wash with 1 L distilled water. Apply vacuum until no more water passes filter. Filter acidified sample. Apply vacuum until no more water passes through filter. Using forceps, transfer entire filter to a watch glass. Add material adhering to edges of muslin cloth disk. Wipe sides and bottom of collecting vessel and Buchner funnel with pieces of filter paper soaked in extraction solvent, taking care to remove all films caused by grease and to collect all solid material. Add pieces of filter paper to material on watch glass. Roll all filter material containing sample and fit into an extraction thimble. Add any pieces of material remaining on watch glass. Wipe watch glass with a filter paper soaked in extraction solvent and place in extraction thimble. Dry filled thimble in a hot-air oven at 103°C for 30 min. Fill thimble with glass wool or small glass beads. Weigh extraction flask and add 100 mL extraction solvent (*n*-hexane, ¶ 3*b*). Extract oil and grease in a Soxhlet apparatus, at a rate of 20 cycles/h for 4 h. Time from first cycle. For stripping and recovery of solvent, cooling extraction flask before weighing, and determining initial sample volume, see Section 5520B.4.

5. Calculation

See Section 5520B.5.

6. Precision and Bias

In analyses of synthetic samples containing various amounts of Crisco and Shell S.A.E. No. 20 oil, an average recovery of 98.7% was obtained, with a standard deviation of 1.86%. Ten replicates each of two wastewater samples yielded standard deviations of 0.76 mg and 0.48 mg.

7. Bibliography

HATFIELD, W.D. & G.E. SYMONS. 1945. The determination of grease in sewage. *Sewage Works J.* 17:16.

GILCREAS, F.W., W.W. SANDERSON & R.P. ELMER. 1953. Two new methods for the determination of grease in sewage. *Sewage Ind. Wastes* 25:1379.

ULLMANN, W.W. & W.W. SANDERSON. 1959. A further study of methods for the determination of grease in sewage. *Sewage Ind. Wastes* 31:8.

5520 E. Extraction Method for Sludge Samples

1. General Discussion

Drying acidified sludge by heating leads to low results. Magnesium sulfate monohydrate is capable of combining with 75% of its own weight in water in forming $MgSO_4 \cdot 7H_2O$ and is used to dry sludge. After drying, the oil and grease can be extracted with *n*-hexane.

2. Apparatus

a. Beaker, 150-mL, glass.
b. Mortar and pestle, porcelain.
c. Extraction apparatus, Soxhlet.
d. Extraction thimble, paper, solvent-extracted.
e. Glass beads or glass wool, solvent-extracted.
f. Electric heating mantle.
g. Vacuum pump or other source of vacuum.
h. Liquid funnel, glass.
i. Grease-free cotton: Extract nonabsorbent cotton with solvent.
j. Water bath, capable of maintaining 85°C.
k. Distilling adapter with drip tip. See 5520B.2*i* and Figure 5520:1.
l. Ice bath.
m. Waste receptacle, for used solvent.
n. Desiccator.

3. Reagents

a. Hydrochloric acid, HCl, conc.
b. n-*Hexane:* See Section 5520B.3*b*.
c. Magnesium sulfate monohydrate: Prepare $MgSO_4 \cdot H_2O$ by drying a thin layer overnight in an oven at 150°C.

4. Procedure

When sample is brought into the laboratory, if it has not been acidified previously (Section 5520A.3), add 1 mL conc HCl/80 g sample. In a 150-mL beaker weigh out a sample of wet sludge, 20 ± 0.5 g, for which the dry-solids content is known. Acidify to pH 2.0 or lower (generally, 0.3 mL conc HCl is sufficient). Add 25 g $MgSO_4 \cdot H_2O$. Stir to a smooth paste and spread on sides of beaker to facilitate subsequent sample removal. Let stand until solidified, 15 to 30 min. Remove solids and grind in a porcelain mortar. Add powder to a paper extraction thimble. Wipe beaker and mortar with small pieces of filter paper moistened with solvent and add to thimble. Fill thimble with glass wool or small glass beads. Tare extraction flask, and add 100 mL *n*-hexane. Extract in a Soxhlet apparatus at a rate of 20 cycles/h for 4 h. If any turbidity or suspended matter is present in the extraction flask, remove by filtering through grease-free cotton into another weighed flask. Rinse flask and cotton with solvent. For solvent stripping and recovery, and cooling the extraction flask before weighing, see Section 5520B.4.

5. Calculation

Oil and grease as % of dry solids

$$= \frac{\text{gain in weight of flask, g} \times 100}{\text{weight of wet solids, g} \times \text{dry solids fraction}}$$

6. Precision

The examination of six replicate samples of sludge yielded a standard deviation of 4.6%.

5520 F. Hydrocarbons

1. General Discussion

Silica gel has the ability to adsorb polar materials. If a solution of hydrocarbons and fatty materials in a nonpolar solvent is mixed with silica gel, the fatty acids are removed selectively from solution. The materials not eliminated by silica gel adsorption are designated hydrocarbons by this test.

2. Apparatus

a. Magnetic stirrer.
b. Magnetic stirring bars, TFE-coated.
c. Liquid funnel, glass.
d. Filter paper, 11-cm diam.*
e. Desiccator.

3. Reagents

a. n-*Hexane:* See Section 5520B.3*b*.
b. Trichlorotrifluoroethane: See Section 5520C.3*b*.
c. Silica gel, 100 to 200 mesh.† Dry at 110°C for 24 h and store in a tightly sealed container.

4. Procedure

Use the oil and grease extracted by Method B, C, D, E, or G for this test. When only hydrocarbons are of interest, introduce this procedure in any of the methods before final measurement. When hydrocarbons are to be determined after total oil and grease has been measured, redissolve the extracted oil and grease in trichlorotrifluoroethane (Method C)

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* Whatman No. 40 or equivalent.

† Davidson Grade 923 or equivalent.

or 100 mL *n*-hexane (Method B, D, E, or G). To 100 mL solvent add 3.0 g silica gel/100 mg total oil and grease, up to a total of 30.0 g silica gel (1000 mg total oil and grease). For samples with more than 1000 mg total oil and grease use a measured volume of the 100 mL solvent dissolved sample, add appropriate amount of silica gel for amount of total oil and grease in the sample portion, and bring volume to 100 mL. Stopper container and stir on a magnetic stirrer for 5 min. For infrared measurement of hydrocarbons no further treatment is required before measurement as described in Method C. For gravimetric determinations, filter solution through filter paper pre-moistened with solvent, wash silica gel and filter paper with 10 mL solvent, and combine with filtrate. For solvent stripping and recovery, and for cooling extraction flask before weighing, see Section 5520B.4.

5. Calculation

Calculate hydrocarbon concentration, in milligrams per liter, as in oil and grease (Method B, C, D, or G).

6. Precision and Bias

The following data, obtained on synthetic samples, are indicative for natural animal, vegetable, and mineral products, but cannot be applied to the specialized industrial products previously discussed.

For hydrocarbon determinations on 10 synthetic solvent extracts containing known amounts of a wide variety of petroleum products, average recovery was 97.2%. Similar synthetic extracts of Wesson oil, olive oil, Crisco, and butter gave 0.0% recovery as hydrocarbons measured by infrared analysis.

Using reagent water fortified with approximately 20 mg/L each of hexadecane and stearic acid, combined data from single-laboratory studies and an interlaboratory method validation study yielded an average recovery of 89% and precision (as relative standard deviation) of 13%.

7. Bibliography

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1995. Report of the Method 1664 Validation Studies. EPA-821-R-95-036, U.S. Environmental Protection Agency, Washington, D.C.

5520 G. Solid-Phase, Partition-Gravimetric Method

1. General Discussion

Dissolved or emulsified oil and grease is extracted from water by passing a sample through a solid-phase extraction (SPE) disk where the oil and grease are adsorbed by the disk and subsequently eluted with *n*-hexane. Some extractables, especially unsaturated fats and fatty acids, oxidize readily; hence, special precautions regarding temperature and solvent vapor displacement are provided. This method is not applicable to materials that volatilize at temperatures below 85°C, or crude and heavy fuel oils containing a significant percentage of material not soluble in *n*-hexane. This method may be a satisfactory alternative to liquid-liquid extraction techniques, especially for samples that tend to form difficult emulsions during the extraction step.[1]

2. Apparatus

a. SPE extraction apparatus, 90 mm.*

b. Oil and grease SPE extraction disk, 90 mm.†

c. Distilling flask, 125 mL.

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

e. Vacuum pump or other source of vacuum.

f. Distilling adapter with drip tip. Setup of distillate recovery apparatus is shown in Figure 5520:1. Alternatively, use commercially available solvent recovery equipment.

g. Ice bath.

h. Waste receptacle, for used solvent.

i. 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. Sodium sulfate, Na_2SO_4, anhydrous crystal.

d. Acetone, residue less than 1 mg/L.

e. Methanol, ACS grade.

f. Hexadecane, 98% minimum purity.

g. Stearic acid, 98% minimum purity.

h. Standard mixture, hexadecane/stearic acid 1:1 w/w, in acetone at 2 mg/mL each.

4. Procedure

When a sample is brought into the laboratory, either mark sample bottle at water meniscus or weigh 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). Assemble SPE extraction apparatus with oil and grease SPE disk installed and condition disk according to apparatus and disk manufacturers' instructions. Do not let disk dry before introducing sample for extraction. Conditioning is a critical step for uniform flow and good recovery. If the sample is high in suspended solids, decant liquid portion after allowing solids to settle. Extract sample at

* SPE extraction apparatus is available from multiple vendors. Currently available equipment includes manual, semi-automated, and automated.
† SPE extraction disks are available from multiple vendors.

an optimum flow rate of 100 mL/min; faster extraction rates will reduce recoveries. After extraction, ensure that the disk dries fully before elution; however, use of an acetone rinse to aid in drying also will result in low recoveries. Carefully control vacuum during elution to let solvent fully penetrate the disk. A good-quality pump capable of controlling the vacuum from 20.3 cm (8 in.) Hg for elution to 63.5 cm (25 in.) Hg for disk drying will reduce variability of results. Pour sample into reservoir. If sample is high in suspended solids or sediment that may clog the disk, decant supernatant into reservoir first. Apply vacuum at an optimum flow rate of 100 mL/min and add sediment-laden residual from decanted sample to reservoir before allowing disk to go dry. After sample extraction is complete, apply full vacuum for 10 min to remove as much residual water as possible from disk and then change receivers. Rinse sample container with a portion of *n*-hexane and add solvent to reservoir, making sure that reservoir walls are rinsed in the process. Apply a slight vacuum to draw a few drops of *n*-hexane through disk, then release. Let solvent soak disk for 2 min. Collect eluent by applying enough vacuum to cause a slow stream of hexane to drip through disk. Let disk dry before repeating elution with a second portion of *n*-hexane. Pour combined eluent through a funnel containing a filter paper and 10 g Na_2SO_4, both of which have been solvent-rinsed, into a clean, tared distilling flask containing boiling chips. Rinse collection vial and filter containing Na_2SO_4 with *n*-hexane, and add to boiling flask. Distill solvent from flask in a water bath at 85°C. Fit distillation flask with a distillation adaptor equipped with a drip tip and collect solvent in an ice-bath-cooled receiver (Figure 5520:1); reduce volume, cool, and weigh to constant weight as described in 5520B.4.

5. Calculations

Calculate oil and grease in sample as follows:

$$\text{mg oil and grease/L} = \frac{W_r}{V_s}$$

where:

W_r = total weight of flask and residue, minus tare weight of flask, mg, and

V_s = initial sample volume, L.

6. Precision and Bias

In a comparison of laboratory-fortified reagent water samples (n = 46) with additions of approximately 20 mg/L each of hexadecane and stearic acid and analyzed by both automated and manual SPE systems, recoveries of 87.1 ± 7.8% and 92.5 ± 4.4% recovery on 10% fortified reagent water samples.‡

Precision for wastewaters may vary by matrix. In a single-laboratory testing replicate (n=4–6) domestic wastewater samples (8 different samples) in the 18- to 60-mg/L range, the CV averaged 9.0%. Another laboratory testing replicates (n = 4) of 16 different types of wastewater in the 4- to 3000-mg/L range reported CVs ranging from 1.1 to 57.1% for 19 wastewater types; pooled results from four laboratories showed an average CV of 17.0% ± 13.8%.‡

Single-laboratory control charts for matrix known additions analysis showed an average relative percent difference of 2.2% and an average percent recovery of 87.2% with a standard deviation of 5.5%.

Two laboratories were able to achieve an MDL of 1.4 mg/L, using either manual or semi-automated SPE apparatus.

7. Reference

1. U.S. Environmental Protection Agency. 1999. Method 1664, Revision A. EPA-821-R-98-002, U.S. Environmental Protection Agency, Washington, D.C.

‡ Data provided by USEPA/DynCorp Systems from the public comment document on EPA Method 1664. Proposed Rule and Notice of Availability (62 Fed. Reg. 51621; FRL-5901-5).

5530 PHENOLS*

5530 A. Introduction

Phenols, defined as hydroxy derivatives of benzene and its condensed nuclei, may occur in domestic and industrial wastewaters, natural waters, and potable water supplies. Chlorination of such waters may produce odorous and objectionable-tasting chlorophenols. Phenol removal processes in water treatment include superchlorination, chlorine dioxide or chloramine treatment, ozonation, and activated carbon adsorption.

1. Selection of Method

The analytical procedures offered here use the 4-aminoantipyrine colorimetric method that determines phenol, ortho- and meta-substituted phenols, and, under proper pH conditions, those para-substituted phenols in which the substitution is a carboxyl, halogen, methoxyl, or sulfonic acid group. The 4-aminoantipyrine method does not determine those para-substituted phenols where the substitution is an alkyl, aryl, nitro, benzoyl, nitroso, or aldehyde group. A typical example of these latter

* Approved by Standard Methods Committee, 2000.

groups is paracresol, which may be present in certain industrial wastewaters and in polluted surface waters.

The 4-aminoantipyrine method is given in two forms: Method C, for extreme sensitivity, is adaptable for use in water samples containing less than 1 mg phenol/L. It concentrates the color in a nonaqueous solution. Method D retains the color in the aqueous solution. Because the relative amounts of various phenolic compounds in a given sample are unpredictable, it is not possible to provide a universal standard containing a mixture of phenols. For this reason, phenol (C_6H_5OH) itself has been selected as a standard for colorimetric procedures and any color produced by the reaction of other phenolic compounds is reported as phenol. Because substitution generally reduces response, this value represents the minimum concentration of phenolic compounds. A gas-liquid chromatographic procedure is included in Section 6420B and may be applied to samples or concentrates to quantify individual phenolic compounds.

2. Interferences

Interferences such as phenol-decomposing bacteria, oxidizing and reducing substances, and alkaline pH values are dealt with by acidification. Some highly contaminated wastewaters may require specialized techniques for eliminating interferences and for quantitative recovery of phenolic compounds.

Eliminate major interferences as follows (see Section 5530B for reagents):

Oxidizing agents, such as chlorine and those detected by the liberation of iodine on acidification in the presence of potassium iodide (KI)—Remove immediately after sampling by adding excess ferrous sulfate ($FeSO_4$). If oxidizing agents are not removed, the phenolic compounds will be oxidized partially.

Sulfur compounds—Remove by acidifying to pH 4.0 with H_3PO_4 and aerating briefly by stirring. This eliminates the interference of hydrogen sulfide (H_2S) and sulfur dioxide (SO_2).

Oils and tars—Make an alkaline extraction by adjusting to pH 12 to 12.5 with NaOH pellets. Extract oil and tar from aqueous solution with 50 mL chloroform ($CHCl_3$). Discard oil- or tar-containing layer. Remove excess $CHCl_3$ in aqueous layer by warming on a water bath before proceeding with the distillation step.

3. Sampling

Sample in accordance with the instructions of Section 1060.

4. Preservation and Storage of Samples

Phenols in concentrations usually encountered in wastewaters are subject to biological and chemical oxidation. Preserve and store samples at 4°C or lower unless analyzed within 4 h after collection.

Acidify with 2 mL conc H_2SO_4/L.

Analyze preserved and stored samples within 28 d after collection.

5. Bibliography

ETTINGER, M.B., S. SCHOTT & C.C. RUCHHOFT. 1943. Preservation of phenol content in polluted river water samples previous to analysis. *J. Amer. Water Works Assoc.* 35:299.

CARTER, M.J. & M.T. HUSTON. 1978. Preservation of phenolic compounds in wastewaters. *Environ. Sci. Technol.* 12:309.

NEUFELD, R.D. & S.B. POLADINO. 1985. Comparison of 4-aminoantipyrine and gas-liquid chromatography techniques for analysis of phenolic compounds. *J. Water Pollut. Control Fed.* 57:1040.

5530 B. Cleanup Procedure

1. Principle

Phenols are distilled from nonvolatile impurities. Because the volatilization of phenols is gradual, the distillate volume must ultimately equal that of the original sample.

2. Apparatus

a. Distillation apparatus, all-glass, consisting of a 1-L borosilicate glass distilling apparatus with Graham condenser.*

b. pH meter.

3. Reagents

Prepare all reagents with distilled water free of phenols and chlorine.

a. Phosphoric acid solution, H_3PO_4, 1 + 9: Dilute 10 mL 85% H_3PO_4 to 100 mL with water.

b. Methyl orange indicator solution.

c. Special reagents for turbid distillates:

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

2) *Sodium chloride,* NaCl.

3) *Chloroform,* $CHCl_3$, or *methylene chloride,* CH_2Cl_2.

4) *Sodium hydroxide,* NaOH, 2.5*N*: Dilute 41.7 mL 6*N* NaOH to 100 mL or dissolve 10 g NaOH pellets in 100 mL water.

4. Procedure

a. Measure 500 mL sample into a beaker, adjust pH to approximately 4.0 with H_3PO_4 solution using methyl orange indicator or a pH meter, and transfer to distillation apparatus. Use a 500-mL graduated cylinder as a receiver. Omit adding H_3PO_4 and adjust pH to 4.0 with 2.5*N* NaOH if sample was preserved as described in 5530A.4.

b. Distill 450 mL, stop distillation and, when boiling ceases, add 50 mL warm water to distilling flask. Continue distillation until a total of 500 mL has been collected.

c. One distillation should purify the sample adequately. Occasionally, however, the distillate is turbid. If so, acidify with

* Corning No. 3360 or equivalent.

H_3PO_4 solution and distill as described in ¶ 4*b*. If second distillate is still turbid, use extraction process described in ¶ 4*d* before distilling sample.

d. Treatment when second distillate is turbid: Extract a 500-mL portion of original sample as follows: Add 4 drops methyl orange indicator and make acidic to methyl orange with 1*N* H_2SO_4. Transfer to a separatory funnel and add 150 g NaCl. Shake with five successive portions of $CHCl_3$, using 40 mL in the first portion and 25 mL in each successive portion. Transfer $CHCl_3$ layer to a second separatory funnel and shake with three successive portions of 2.5*N* NaOH solution, using 4.0 mL in the first portion and 3.0 mL in each of the next two portions. Combine alkaline extracts, heat on a water bath until $CHCl_3$ has been removed, cool, and dilute to 500 mL with distilled water. Proceed with distillation as described in ¶s 4*a* and *b*.

NOTE: CH_2Cl_2 may be used instead of $CHCl_3$, especially if an emulsion forms when the $CHCl_3$ solution is extracted with NaOH.

5530 C. Chloroform Extraction Method

1. General Discussion

a. Principle: Steam-distillable phenols react with 4-aminoantipyrine at pH 7.9 ± 0.1 in the presence of potassium ferricyanide to form a colored antipyrine dye. This dye is extracted from aqueous solution with $CHCl_3$ and the absorbance is measured at 460 nm. This method covers the phenol concentration range from 1.0 μg/L to over 250 μg/L with a sensitivity of 1 μg/L.

b. Interference: All interferences are eliminated or reduced to a minimum if the sample is preserved, stored, and distilled in accordance with the foregoing instructions.

c. Minimum detectable quantity: The minimum detectable quantity for clean samples containing no interferences is 0.5 μg phenol when a 25-mL $CHCl_3$ extraction with a 5-cm cell or a 50-mL $CHCl_3$ extraction with a 10-cm cell is used in the photometric measurement. This quantity is equivalent to 1 μg phenol/L in 500 mL distillate.

2. Apparatus

a. Photometric equipment: A spectrophotometer for use at 460 nm equipped with absorption cells providing light paths of 1 to 10 cm, depending on the absorbances of the colored solutions and the individual characteristics of the photometer.

b. Filter funnels: Buchner type with fritted disk.*

c. Filter paper: Alternatively use an appropriate 11-cm filter paper for filtering $CHCl_3$ extracts instead of the Buchner-type funnels and anhydrous Na_2SO_4.

d. pH meter.

e. Separatory funnels, 1000-mL, Squibb form, with ground-glass stoppers and TFE stopcocks. At least eight are required.

3. Reagents

Prepare all reagents with distilled water free of phenols and chlorine.

a. Stock phenol solution: Dissolve 100 mg phenol in freshly boiled and cooled distilled water and dilute to 100 mL. CAUTION—*Toxic; handle with extreme care.* Ordinarily this direct weighing yields a standard solution; if extreme accuracy is required, standardize as follows:

1) To 100 mL water in a 500-mL glass-stoppered conical flask, add 50.0 mL stock phenol solution and 10.0 mL bromate-bromide solution. Immediately add 5 mL conc HCl and swirl gently. If brown color of free bromine does not persist, add 10.0-mL portions of bromate-bromide solution until it does. Keep flask stoppered and let stand for 10 min; then add approximately 1 g KI. Usually four 10-mL portions of bromate-bromide solution are required if the stock phenol solution contains 1000 mg phenol/L.

2) Prepare a blank in exactly the same manner, using distilled water and 10.0 mL bromate-bromide solution. Titrate blank and sample with 0.025*M* sodium thiosulfate, using starch solution indicator.

3) Calculate the concentration of phenol solution as follows:

$$\text{mg phenol/L} = 7.842\,[(A \times B) - C]$$

where:

A = mL thiosulfate for blank,
B = mL bromate-bromide solution used for sample divided by 10, and
C = mL thiosulfate used for sample.

b. Intermediate phenol solution: Dilute 1.00 mL stock phenol solution in freshly boiled and cooled distilled water to 100 mL; 1 mL = 10.0 μg phenol. Prepare daily.

c. Standard phenol solution: Dilute 50.0 mL intermediate phenol solution to 500 mL with freshly boiled and cooled distilled water; 1 mL = 1.0 μg phenol. Prepare within 2 h of use.

d. Bromate-bromide solution: Dissolve 2.784 g anhydrous $KBrO_3$ in water, add 10 g KBr crystals, dissolve, and dilute to 1000 mL.

e. Hydrochloric acid, HCl, conc.

f. Standard sodium thiosulfate titrant, 0.025*M*: See Section 4500-O.C.2*e*.

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

h. Ammonium hydroxide, NH_4OH, 0.5*N*: Dilute 35 mL fresh, conc NH_4OH to 1 L with water.

i. Phosphate buffer solution: Dissolve 104.5 g K_2HPO_4 and 72.3 g KH_2PO_4 in water and dilute to 1 L. The pH should be 6.8.

j. 4-Aminoantipyrine solution: Dissolve 2.0 g 4-aminoantipyrine in water and dilute to 100 mL. Prepare daily.

k. Potassium ferricyanide solution: Dissolve 8.0 g $K_3Fe(CN)_6$ in water and dilute to 100 mL. Filter if necessary. Store in a brown glass bottle. Prepare fresh weekly.

* 15-mL Corning No. 36060 or equivalent.

l. Chloroform, $CHCl_3$.

m. Sodium sulfate, anhydrous Na_2SO_4, granular.

n. Potassium iodide, KI, crystals.

4. Procedure

Ordinarily, use Procedure *a;* however, Procedure *b* may be used for infrequent analyses.

a. Place 500 mL distillate, or a suitable portion containing not more than 50 μg phenol, diluted to 500 mL, in a 1-L beaker. Prepare a 500-mL distilled water blank and a series of 500-mL phenol standards containing 5, 10, 20, 30, 40, and 50 μg phenol.

Treat sample, blank, and standards as follows: Add 12.0 mL 0.5*N* NH_4OH and *immediately* adjust pH to 7.9 $\pm$ 0.1 with phosphate buffer. Under some circumstances, a higher pH may be required.† About 10 mL phosphate buffer are required. Transfer to a 1-L separatory funnel, add 3.0 mL aminoantipyrine solution, mix well, add 3.0 mL $K_3Fe(CN)_6$ solution, mix well, and let color develop for 15 min. The solution should be clear and light yellow.

Extract immediately with $CHCl_3$, using 25 mL for 1- to 5-cm cells and 50 mL for a 10-cm cell. Shake separatory funnel at least 10 times, let $CHCl_3$ settle, shake again 10 times, and let $CHCl_3$ settle again. Filter each $CHCl_3$ extract through filter paper or fritted glass funnels containing a 5-g layer of anhydrous Na_2SO_4. Collect dried extracts in clean cells for absorbance measurements; do not add more $CHCl_3$ or wash filter papers or funnels with $CHCl_3$.

Read absorbance of sample and standards against the blank at 460 nm. Plot absorbance against micrograms phenol concentration. Construct a separate calibration curve for each photometer and check each curve periodically to insure reproducibility.

b. For infrequent analyses prepare only one standard phenol solution. Prepare 500 mL standard phenol solution of a strength approximately equal to the phenolic content of that portion of original sample used for final analysis. Also prepare a 500-mL distilled water blank.

Continue as described in ¶ *a*, above, but measure absorbances of sample and standard phenol solution against the blank at 460 nm.

5. Calculation

a. For Procedure *a:*

$$\mu\text{g phenol/L} = \frac{A}{B} \times 1000$$

where:

A = μg phenol in sample, from calibration curve, and
B = mL original sample.

b. For Procedure *b,* calculate the phenol content of the original sample:

$$\mu\text{g phenol/L} = \frac{C \times D \times 1000}{E \times B}$$

where:

C = μg standard phenol solution,
D = absorbance reading of sample,
E = absorbance of standard phenol solution, and
B = mL original sample.

6. Precision and Bias

Because the "phenol" value is based on C_6H_5OH, this method yields only an approximation and represents the minimum amount of phenols present. This is true because the phenolic reactivity to 4-aminoantipyrine varies with the types of phenols present.

In a study of 40 refinery wastewaters analyzed in duplicate at concentrations from 0.02 to 6.4 mg/L the average relative standard deviation was $\pm$ 12%. Data are not available for precision at lower concentrations.

7. Bibliography

SCOTT, R.D. 1931. Application of a bromine method in the determination of phenols and cresols. *Ind. Eng. Chem.*, Anal. Ed. 3:67.

EMERSON, E., H.H. BEACHAM & L.C. BEEGLE. 1943. The condensation of aminoantipyrine. II. A new color test for phenolic compounds. *J. Org. Chem.* 8:417.

ETTINGER, M.B. & R.C. KRONER. 1949. The determination of phenolic materials in industrial wastes. *Proc. 5th Ind. Waste Conf.*, Purdue Univ., p. 345.

ETTINGER, M.B., C.C. RUCHHOFT & R.J. LISHKA. 1951. Sensitive 4-aminoantipyrine method for phenolic compounds. *Anal. Chem.* 23: 1783.

DANNIS, M. 1951. Determination of phenols by the aminoantipyrine method. *Sewage Ind. Wastes* 23:1516.

MOHLER, E.F., JR. & L.N. JACOB. 1957. Determination of phenolic-type compounds in water and industrial waste waters: Comparison of analytical methods. *Anal. Chem.* 29:1369.

BURTSCHELL, R.H., A.A. ROSEN, F.M. MIDDLETON & M.B. ETTINGER. 1959. Chlorine derivatives of phenol causing taste and odor. *J. Amer. Water Works Assoc.* 51:205.

GORDON, G.E. 1960. Colorimetric determination of phenolic materials in refinery waste waters. *Anal. Chem.* 32:1325.

OCHYNSKI, F.W. 1960. The absorptiometric determination of phenol. *Analyst* 85:278.

FAUST, S.D. & O.M. ALY. 1962. The determination of 2,4-dichlorophenol in water. *J. Amer. Water Works Assoc.* 54:235.

FAUST, S.D. & E.W. MIKULEWICZ. 1967. Factors influencing the condensation of 4-aminoantipyrine with derivatives of hydroxybenzene. II. Influence of hydronium ion concentration on absorptivity. *Water Res.* 1:509.

FAUST, S.D. & P.W. ANDERSON. 1968. Factors influencing the condensation of 4-aminoantipyrine with derivations of hydroxy benzene. III. A study of phenol content in surface waters. *Water Res.* 2:515.

SMITH, L.S. 1976. Evaluation of Instrument for the (Ultraviolet) Determination of Phenol in Water. EPA-600/4-76-048, U.S. Environmental Protection Agency, Cincinnati, Ohio.

† For NPDES permit analyses, pH 10 $\pm$ 0.1 is required.

5530 D. Direct Photometric Method

1. General Discussion

a. Principle: Steam-distillable phenolic compounds react with 4-aminoantipyrine at pH 7.9 ± 0.1 in the presence of potassium ferricyanide to form a colored antipyrine dye. This dye is kept in aqueous solution and the absorbance is measured at 500 nm.

b. Interference: Interferences are eliminated or reduced to a minimum by using the distillate from the preliminary distillation procedure.

c. Minimum detectable quantity: This method has less sensitivity than Method C. The minimum detectable quantity is 10 μg phenol when a 5-cm cell and 100 mL distillate are used.

2. Apparatus

a. Photometric equipment: Spectrophotometer equipped with absorption cells providing light paths of 1 to 5 cm for use at 500 nm.

b. pH meter.

3. Reagents

See Section 5530C.3.

4. Procedure

Place 100 mL distillate, or a portion containing not more than 0.5 mg phenol diluted to 100 mL, in a 250-mL beaker. Prepare a 100-mL distilled water blank and a series of 100-mL phenol standards containing 0.1, 0.2, 0.3, 0.4, and 0.5 mg phenol. Treat sample, blank, and standards as follows: Add 2.5 mL 0.5*N* NH_4OH solution and immediately adjust to pH 7.9 ± 0.1 with phosphate buffer. Add 1.0 mL 4-aminoantipyrine solution, mix well, add 1.0 mL $K_3Fe(CN)_6$ solution, and mix well.

After 15 min, transfer to cells and read absorbance of sample and standards against the blank at 500 nm.

5. Calculation

a. Use of calibration curve: Estimate sample phenol content from photometric readings by using a calibration curve constructed as directed in Section 5530C.4*a*.

$$\text{mg phenol/L} = \frac{A}{B} \times 1000$$

where:
A = mg phenol in sample, from calibration curve, and
B = mL original sample.

b. Use of single phenol standard:

$$\text{mg phenol/L} = \frac{C \times D \times 1000}{E \times B}$$

where:
C = mg standard phenol solution,
D = absorbance of sample, and
E = absorbance of standard phenol solution.

6. Precision and Bias

Precision and bias data are not available.

5540 SURFACTANTS*

5540 A. Introduction

1. Occurrence and Significance

Surfactants enter waters and wastewaters mainly by discharge of aqueous wastes from household and industrial laundering and other cleansing operations. A surfactant combines in a single molecule a strongly hydrophobic group with a strongly hydrophilic one. Such molecules tend to congregate at the interfaces between the aqueous medium and the other phases of the system such as air, oily liquids, and particles, thus imparting properties such as foaming, emulsification, and particle suspension.

The surfactant hydrophobic group generally is a hydrocarbon radical (R) containing about 10 to 20 carbon atoms. The hydrophilic groups are of two types, those that ionize in water and those that do not. Ionic surfactants are subdivided into two categories, differentiated by the charge. An anionic surfactant ion is negatively charged, e.g., $(RSO_3)^-Na^+$, and a cationic one is positively charged, e.g., $(RMe_3N)^+Cl^-$. Nonionizing (nonionic) surfactants commonly contain a polyoxyethylene hydrophilic group ($ROCH_2CH_2OCH_2CH_2......OCH_2CH_2OH$, often abbreviated RE_n, where n is the average number of $-OCH_2CH_2-$ units in the hydrophilic group). Hybrids of these types exist also.

In the United States, ionic surfactants amount to about two thirds of the total surfactants used and nonionics to about one third. Cationic surfactants amount to less than one tenth of the

*Approved by Standard Methods Committee, 2000.

ionics and are used generally for disinfecting, fabric softening, and various cosmetic purposes rather than for their detersive properties. At current detergent and water usage levels the surfactant content of raw domestic wastewater is in the range of about 1 to 20 mg/L. Most domestic wastewater surfactants are dissolved in equilibrium with proportional amounts adsorbed on particulates. Primary sludge concentrations range from 1 to 20 mg adsorbed anionic surfactant per gram dry weight.[1] In environmental waters the surfactant concentration generally is below 0.1 mg/L except in the vicinity of an outfall or other point source of entry.[2]

2. Analytical Precautions

Because of inherent properties of surfactants, special analytical precautions are necessary. Avoid foam formation because the surfactant concentration is higher in the foam phase than in the associated bulk aqueous phase and the latter may be significantly depleted. If foam is formed, let it subside by standing, or collapse it by other appropriate means, and remix the liquid phase before sampling. Adsorption of surfactant from aqueous solutions onto the walls of containers, when concentrations below about 1 mg/L are present, may seriously deplete the bulk aqueous phase. Minimize adsorption errors, if necessary, by rinsing container with sample, and for anionic surfactants by adding alkali phosphate (e.g., 0.03*N* KH_2PO_4).[3]

3. References

1. SWISHER, R.D. 1987. Surfactant Biodegradation, 2nd ed. Marcel Dekker, New York, N.Y.
2. ARTHUR D. LITTLE, INC. 1977. Human Safety and Environmental Aspects of Major Surfactants. Rep. No. PB-301193, National Technical Information Serv., Springfield, Va.
3. WEBER, W.J., JR., J.C. MORRIS & W. STUMM. 1962. Determination of alkylbenzenesulfonate by ultraviolet spectrophotometry. *Anal. Chem.* 34:1844.

5540 B. Surfactant Separation by Sublation

1. General Discussion

a. Principle: The sublation process isolates the surfactant, regardless of type, from dilute aqueous solution, and yields a dried residue relatively free of nonsurfactant substances. It is accomplished by bubbling a stream of nitrogen up through a column containing the sample and an overlying layer of ethyl acetate. The surfactant is adsorbed at the water-gas interfaces of the bubbles and is carried into the ethyl acetate layer. The bubbles escape into the atmosphere leaving behind the surfactant dissolved in ethyl acetate. The solvent is separated, dehydrated, and evaporated, leaving the surfactant as a residue suitable for analysis. This procedure is the same as that used by the Organization for Economic Co-operation and Development (OECD),[1] following the development by Wickbold.[2,3]

b. Interferences: The sublation method is specific for surfactants, because any substance preferentially adsorbed at the water-gas interface is by definition a surfactant. Although nonsurfactant substances largely are rejected in this separation process, some amounts will be carried over mechanically into the ethyl acetate.

c. Limitations: The sublation process separates only dissolved surfactants. If particulate matter is present it holds back an equilibrium amount of adsorbed surfactant. As sublation removes the initially dissolved surfactant, the particulates tend to reequilibrate and their adsorbed surfactants redissolve. Thus, continued sublation eventually should remove substantially all adsorbed surfactant. However, if the particulates adsorb the surfactant tightly, as sewage particulates usually do, complete removal may take a very long time. The procedure given herein calls for preliminary filtration and measures only dissolved surfactant. Determine adsorbed surfactant content by analyzing particulates removed by filtration; no standard method is available now.

d. Operating conditions: Make successive 5-min sublations from 1 L of sample containing 5 g $NaHCO_3$ and 100 g NaCl. Under the conditions specified, extensive transfer of surfactant occurs in the first sublation and is substantially complete in the second.[2–4]

e. Quantitation: Quantitate the surfactant residue by the procedures in 5540C or D. Direct weighing of the residue is not useful because the weight of surfactant isolated generally is too low, less than a milligram, and varied amounts of mechanically entrained nonsurfactants may be present. The procedure is applicable to water and wastewater samples.

2. Apparatus

a. Sublator: A glass column with dimensions as shown in Figure 5540:1.* For the sintered glass disk use a coarse-porosity frit (designation "c"–nominal maximum pore diam 40 to 60 μm as measured by ASTM E-128) of the same diameter as the column internal diameter. Volume between disk and upper stopcock should be approximately 1 L.

b. Gas washing bottle, as indicated in Figure 5540:1, working volume 100 mL or more.

c. Separatory funnel, working volume 250 mL, preferably with inert TFE stopcock.

d. Filtration equipment, suitable for 1-L samples, using medium-porosity qualitative-grade filter paper.

e. Gas flowmeter, for measuring flows up to 1 L/min.

3. Reagents

a. Nitrogen, standard commercial grade.

b. Ethyl acetate: CAUTION: *Ethyl acetate is flammable and its vapors can form explosive mixtures with air.*

c. Sodium bicarbonate, $NaHCO_3$.

d. Sodium chloride, NaCl.

e. Water, surfactant-free.

*This apparatus is available from Kontes at kontes.com; Cal Glass, Costa Mesa, CA; or similar suppliers.

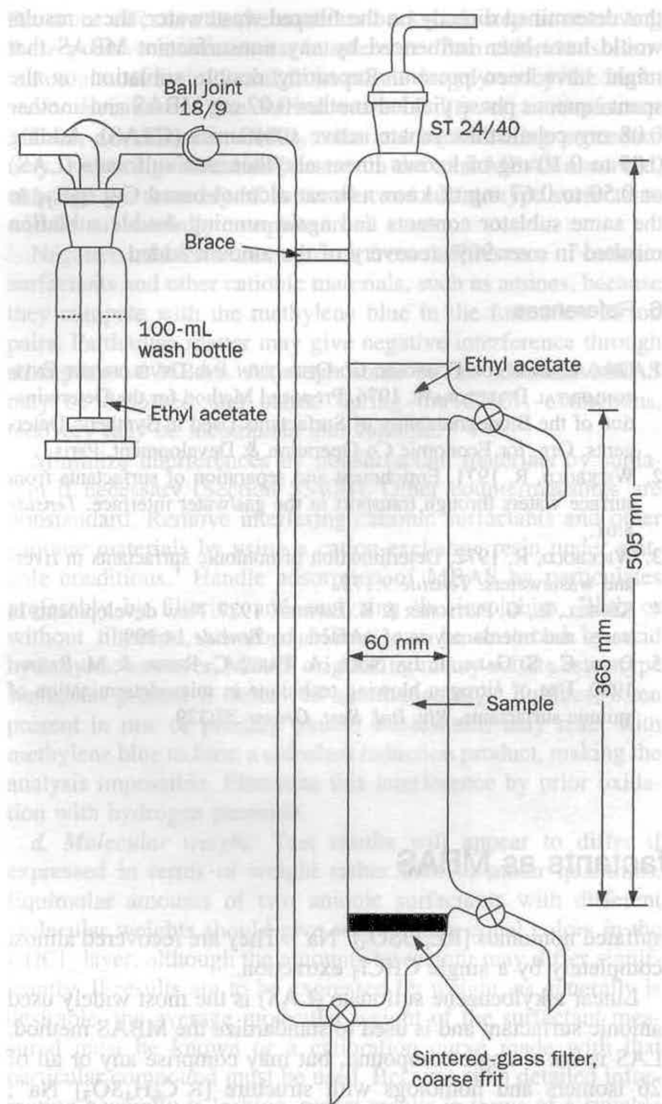

Figure 5540:1. Sublation apparatus.[1] See Section 5540B.2*a* and *b* and 4*c*. Bottom stopcock: TFE plug, 4-mm bore; side stopcocks: TFE plug, 2-mm bore.

4. Procedure

a. Sample size: Select a sample to contain not more than 1 to 2 mg surfactant.[4] For most waters the sample volume will be about 1 L; for wastewater use a smaller volume.

b. Filtration: Filter sample through medium-porosity qualitative filter paper. Wash filter paper by discarding the first few hundred milliliters of filtrate.

c. Assembly: Refer to Figure 5540:1.

Connect nitrogen cylinder through flowmeter to inlet of gas washing bottle. Connect gas outlet at top of sublator to a gas scrubber or other means for disposing of ethyl acetate vapor (e.g., vent to a hood or directly outdoors). In the absence of a flowmeter, ensure proper gas flow rate by measuring volume of gas leaving the sublator, with a water-displacement system.

d. Charging: Fill gas washing bottle about two-thirds full with ethyl acetate. Rinse sublation column with ethyl acetate and discard rinse. Place measured filtered sample in sublator and add 5 g $NaHCO_3$, 100 g NaCl, and sufficient water to bring the level up to or slightly above the upper stopcock (about 1 L total volume). If sample volume permits, add salts as a solution in 400 mL water or dissolve them in the sample and quantitatively transfer to the sublator. Add 100 mL ethyl acetate by running it carefully down the wall of the sublator to form a layer on top of the sample.

e. Sublation: Start the nitrogen flow, increasing the rate carefully to 1 L/min initially but do not exceed a rate at which the liquid phases begin vigorous intermixing at their interface. Avoid overly vigorous intermixing, which will lead to back-extraction of the surfactant into the aqueous phase and to dissolution of ethyl acetate. Continue sublation for 5 min at 1 L/min. If a lower flow rate is necessary to avoid phase intermixing, prolong sublation time proportionally. If the volume of the upper phase has decreased by more than about 20%, repeat the operation on a new sample but avoid excessive intermixing at the interface. Draw off entire ethyl acetate layer through upper stopcock into the separatory funnel; return any transferred water layer to the sublator. Filter ethyl acetate layer into a 250-mL beaker through a dry, medium-porosity, qualitative filter paper (prewashed with ethyl acetate to remove any adventitious surfactant) to remove any remaining aqueous phase.

Repeat process of preceding paragraph with a second 100-mL layer of ethyl acetate, using the same separatory funnel and filter, and finally rinse sublator wall with another 20 mL, all into the original beaker.

Evaporate ethyl acetate from the beaker on a steam bath in a hood, blowing a gentle stream of nitrogen or air over the liquid surface to speed evaporation and to minimize active boiling. Evaporate the first 100 mL during the second sublation to avoid overfilling the beaker. To avoid possible solute volatilization, discontinue heating after removing the ethyl acetate. The sublated surfactant remains in the beaker as a film of residue.

Draw off aqueous layer in the sublator and discard, using the stopcock just above the sintered disk to minimize disk fouling.

5. Precision and Bias

Estimates of the efficiency of surfactant transfer and recovery in the sublation process include the uncertainties of the analytical methods used in quantitating the surfactant. At present the analytical methods are semiquantitative for surfactant at levels below 1 mg/L in environmental samples.

With various known surfactants at 0.2 to 2 mg/L and appropriate analytical methods, over 90% of added surfactant was recovered in one 5-min sublation from 10% NaCl. Without NaCl, recovery of nonionics was over 90% but recovery of anionics and cationics was only 2 to 25%.[4]

Five laboratories studied the recovery of five anionic surfactant types from concentrations of 0.05, 0.2, 1.0, and 5.0 mg/L in aqueous solutions.[5] The amount in each solution was determined directly by methylene blue analysis and compared with the amount recovered in the sublation process, also analyzed by methylene blue. The overall average recovery was 95.9% with a standard deviation of $\pm$ 7.4 ($n = 100$). The extreme individual values for recovery were 65% and 115% and the other 98 values ranged from 75% to 109%. Recovery did not depend on surfactant concentration (average recoveries ranging from 94.7% at 5.0

TABLE 5540:I. SURFACTANT RECOVERY BY SUBLATION

Variable	MBAS	CTAS
Sample volume, mL	200–300	500
Concentration without sublation, mg/L	2.2–4.7	—
Concentration found in sublate,* mg/L	1.8–4.4	0.3–0.6
Recovery in sublate, %	87 ± 16†	—
Amount in second sublate,‡ mg	0.02 ± 0.02†	0.08 ± 0.01†
Amount added, mg	0.05–0.10§	0.50–0.67‖
Recovery in sublation,# %	94 ± 17†	92 ± 6†

* Two 5-min sublations.
† Average ± SD (n = 8).
‡ Two more 5-min sublations.
§ Reference LAS.
‖ Linear alcohol ethoxylate $C_{12-18}E_{11}$.
Fifth and sixth 5-min sublations.

mg/L to 96.8% at 1.0 mg/L) nor on the surfactant type (average recoveries ranging from 94.7% to 96.6%). Average recoveries at the five laboratories ranged from 90.0% to 98.0%.

Application of the sublation method in three laboratories to eight different samples of raw wastewater in duplicate gave the results shown in Table 5540:I. Methylene blue active substances (MBAS) recovery in double sublation averaged 87 ± 16% of that determined directly on the filtered wastewater; these results would have been influenced by any nonsurfactant MBAS that might have been present. Repeating double sublation on the spent aqueous phase yielded another 0.02 mg MBAS and another 0.08 mg cobalt thiocyanate active substances (CTAS). Adding 0.05 to 0.10 mg of known linear alkylbenzene sulfonate (LAS) or 0.50 to 0.67 mg of known linear alcohol-based $C_{12-18}E_{11}$ to the same sublator contents and again running double sublation resulted in over 90% recovery of the amount added.

6. References

1. ORGANIZATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT ENVIRONMENTAL DIRECTORATE. 1976. Proposed Method for the Determination of the Biodegradability of Surfactants Used in Synthetic Detergents. Org. for Economic Co-Operation & Development, Paris.
2. WICKBOLD, R. 1971. Enrichment and separation of surfactants from surface waters through transport in the gas/water interface. *Tenside* 8:61.
3. WICKBOLD, R. 1972. Determination of nonionic surfactants in river- and wastewaters. *Tenside* 9:173.
4. KUNKEL, E., G. PEITSCHER & K. ESPETER. 1977. New developments in trace- and microanalysis of surfactants. *Tenside* 14:199.
5. DIVO, C., S. GAFA, T. LA NOCE, A. PARIS, C. RUFFO & M. SANNA. 1980. Use of nitrogen blowing technique in microdetermination of anionic surfactants. *Riv. Ital. Sost. Grasse* 57:329.

5540 C. Anionic Surfactants as MBAS

1. General Discussion

a. Definition and principle: Methylene blue active substances (MBAS) bring about the transfer of methylene blue, a cationic dye, from an aqueous solution into an immiscible organic liquid upon equilibration. This occurs through ion pair formation by the MBAS anion and the methylene blue cation. The intensity of the resulting blue color in the organic phase is a measure of MBAS. Anionic surfactants are among the most prominent of many substances, natural and synthetic, showing methylene blue activity. The MBAS method is useful for estimating the anionic surfactant content of waters and wastewaters, but the possible presence of other types of MBAS always must be kept in mind.

This method is relatively simple and precise. It comprises three successive extractions from acid aqueous medium containing excess methylene blue into chloroform ($CHCl_3$), followed by an aqueous backwash and measurement of the blue color in the $CHCl_3$ by spectrophotometry at 652 nm. The method is applicable at MBAS concentrations down to about 0.025 mg/L.

b. Anionic surfactant responses: Soaps do not respond in the MBAS method. Those used in or as detergents are alkali salts of C_{10-20} fatty acids $[RCO_2]^- Na^+$, and though anionic in nature they are so weakly ionized that an extractable ion pair is not formed under the conditions of the test. Nonsoap anionic surfactants commonly used in detergent formulations are strongly responsive. These include principally surfactants of the sulfonate type $[RSO_3]^- Na^+$, the sulfate ester type $[ROSO_3]^- Na^+$, and sulfated nonionics $[RE_nOSO_3]^- Na^+$. They are recovered almost completely by a single $CHCl_3$ extraction.

Linear alkylbenzene sulfonate (LAS) is the most widely used anionic surfactant and is used to standardize the MBAS method. LAS is not a single compound, but may comprise any or all of 26 isomers and homologs with structure $[R'C_6H_4SO_3]^- Na^+$, where R′ is a linear secondary alkyl group ranging from 10 to 14 carbon atoms in length. The manufacturing process defines the mixture, which may be modified further by the wastewater treatment process.

Sulfonate- and sulfate-type surfactants respond together in MBAS analysis, but they can be differentiated by other means. The sulfate type decomposes upon acid hydrolysis; the resulting decrease in MBAS corresponds to the original sulfate surfactant content while the MBAS remaining corresponds to the sulfonate surfactants. Alkylbenzene sulfonate can be identified and quantified by infrared spectrometry after purification.[1] LAS can be distinguished from other alkylbenzene sulfonate surfactants by infrared methods.[2] LAS can be identified unequivocally and its detailed isomer-homolog composition determined by desulfonation-gas chromatography.[3]

c. Interferences: Positive interferences result from all other MBAS species present; if a direct determination of any individual MBAS species, such as LAS, is sought, all others interfere. Substances such as organic sulfonates, sulfates, carboxylates and phenols, and inorganic thiocyanates, cyanates, nitrates, and chlorides also may transfer more or less methylene blue into the chloroform phase. The poorer the extractability of their ion pairs,

the more effective is the aqueous backwash step in removing these positive interferences; interference from chloride is eliminated almost entirely and from nitrate largely so by the backwash. Because of the varied extractability of nonsurfactant MBAS, deviations in $CHCl_3$ ratio and backwashing procedure may lead to significant differences in the total MBAS observed, although the recovery of sulfonate- and sulfate-type surfactants will be substantially complete in all cases.

Negative interferences can result from the presence of cationic surfactants and other cationic materials, such as amines, because they compete with the methylene blue in the formation of ion pairs. Particulate matter may give negative interference through adsorption of MBAS. Although some of the adsorbed MBAS may be desorbed and paired during the $CHCl_3$ extractions, recovery may be incomplete and variable.

Minimize interferences by nonsurfactant materials by sublation if necessary (Section 5540B). Other countermeasures are nonstandard. Remove interfering cationic surfactants and other cationic materials by using a cation-exchange resin under suitable conditions.[3] Handle adsorption of MBAS by particulates preferably by filtering and analyzing the insolubles. With or without filtration, adsorbed MBAS can be desorbed by acid hydrolysis; however, MBAS originating in any sulfate ester-type surfactant present is destroyed simultaneously.[1] Sulfides, often present in raw or primary treated wastewater, may react with methylene blue to form a colorless reduction product, making the analysis impossible. Eliminate this interference by prior oxidation with hydrogen peroxide.

d. Molecular weight: Test results will appear to differ if expressed in terms of weight rather than in molar quantities. Equimolar amounts of two anionic surfactants with different molecular weights should give substantially equal colors in the $CHCl_3$ layer, although the amounts by weight may differ significantly. If results are to be expressed by weight, as generally is desirable, the average molecular weight of the surfactant measured must be known or a calibration curve made with that particular compound must be used. Because such detailed information generally is lacking, report results in terms of a suitable standard calibration curve, for example "0.65 mg MBAS/L (calculated as LAS, mol wt 318)."

e. Minimum detectable quantity: About 10 μg MBAS (calculated as LAS).

f. Application: The MBAS method has been applied successfully to drinking water samples. In wastewater, industrial wastes, and sludge, numerous materials normally present can interfere seriously if direct determination of MBAS is attempted. Most nonsurfactant aqueous-phase interferences can be removed by sublation. The method is linear over an approximate range of 10 to 200 μg of MBAS standard. This may vary somewhat, depending on source of standard material.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 652 nm, providing a light path of 1 cm or longer.

2) *Filter photometer,* providing a light path of 1 cm or longer and equipped with a red color filter exhibiting maximum transmittance near 652 nm.

b. Separatory funnels: 500-mL, preferably with inert TFE stopcocks and stoppers.

3. Reagents

a. Stock LAS solution: Weigh an amount of the reference material* equal to 1.00 g LAS on a 100% active basis. Dissolve in water and dilute to 1000 mL; 1.00 mL = 1.00 mg LAS. Store in a refrigerator to minimize biodegradation. If necessary, prepare weekly.

b. Standard LAS solution: Dilute 10.00 mL stock LAS solution to 1000 mL with water; 1.00 mL = 10.0 μg LAS. Prepare daily.

c. Phenolphthalein indicator solution, alcoholic.

d. Sodium hydroxide, NaOH, 1*N*.

e. Sulfuric acid, H_2SO_4, 1*N* and 6*N*.

f. Chloroform, $CHCl_3$: CAUTION: *Chloroform is toxic and a suspected carcinogen. Take appropriate precautions against inhalation and skin exposure.*

g. Methylene blue reagent: Dissolve 100 mg methylene blue† in 100 mL water. Transfer 30 mL to a 1000-mL flask. Add 500 mL water, 41 mL 6*N* H_2SO_4, and 50 g sodium phosphate, monobasic, monohydrate, $NaH_2PO_4 \cdot H_2O$. Shake until dissolved. Dilute to 1000 mL.

h. Wash solution: Add 41 mL 6*N* H_2SO_4 to 500 mL water in a 1000-mL flask. Add 50 g $NaH_2PO_4 \cdot H_2O$ and shake until dissolved. Dilute to 1000 mL.

i. Methanol, CH_3OH. CAUTION: *Methanol vapors are flammable and toxic; take appropriate precautions.*

j. Hydrogen peroxide, H_2O_2, 30%.

k. Glass wool: Pre-extract with $CHCl_3$ to remove interferences.

l. Water, reagent-grade, MBAS-free. Use for making all reagents and dilutions.

4. Procedure

a. Preparation of calibration curve: Prepare an initial calibration curve consisting of at least five standards covering the referenced (¶ 1*f*) or desired concentration range. Provided that linearity is demonstrated over the range of interest ($r = 0.995$ or better) run daily check standards at the reporting limit and a concentration above the expected samples' concentration. Check standard results should be within 25% of original value at the reporting limit and 10% of original value for all others. Otherwise, prepare a new calibration curve.

Prepare a series of separatory funnels for a reagent blank and selected standards. Pipet portions of standard LAS solution (¶ 3*b*) into funnels. Add sufficient water to make the total volume 100 mL in each separatory funnel. Treat each standard as described in ¶s 4*d* and *e* following, and plot a calibration curve of absorbance vs. micrograms LAS taken, specifying the molecular weight of the LAS used.

b. Sample size: For direct analysis of waters and wastewaters, select sample volume on the basis of expected MBAS concentration:

* For sources of suitable reference material, contact *Standard Methods* manager, American Water Works Association, 6666 West Quincy Ave., Denver, CO 80235.
† Eastman No. P573 or equivalent.

Expected MBAS Concentration *mg/L*	Sample Taken *mL*
0.025–0.080	400
0.08 –0.40	250
0.4 –2.0	100

If expected MBAS concentration is above 2 mg/L, dilute sample containing 40 to 200 μg MBAS to 100 mL with water.

For analysis of samples purified by sublation, dissolve sublate residue (Section 5540B.4*e*) in 10 to 20 mL methanol, quantitatively transfer the entire amount (or a suitable portion if more than 200 μg MBAS is expected) to 25 to 50 mL water, evaporate without boiling until methanol is gone, adding water as necessary to avoid going to dryness, and dilute to about 100 mL with water.

c. Peroxide treatment: If necessary to avoid decolorization of methylene blue by sulfides, add a few drops of 30% H_2O_2.

d. Ion pairing and extraction:

1) Add sample to a separatory funnel. Make alkaline by dropwise addition of 1*N* NaOH, using phenolphthalein indicator. Discharge pink color by dropwise addition of 1*N* H_2SO_4.

2) Add 10 mL $CHCl_3$ and 25 mL methylene blue reagent. Rock funnel vigorously for 30 s and let phases separate. Alternatively, place a magnetic stirring bar in the separatory funnel; lay funnel on its side on a magnetic mixer and adjust speed of stirring to produce a rocking motion. Excessive agitation may cause emulsion formation. To break persistent emulsions add a small volume of isopropyl alcohol (<10 mL); add same volume of isopropyl alcohol to all standards. Some samples require a longer period of phase separation than others. Before draining $CHCl_3$ layer, swirl gently, then let settle.

3) Draw off $CHCl_3$ layer into a second separatory funnel. Rinse delivery tube of first separatory funnel with a small amount of $CHCl_3$. Repeat extraction two additional times, using 10 mL $CHCl_3$ each time. If blue color in water phase becomes faint or disappears, discard and repeat, using a smaller sample.

4) Combine all $CHCl_3$ extracts in the second separatory funnel. Add 50 mL wash solution and shake vigorously for 30 s. Emulsions do not form at this stage. Let settle, swirl, and draw off $CHCl_3$ layer through a funnel containing a plug of glass wool into a 100-mL volumetric flask; filtrate must be clear. Extract wash solution twice with 10 mL $CHCl_3$ each and add to flask through the glass wool. Rinse glass wool and funnel with $CHCl_3$. Collect washings in volumetric flask, dilute to mark with $CHCl_3$, and mix well.

e. Measurement: Determine absorbance at 652 nm against a blank of $CHCl_3$.

5. Calculation

From the calibration curve (¶ 4*a*) read micrograms of apparent LAS (mol wt ___) corresponding to the measured absorbance.

$$\text{mg MBAS/L} = \frac{\mu\text{g apparent LAS}}{\text{mL original sample}}$$

Report as "MBAS, calculated as LAS, mol wt ___."

6. Precision and Bias

A synthetic sample containing 270 μg LAS/L in distilled water was analyzed in 110 laboratories with a relative standard deviation of 14.8% and a relative error of 10.6%.

A tap water sample to which was added 480 μg LAS/L was analyzed in 110 laboratories with a relative standard deviation of 9.9% and a relative error of 1.3%.

A river water sample with 2.94 mg LAS/L added was analyzed in 110 laboratories with a relative standard deviation of 9.1% and a relative error of 1.4%.[4]

7. References

1. American Public Health Association, American Water Works Association & Water Pollution Control Federation. 1981. Carbon adsorption-infrared method. *In* Standard Methods for the Examination of Water and Wastewater, 15th ed. American Public Health Assoc., Washington, D.C.
2. Ogden, C.P., H.L. Webster & J. Halliday. 1961. Determination of biologically soft and hard alkylbenzenesulfonates in detergents and sewage. *Analyst* 86:22.
3. Osburn, Q.W. 1986. Analytical methodology for LAS in waters and wastes. *J. Amer. Oil Chem. Soc.* 63:257.
4. Lishka, R.J. & J.H. Parker. 1968. Water Surfactant No. 3, Study No. 32. U.S. Public Health Serv. Publ. No. 999-UIH-11, Cincinnati, Ohio.

8. Bibliography

Barr, T., J. Oliver & W.V. Stubbings. 1948. The determination of surface-active agents in solution. *J. Soc. Chem. Ind.* (London) 67:45.

Epton, S.R. 1948. New method for the rapid titrimetric analysis of sodium alkyl sulfates and related compounds. *Trans. Faraday Soc.* 44:226.

Evans, H.C. 1950. Determination of anionic synthetic detergents in sewage. *J. Soc. Chem. Ind.* (London) 69:Suppl. 2, S76.

Degens, P.N., Jr., H.C. Evans, J.D. Kommer & P.A. Winsor. 1953. Determination of sulfate and sulfonate anion-active detergents in sewage. *J. Appl. Chem.* (London) 3:54.

American Water Works Association. 1954. Task group report. Characteristics and effects of synthetic detergents. *J. Amer. Water Works Assoc.* 46:751.

Edwards, G.P. & M.E. Ginn. 1954. Determination of synthetic detergents in sewage. *Sewage Ind. Wastes* 26:945.

Longwell, J. & W.D. Maniece. 1955. Determination of anionic detergents in sewage, sewage effluents, and river water. *Analyst* 80:167.

Moore, W.A. & R.A. Kolbeson. 1956. Determination of anionic detergents in surface waters and sewage with methyl green. *Anal. Chem.* 28:161.

Rosen, A.A., F.M. Middleton & N. Taylor. 1956. Identification of synthetic detergents in foams and surface waters. *J. Amer. Water Works Assoc.* 48:1321.

Sallee, E.M., et al. 1956. Determination of trace amounts of alkyl benzenesulfonates in water. *Anal. Chem.* 28:1822.

American Water Works Association. 1958. Task group report. Determination of synthetic detergent content of raw water supplies. *J. Amer. Water Works Assoc.* 50:1343.

McGuire, O.E., F. Kent, LaR. L. Miller & G.J. Papenmeier. 1962. Field test for analysis of anionic detergents in well waters. *J. Amer. Water Works Assoc.* 54:665.

Abbott, D.C. 1962. The determination of traces of anionic surface-active materials in water. *Analyst* 87:286.

ABBOTT, D.C. 1963. A rapid test for anionic detergents in drinking water. *Analyst* 88:240.

REID, V.W., G.F. LONGMAN & E. HEINERTH. 1967. Determination of anionic-active detergents by two-phase titration. *Tenside* 4:292.

WANG, L.K., P.J. PANZARDI, W.W. SHUSTER & D. AULENBACH. 1975. Direct two-phase titration method for analyzing anionic nonsoap surfactants in fresh and saline waters. *J. Environ. Health* 38:159.

5540 D. Nonionic Surfactants as CTAS

1. General Discussion

a. Definition and principle: Cobalt thiocyanate active substances (CTAS) are those that react with aqueous cobalt thiocyanate solution to give a cobalt-containing product extractable into an organic liquid in which it can be measured. Nonionic surfactants exhibit such activity, as may other natural and synthetic materials; thus, estimation of nonionic surfactants as CTAS is possible only if substantial freedom from interfering CTAS species can be assured.

The method requires sublation to remove nonsurfactant interferences and ion exchange to remove cationic and anionic surfactants, partition of CTAS into methylene chloride from excess aqueous cobalt thiocyanate by a single extraction, and measurement of CTAS in the methylene chloride by spectrophotometry at 620 nm. Lower limit of detectability is around 0.1 mg CTAS, calculated as $C_{12-18}E_{11}$. Beyond the sublation step the procedure is substantially identical to that of the Soap and Detergent Association (SDA).[1]

b. Nonionic surfactant responses: For pure individual molecular species the CTAS response is negligible up to about RE_5, where it increases sharply and continues to increase more gradually for longer polyether chains.[2,3] Fewer than about six oxygens in the molecule do not supply enough cumulative coordinate bond strength to hold the complex together. Commercial nonionic surfactants generally range from about RE_7 to RE_{15}; however, each such product, because of synthesis process constraints, is actually a mixture of many individual species ranging from perhaps RE_0 to RE_{2n} in a Poisson distribution averaging RE_n.

The hydrophobes used for nonionic surfactants in the U.S. household detergent industry are mainly linear primary and linear secondary alcohols with chain lengths ranging from about 12 to about 18 carbon atoms. Nonionics used in industrial operations include some based on branched octyl- and nonylphenols. These products give strong CTAS responses that may differ from each other, on a weight basis, by as much as a factor of 2. Specifically, eight such products showed responses from 0.20 to 0.36 absorbance units/mg by the SDA procedure.[1]

As with anionic surfactants measured as MBAS, the nonionic surfactants found in water and wastewater might have CTAS responses at least as varied as their commercial precursors because the proportions of the individual molecular species will have been changed by biochemical and physicochemical removal at varied rates, and further because their original molecular structures may have been changed by biodegradation processes.

c. Reference nonionic surfactant: Until it is practical to determine the nature and molecular composition of an unknown mixed CTAS, and to calculate or determine the CTAS responses of its component species, exact quantitation of uncharacterized CTAS in a sample in terms of weight is not possible. Instead, express the analytical result in terms of some arbitrarily chosen reference nonionic surfactant, i.e., as the weight of the reference that gives the same amount of CTAS response. The reference is the nonionic surfactant $C_{12-18}E_{11}$, derived from a mixture of linear primary alcohols ranging from 12 to 18 carbon atoms in chain length by reaction with ethylene oxide in a molar ratio of 1:11. $C_{12-18}E_{11}$ is reasonably representative of nonionic surfactants in commercial use; its CTAS response is about 0.21 absorbance units/mg.

If the identity of the nonionic surfactant in the sample is known, use that same material in preparing the calibration curve.

d. Interferences: Both anionic and cationic surfactants may show positive CTAS response[1,4] but both are removed in the ion-exchange step. Sublation removes nonsurfactant interferences. Physical interferences occur if some of the CTAS is adsorbed on particulate matter. Avoid such interference by filtering out the particulates for the sublation step; this will measure only dissolved CTAS.

e. Minimum detectable quantity: About 0.1 mg CTAS, calculated as $C_{12-18}E_{11}$, which corresponds to 0.1 mg/L in a 1-L sample.

f. Application: The method is suitable for determining dissolved nonionic surfactants of the ethoxylate type in most aqueous systems.

2. Apparatus

a. Sublation apparatus: See Section 5540B.2.

b. Ion-exchange column, glass, about 1- × 30-cm. Slurry anion-exchange resin in methanol and pour into column to give a bed about 10 cm deep. Insert plug of glass wool and then add a 10-cm bed of cation-exchange resin on top in the same manner. One column may be used for treating up to six sublated samples before repacking.

c. Spectrophotometer and 2.0-cm stoppered cells, suitable for measuring absorbance at 620 nm.

d. Separatory funnels, 125-mL, preferably with TFE stopcock and stopper.

e. Extraction flasks, Soxhlet type, 150-mL.

3. Reagents

a. Sublation reagents: See Section 5540B.3.

b. Anion-exchange resin, polystyrene-quaternary ammonium-type,* 50- to 100-mesh, hydroxide form. To convert chloride form to hydroxide, elute with 20 bed volumes of 1*N* NaOH and wash with methanol until free alkali is displaced.

c. Cation-exchange resin, polystyrene-sulfonate type,† 50- to 100-mesh, hydrogen form.

d. Cobaltothiocyanate reagent: Dissolve 30 g $Co(NO_3)_2 \cdot 6H_2O$ and 200 g NH_4SCN in water and dilute to 1 L. This reagent is stable for at least 1 month at room temperature.

e. Reference nonionic surfactant, $C_{12-18}E_{11}$: Reaction product of C_{12-18} linear primary alcohol with ethylene oxide in 1:11 molar ratio.‡

f. Reference nonionic surfactant stock solution, methanolic, approximately 2 mg nonionic/mL methanol: Quantitatively transfer entire contents (approximately 1 g nonionic) from preweighed ampule into 500-mL volumetric flask, thoroughly rinse ampule with methanol, make up to volume with methanol, and reweigh dried ampule. Calculate concentration in milligrams per milliter as in ¶ 5*a*. Because of possible phase separation, use all material in the ampule.

g. Reference nonionic surfactant standard solution, methanolic, approximately 0.1 mg nonionic/mL methanol: Dilute 10.00 mL stock solution to 200 mL with methanol. Exact concentration is 1/20 that of the stock solution.

h. Sodium hydroxide, NaOH: 1*N*.

i. Glass wool: Pre-extract with chloroform or methylene chloride.

j. Methanol, CH_3OH: CAUTION: *Methanol vapors are flammable and toxic; take appropriate precautions.*

k. Methylene chloride, CH_2Cl_2: CAUTION: *Methylene chloride vapors are toxic; take adequate precautions.*

l. Water: Use distilled or deionized, CTAS-free water for making reagents and dilutions.

4. Procedure

a. Purification by sublation: Proceed according to Section 5540B, using sample containing no more than 2 mg CTAS. (NOTE: For samples of known character containing no interfering materials, omit this step.)

b. Ion-exchange removal of anionic and cationic surfactants: Dissolve sublation residue in 5 to 10 mL methanol and transfer quantitatively to ion-exchange column. Elute with methanol at 1 drop/s into a clean, dry 150-mL extraction flask until about 125 mL is collected. Evaporate methanol on a steam bath aided by a gentle stream of clean, dry nitrogen or air, taking care to avoid loss by entrainment; remove from heat as soon as the methanol is completely evaporated. (NOTE: With samples of known character containing no anionic or cationic materials, omit step *b*.)

c. CTAS calibration curve: Into a series of 150-mL extraction flasks containing 10 to 20 mL methanol place 0.00, 5.00, 10.00, 20.00, and 30.00 mL reference nonionic surfactant standard solution and evaporate just to dryness. Continue as in ¶s 4*d* and *e*, below, and plot a calibration curve of absorbance against milligrams of reference nonionic taken, specifying its identity (e.g., $C_{12-18}E_{11}$ and lot number).

d. Cobalt complexing and extraction: Charge a 125-mL separatory funnel with 5 mL cobaltothiocyanate reagent. With precautions against excessive and variable evaporation of the methylene chloride, dissolve residue from ion-exchange operation, ¶ 4*b*, by adding 10.00 mL methylene chloride and swirling for a few seconds. Immediately transfer by pouring into the separatory funnel. *Do not rinse flask.* (NOTE: Because of the volatility of methylene chloride, rigidly standardize these operations with respect to handling and elapsed time; alternatively, evaporate the methanol in 200-mL erlenmeyer flasks to be stoppered with glass or TFE stoppers during dissolution. Transfer as directed here is incomplete, but in this case it will not introduce error because the loss of nonionics is exactly compensated for by the diminished volume of the organic layer in the extraction.) Shake separatory funnel vigorously for 60 s and let layers separate. Run lower layer into a 2.0-cm cell through a funnel containing a plug of pre-extracted glass wool and stopper. Be sure filtrate is absolutely clear. (NOTE: If desired, clarify by running the lower layer into a 12-mL centrifuge tube, stopper, spin at or above 1000 × *g* for 3 min, and transfer to the cell by a Pasteur pipet; use same procedure for both calibration and samples.)

e. Measurement: Determine absorbance at 620 nm against a blank of methylene chloride. (NOTE: If haze develops in the cell, warm slightly with a hot air gun or heat lamp to clarify.)

5. Calculations

a. Nonionic surfactant in reference nonionic stock solution ¶ 3*f*:

$$\text{mg nonionic/mL methanol} = \text{mg reference sample/500 mL}$$

b. Nonionic surfactant in sample: From the calibration curve read milligrams of reference nonionic corresponding to the measured absorbance:

$$\text{mg CTAS/L} = \text{mg apparent nonionic/L sample}$$

Report as "CTAS, calculated as nonionic surfactant $C_{12-18}E_{11}$."

6. Precision and Bias

Twenty-four samples of 6.22% w/v solution of reference nonionic surfactant $C_{12-18}E_{11}$ were analyzed in three laboratories by CTAS alone, without sublation or ion exchange. The overall relative standard deviation was about 3%. Results of the three laboratories individually were:

Laboratory	% w/w ± SD
A	6.08 ± 0.14 (n = 36)
B	6.56 ± 0.17 (n = 6)
C	6.25 ± 0.14 (n = 36)
Overall	6.20 ± 0.19 (n = 78)

Samples of raw wastewater were freed of surfactants by four successive sublations, then 0.50 or 0.67 mg reference nonionic surfactant $C_{12-18}E_{11}$ was added and carried through the entire sequence of sublation, ion exchange, and CTAS extraction.

* Bio-Rad, AGl-X2, or equivalent.
† Bio-Rad AG 50W-X8, or equivalent.
‡ For sources of suitable reference material, contact *Standard Methods* manager.

Recoveries averaged 92% with overall standard deviation around 6%:

Laboratory	% Recovery ± SD
A	87 ± 4 ($n = 4$)
B	97 ± 1 ($n = 4$)
Overall	92 ± 6 ($n = 8$)

The above data relate to the bias and precision of the method when applied to a known nonionic surfactant. When the nature of the nonionic surfactant is unknown, there is greater uncertainty. The response of the reference $C_{12-18}E_{11}$ is about 0.21 absorbance units/mg, while that of the eight nonionic types mentioned under ¶ 1*b* ranged from 0.20 to 0.36, and environmental nonionics might differ still more. If the nonionic surfactant in the sample has a response of 0.42, the result calculated in terms of milligrams $C_{12-18}E_{11}$ would be double the actual milligrams of the unknown nonionic.

7. References

1. SOAP AND DETERGENT ASSOCIATION ANALYTICAL SUBCOMMITTEE. 1977. Analytical methods for nonionic surfactants in laboratory biodegradation and environmental studies. *Environ. Sci. Technol.* 11:1167.
2. CRABB, N.T. & H.E. PERSINGER. 1964. The determination of polyoxyethylene nonionic surfactants in water at the parts per million level. *J. Amer. Oil Chem. Soc.* 41:752.
3. CRABB, N.T. & H.E. PERSINGER. 1968. A determination of the apparent molar absorption coefficients of the cobalt thiocyanate complexes of nonylphenol ethylene oxide adducts. *J. Amer. Oil Chem. Soc.* 45:611.
4. GREFF, R.A., E.A. SETZKORN & W.D. LESLIE. 1965. A colorimetric method for the determination of parts per million of nonionic surfactants. *J. Amer Oil Chem. Soc.* 42:180.

8. Bibliography

TABAK, H.H. & R.L. BUNCH. 1981. Measurement of non-ionic surfactants in aqueous environments. Proc. 36th Ind. Waste Conf., p. 888. Purdue Univ., Lafayette, Ind.

5550 TANNIN AND LIGNIN*

5550 A. Introduction

Lignin is a plant constituent that often is discharged as a waste during the manufacture of paper pulp. Another plant constituent, tannin, may enter the water supply through the process of vegetable matter degradation or through the wastes of the tanning industry. Tannin also is applied in the so-called internal treatment of boiler waters, where it reduces scale formation by causing the production of a more easily handled sludge.

* Approved by Standard Methods Committee, 2000.

5550 B. Colorimetric Method

1. General Discussion

a. Principle: Both lignin and tannin contain aromatic hydroxyl groups that react with Folin phenol reagent (tungstophosphoric and molybdophosphoric acids) to form a blue color suitable for estimation of concentrations up to at least 9 mg/L. However, the reaction is not specific for lignin or tannin, nor for compounds containing aromatic hydroxyl groups, inasmuch as many other reducing materials, both organic and inorganic, respond similarly.

b. Applicability: This method is generally suitable for the analysis of any organic chemical that will react with Folin phenol reagent to form measurable blue color at the concentration of interest. However, many compounds are reactive (see ¶ 1*c*) and each yields a different molar extinction coefficient (color intensity). Hence, the analyst must demonstrate conclusively the absence of interfering substances.

c. Interferences: Any substance able to reduce Folin phenol reagent will produce a false positive response. Organic chemicals known to interfere include hydroxylated aromatics, proteins, humic substances, nucleic acid bases, fructose, and amines. Inorganic substances known to interfere include iron (II), manganese (II), nitrite, cyanide, bisulfite, sulfite, sulfide, hydrazine, and hydroxylamine hydrochloride. Both 2 mg ferrous iron/L and 125 mg sodium sulfite/L individually produce a color equivalent to 1 mg tannic acid/L.

d. Minimum detectable concentrations: Approximately 0.025 mg/L for phenol and tannic acid and 0.1 mg/L for lignin with a 1-cm-path-length spectrophotometer.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 700 nm. A light path of 1 cm or longer yields satisfactory results.

b. Filter photometer, provided with a red filter exhibiting maximum transmittance in the wavelength range of 600 to 700 nm. Sensitivity improves with increasing wavelength. A light path of 1 cm or longer yields satisfactory results.

c. Nessler tubes, matched, 100-mL, tall form, marked at 50-mL volume.

3. Reagents

a. Folin phenol reagent: Transfer 100 g sodium tungstate, $Na_2WO_4 \cdot 2H_2O$, and 25 g sodium molybdate, $Na_2MoO_4 \cdot 2H_2O$, together with 700 mL distilled water, to a 2000-mL flat-bottom boiling flask. Add 50 mL 85% H_3PO_4 and 100 mL conc HCl. Connect to a reflux condenser and boil gently for 10 h. Add 150 g Li_2SO_4, 50 mL distilled water, and a few drops of liquid bromine. Boil without condenser for 15 min to remove excess bromine. Cool to 25°C, dilute to 1 L, and filter. Store finished reagent, which should have no greenish tint, in a tightly stoppered bottle to protect against reduction by air-borne dust and organic materials.

Alternatively, purchase commercially prepared Folin phenol reagent and use before the recommended expiration date.

b. Carbonate-tartrate reagent: Dissolve 200 g Na_2CO_3 and 12 g sodium tartrate, $Na_2C_4H_4O_6 \cdot 2H_2O$, in 750 mL hot distilled water, cool to 20°C, and dilute to 1 L.

c. Stock solution: The nature of the substance present in the sample dictates the choice of chemical used to prepare the standard, because each substance produces a different color intensity. Weigh 1.000 g tannic acid, tannin, lignin, or other compound being used for boiler water treatment or known to be a contaminant of the water sample. Dissolve in distilled water and dilute to 1000 mL. If the identity of the compound in the water sample is not known, use phenol and report results as "substances reducing Folin phenol reagent" in mg phenol/L. Interpret such results with caution.

Note that tannin and lignin are not individual chemical species of known molecular weight and structure; rather, they are substances containing a spectrum of chemicals of different molecular weights. Their chemical properties depend on source and method of isolation. If a particular substance is being added to the water, use it to prepare the stock solution.

d. Standard solution: Dilute 10.00 mL or 50.00 mL stock solution to 1000 mL with distilled water; 1.00 mL = 10.0 or 50.0 μg active ingredient.

4. Procedure

Bring 50-mL portions of clear sample and standards to a temperature above 20°C and maintain within a ± 2°C range. Add in rapid succession 1 mL Folin phenol reagent and 10 mL carbonate-tartrate reagent. Allow 30 min for color development. Compare visually against simultaneously prepared standards in matched Nessler tubes or make photometric readings against a reagent blank prepared at the same time. Use the following guide for instrumental measurement at a wavelength of 700 nm:

Tannic Acid in 61-mL Final Volume *μg*	Lignin in 61-mL Final Volume *μg*	Light Path *cm*
50–600	100–1500	1
10–150	30–400	5

Report results in mg/L of the compound known to be present or as "substances reducing Folin phenol reagent" in mg phenol/L.

5. Precision and Bias

In a single laboratory analyzing seven replicates for phenol at 0.1 mg/L the precision was ± 7% and recovery was 107%.

6. Bibliography

FOLIN, O. & V. CIOCALTEU. 1927. On tyrosine and tryptophane determinates in proteins. *J. Biol. Chem.* 73:627.

BERK, A.A. & W.C. SCHROEDER. 1942. Determination of tannin substances in boiler water. *Ind. Eng. Chem.,* Anal. Ed. 14:456.

KLOSTER, M.B. 1974. Determination of tannin and lignin. *J. Amer. Water Works Assoc.* 66:44.

BOX, J.D. 1983. Investigation of the Folin-Ciocalteu phenol reagent for the determination of polyphenolic substances in natural waters. *Water Res.* 17:511; discussion by S.J. Randtke & R.A. Larson. 1984. *Water Res.* 18:1597.

5560 ORGANIC AND VOLATILE ACIDS*

5560 A. Introduction

The measurement of organic acids, by adsorption and elution from a chromatographic column, by gas chromatography, or by distillation, can be used as a control test for anaerobic digestion. The chromatographic separation method is presented for organic acids (B), while a method using distillation (C) and a gas chromatographic method (D) are presented for volatile acids.

Volatile fatty acids are classified as water-soluble fatty acids that can be distilled at atmospheric pressure. These volatile acids

* Approved by Standard Methods Committee, 2001.
Joint Task Group: Theadore C. Heesen (chair), Carlos De Leon, Peter J. Morrissey.

can be removed from aqueous solutions by distillation, despite their high boiling points, because of co-distillation with water. This group includes water-soluble fatty acids with up to six carbon atoms.

The distillation method is empirical and gives incomplete and somewhat variable recovery. Factors such as heating rate and proportion of sample recovered as distillate affect the result, requiring the determination of a recovery factor for each apparatus and set of operating conditions. However, it is suitable for routine control purposes. Removing sludge solids from the sample reduces the possibility of hydrolysis of complex materials to volatile acids.

The gas chromatographic method determines individual concentrations of many of the fatty acids, giving additional information about the sample.

5560 B. Chromatographic Separation Method for Organic Acids

1. General Discussion

a. Principle: An acidified aqueous sample containing organic acids is adsorbed on a column of silicic acid and the acids are eluted with *n*-butanol in chloroform ($CHCl_3$). The eluate is collected and titrated with standard base. All short-chain (C_1 to C_6) organic acids are eluted by this solvent system and are reported collectively as total organic acids.

b. Interference: The $CHCl_3$-butanol solvent system is capable of eluting organic acids other than the volatile acids and also some synthetic detergents. Besides the so-called volatile acids, crotonic, adipic, pyruvic, phthalic, fumaric, lactic, succinic, malonic, gallic, aconitic, and oxalic acids; alkyl sulfates; and alkylaryl sulfonates are adsorbed by silicic acid and eluted.

c. Precautions: Basic alcohol solutions decrease in strength with time, particularly when exposed repeatedly to the atmosphere. These decreases usually are accompanied by the appearance of a white precipitate. The magnitude of such changes normally is not significant in process control if tests are made within a few days of standardization. To minimize this effect, store standard sodium hydroxide (NaOH) titrant in a tightly stoppered borosilicate glass bottle and protect from atmospheric carbon dioxide (CO_2) by attaching a tube of CO_2-absorbing material, as described in the inside front cover. For more precise analyses, standardize titrant or prepare before each analysis.

Although the procedure is adequate for routine analysis of most sludge samples, volatile-acids concentrations above 5000 mg/L may require an increased amount of organic solvent for quantitative recovery. Elute with a second portion of solvent and titrate to reveal possible incomplete recoveries.

2. Apparatus

a. Centrifuge or filtering assembly.

b. Crucibles, Gooch or medium-porosity fritted-glass, with filtering flask and vacuum source. Use crucibles of sufficient size (30 to 35 mL) to hold 12 g silicic acid.

c. Separatory funnel, 1000-mL.

3. Reagents

a. Silicic acid, specially prepared for chromatography, 50 to 200 mesh: Remove fines by slurrying in distilled water and decanting supernatant after settling for 15 min. Repeat several times. Dry washed acid in an oven at 103°C until *absolutely dry,* then store in a desiccator.

b. Chloroform-butanol reagent: Mix 300 mL reagent-grade $CHCl_3$, 100 mL *n*-butanol, and 80 mL 0.5*N* H_2SO_4 in a separatory funnel. Let water and organic layers separate. Drain off lower organic layer through a fluted filter paper into a dry bottle. CAUTION: *Chloroform has been classified as a cancer suspect agent. Use hood for preparation of reagent and conduct of test.*

c. Thymol blue indicator solution: Dissolve 80 mg thymol blue in 100 mL absolute methanol.

d. Phenolphthalein indicator solution: Dissolve 80 mg phenolphthalein in 100 mL absolute methanol.

e. Sulfuric acid, H_2SO_4, conc.

f. Standard sodium hydroxide, NaOH, 0.02*N:* Dilute 20 mL 1.0*N* NaOH stock solution to 1 L with absolute methanol. Prepare stock in water and standardize in accordance with the methods outlined in Section 2310B.3*d*.

4. Procedure

a. Pretreatment of sample: Centrifuge or vacuum-filter enough sludge to obtain 10 to 15 mL clear sample in a small test tube or beaker. Add a few drops of thymol blue indicator solution, then conc H_2SO_4 dropwise, until definitely red to thymol blue (pH = 1.0 to 1.2).

b. Column chromatography: Place 12 g silicic acid in a Gooch or fritted-glass crucible and apply suction to pack column. Tamp column while applying suction to reduce channeling when the sample is applied. With a pipet, distribute 5.0 mL acidified sample as uniformly as possible over column surface. Apply suction momentarily to draw sample into silicic acid. Release vacuum as soon as last portion of sample has entered column. Quickly add 65 mL $CHCl_3$-butanol reagent and apply suction. Discontinue suction just before the last of reagent enters column. Do not reuse columns.

c. Titration: Remove filter flask and purge eluted sample with N_2 gas or CO_2-free air immediately before titrating. (Obtain CO_2-free air by passing air through a CO_2 absorbant.*)

Titrate sample with standard 0.02*N* NaOH to phenolphthalein end point, using a fine-tip buret and taking care to avoid aeration. The fine-tip buret aids in improving accuracy and precision of the titration. Use N_2 gas or CO_2-free air delivered through a

* Ascarite or equivalent.

small glass tube to purge and mix sample and to prevent contact with atmospheric CO_2 during titration.

d. Blank: Carry a distilled water blank through steps ¶s 4*a* through 4*c*.

5. Calculation

$$\text{Total organic acids (mg as acetic acid/L)} = \frac{(a - b) \times N \times 60\,000}{\text{mL sample}}$$

where:

a = mL NaOH used for sample,
b = mL NaOH used for blank, and
N = normality of NaOH.

6. Precision

Average recoveries of about 95% are obtained for organic acid concentrations above 200 mg as acetic acid/L. Individual tests generally vary from the average by approximately 3%. A greater variation results when lower concentrations of organic acids are present. Titration precision expressed as the standard deviation is about ±0.1 mL (approximately ±24 mg as acetic acid/L).

7. Bibliography

MUELLER, H.F., A.M. BUSWELL & T.E. LARSON. 1956. Chromatographic determination of volatile acids. *Sewage Ind. Wastes* 28:255.

MUELLER, H.F., T.E. LARSON & M. FERRETTI. 1960. Chromatographic separation and identification of organic acids. *Anal. Chem.* 32:687.

WESTERHOLD, A.F. 1963. Organic acids in digester liquor by chromatography. *J. Water Pollut. Control Fed.* 35:1431.

HATTINGH, W.H.J. & F.V. HAYWARD. 1964. An improved chromatographic method for the determination of total volatile fatty acid content in anaerobic digester liquors. *Int. J. Air Water Pollut.* 8:411.

POHLAND, F.G. & B.H. DICKSON, JR. 1964. Organic acids by column chromatography. *Water Works Wastes Eng.* 1:54.

5560 C. Distillation Method

1. General Discussion

a. Principle: This technique recovers acids containing up to six carbon atoms. Fractional recovery of each acid increases with increasing molecular weight. Calculations and reporting are on the basis of acetic acid. The method often is applicable for control purposes. Because it is empirical, carry it out exactly as described. Because the still-heating rate, presence of sludge solids, and final distillate volume affect recovery, determine a recovery factor.

b. Interference: Hydrogen sulfide (H_2S) and CO_2 are liberated during distillation and will be titrated to give a positive error. Eliminate this error by discarding the first 15 mL of distillate and account for this in the recovery factor. Residues on glassware from some synthetic detergents have been reported to interfere; use water and dilute acid rinse cycles to prevent this problem.

2. Apparatus

a. Centrifuge, with head to carry four 50-mL tubes or 250-mL bottles.

b. Distillation flask, 500-mL capacity.

c. Condenser, about 76 cm long.

d. Adapter tube.

e. pH meter or recording titrator: See Section 2310B.2*a*.

f. Distillation assembly: Use a conventional distilling apparatus. To minimize fluctuations in distillation rate, supply heat with a variable-wattage electrical heater.

3. Reagents

a. Sulfuric acid, H_2SO_4, 1 + 1.

b. Standard sodium hydroxide titrant, 0.1*N*: See Section 2310B.3*c*.

c. Phenolphthalein indicator solution.

d. Acetic acid stock solution, 2000 mg/L: Dilute 1.9 mL conc CH_3COOH to 1000 mL with deionized water. Standardize against 0.1*N* NaOH.

4. Procedure

a. Recovery factor: To determine the recovery factor, *f*, for a given apparatus, dilute an appropriate volume of acetic acid stock solution to 250 mL in a volumetric flask to approximate the expected sample concentration and distill as for a sample. Calculate the recovery factor

$$f = \frac{a}{b}$$

where:

a = volatile acid concentration recovered in distillate, mg/L, and
b = volatile acid concentration in standard solution used, mg/L.

b. Sample analysis: Centrifuge 200 mL sample for 5 min. Pour off and combine supernatant liquors. Place 100 mL supernatant liquor, or smaller portion diluted to 100 mL, in a 500-mL distillation flask. Add 100 mL distilled water, four to five clay chips or similar material to prevent bumping, and 5 mL H_2SO_4. Mix so that acid does not remain on bottom of flask. Connect flask to a condenser and adapter tube and distill at the rate of about 5 mL/min. Discard the first 15 mL and collect exactly 150 mL distillate in a 250-mL graduated cylinder. Titrate with 0.1*N* NaOH, using phenolphthalein indicator, a pH meter, or an automatic titrator. The end points of these three methods are, respectively, the first pink coloration that persists on standing a

short time, pH 8.3, and the inflection point of the titration curve (see Section 2310). Titration at 95°C produces a stable end point.

Distill and analyze a blank and reference standard with each sample batch to insure system performance.

5. Calculation

$$\text{mg volatile acids as acetic acid/L} = \frac{\text{mL NaOH} \times N \times 60\,000}{\text{mL sample} \times f}$$

where:

N = normality of NaOH, and

f = recovery factor.

6. Bibliography

OLMSTEAD, W.H., W.M. WHITAKER & C.W. DUDEN. 1929–1930. Steam distillation of the lower volatile fatty acids from a saturated salt solution. *J. Biol. Chem.* 85:109.

OLMSTEAD, W.H., C.W. DUDEN, W.M. WHITAKER & R.F. PARKER. 1929–1930. A method for the rapid distillation of the lower volatile fatty acids from stools. *J. Biol. Chem.* 85:115.

BUSWELL, A.M. & S.L. NEAVE. 1930. Laboratory studies of sludge digestion. *Ill. State Water Surv. Bull.* 30:76.

HEUKELEKIAN, H. & A.J. KAPLOVSKY. 1949. Improved method of volatile-acid recovery from sewage sludges. *Sewage Works J.* 21:974.

KAPLOVSKY, A.J. 1951. Volatile-acid production during the digestion of seeded, unseeded, and limed fresh solids. *Sewage Ind. Wastes* 23:713.

5560 D. Gas Chromatographic Method

1. General Discussion

a. Principle: This gas chromatographic procedure may be used to determine the individual concentrations of the following fatty acids: acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids in digester sludge samples. Caproic and heptanoic acids also may be determined, but with less certainty because of their reduced solubility in water. Volatile fatty acids are generally soluble in digester sludge and exist in either the protonated or unprotonated form, depending on the pH of the sludge sample. Acetic and propionic acids usually are in higher concentrations than the other fatty acids in digester sludge. All acids must be converted to the protonated (volatile) form before injection into the gas chromatograph (GC) so that vaporization may occur. The fatty acids must also be separated from the solids material in the sample before introduction into the GC to minimize degradation of the GC column. Digester sludge samples are prepared by acidification, centrifugation, and filtration. The sample is analyzed by direct injection into a gas chromatograph equipped with a flame ionization detector after both centrifugation and filtration.

b. Interference: The blank amount may be high relative to sample analyte. High blanks may be caused by a buildup of contaminants in the injector and guard column or by sample carry-over. The analyst must be aware of the blank levels and correct unacceptably high blank levels that are above the reporting limits.

c. Minimum detectable concentration: The method detection level has been determined by the USEPA method[1] in reagent water to be about 3 mg/L for acetic acid and about 1 mg/L for all other target compounds.

2. Apparatus

a. Gas chromatograph, with a flame ionization detector (FID) and programmable column oven. An on-column capillary injector, a detector make-up tee, and a gas chromatographic data system are recommended.

b. Chromatographic columns, preferably fused silica, bonded polyethylene glycol capillary columns 30 m long × 0.53 mm ID with a film thickness of 0.10 to 0.25 μm. An example of a chromatogram is presented in Figure 5560:1.*

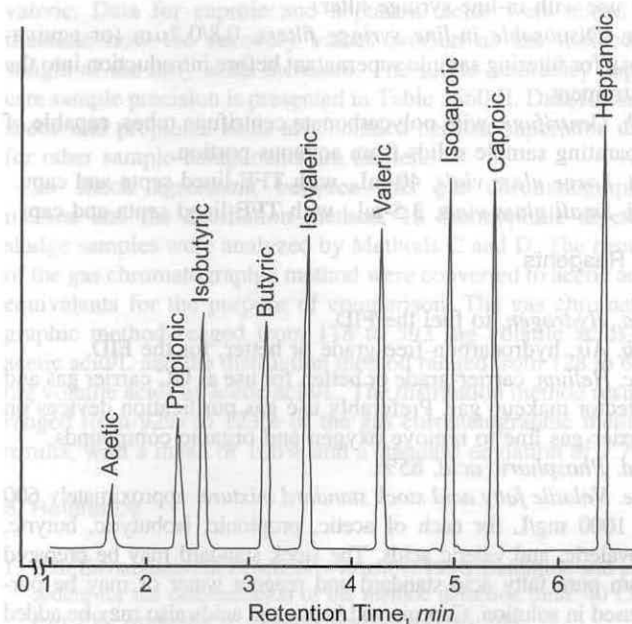

Figure 5560:1. Gas chromatogram of a fatty acid standard. Column DB-FFAP, 0.53-mm-ID, 30-m, 0.50-μm film thickness, temperature-programmed as described in ¶ 2*d*.

c. Guard column: Preferably use a 2- to 5-m 0.53-mm-ID deactivated fused silica guard column if an on-column injector is used. Attach guard column to analytical column with a press-fit connector. Service guard column periodically by breaking off the front section or replace guard column when system performance is degraded.

d. Gas chromatograph operating conditions: Use temperature programming to achieve optimal separation and desirably short

* Several manufacturers of suitable bonded-phase capillary columns may be located on the internet. For example, a tabulation of several brands may be found at www.restekcorp.com/byphase.htm.

run times. Optimize temperatures and flow rates for the particular gas chromatograph and chromatographic column. Optimal injector temperature will allow vaporization of the prepared sample and provide good peak shape. Incorrect injector temperature may cause splitting of the acetic acid peak. Typical conditions are as follows:

Injector temperature: 150°C

Oven temperature program: 95°C hold for 2 min, ramp to 140°C at 10°C/min, no hold. Ramp to 200°C at 40°C/min, 5 min hold. Cool to initial temperature.

Detector conditions: temperature 240°C, hydrogen flow rate 30 mL/min, and air flow rate 300 mL/min.

Carrier gas flow rate: 18 mL/min.

Detector make-up gas flow rate: 12 mL/min, for a total of 30 mL/min from the column into the detector (or as recommended by the manufacturer).

e. Syringe, for sample introduction into GC. Syringe may be for manual injection or used in conjunction with an autosampler.

f. Disposable syringes, 10-mL, plastic with detachable tip,† for use with in-line syringe filter.

g. Disposable in-line syringe filters, 0.8/0.2μm (or equivalent), for filtering sample supernatant before introduction into the instrument.

h. Centrifuge, with polycarbonate centrifuge tubes, capable of separating sample solids from aqueous portion.

i. Large glass vials, 40-mL, with TFE-lined septa and caps.

j. Small glass vials, 3.5-mL, with TFE-lined septa and caps.

3. Reagents

a. Hydrogen, to fuel the FID.

b. Air, hydrocarbon-free-grade or better, for the FID.

c. Helium, carrier-grade or better, for use as GC carrier gas and detector makeup gas. Preferably use gas purification devices on carrier-gas line to remove oxygen and organic compounds.

d. Phosphoric acid, 85%.

e. Volatile fatty acid stock standard mixture, approximately 600 to 1000 mg/L for each of acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids. The stock standard may be prepared from pure fatty acid standard and reagent water or may be purchased in solution. Caproic and heptanoic acids also may be added if desired. Store at 4°C. Replace as needed, or every 6 months.

f. Volatile fatty acid calibration standard mixture, diluted from the stock volatile acid standard mixture to four levels of dilution to span a broad range of concentrations. Typical calibration standard concentrations are 350 mg/L, 140 mg/L, 35 mg/L, and 3.5 mg/L. Prepare fresh monthly or as needed by diluting volatile acid standard mixture with reagent water acidified with phosphoric acid. Store at 4°C.

g. Reagent water: Use any water that is free of volatile fatty acids. Prepare by passing house deionized water through an activated carbon column. Analyze to ensure an acceptable blank level well below method reporting limits.

4. Procedure

a. Sample preparation: Collect digester sludge samples on the day of analysis and refrigerate at 4°C immediately. Transfer a sample portion (10 to 30 mL) to a 40-mL glass vial and acidify to approximately pH 2 with phosphoric acid while stirring with a glass rod. Check pH with pH paper after mixing. Transfer acidified sludge sample into a polycarbonate centrifuge tube and centrifuge until centrate is separated from supernatant. Withdraw supernatant from centrifuge tube with a disposable syringe, filter through a disposable in-line filter, and place in a 3.5-mL glass vial (¶ 2*j*).

Perform one duplicate sample analysis for each set of 10 samples. Also fortify, and analyze, samples analyzed in duplicate as follows: Place 10-mL portion of digester sludge in a 40-mL vial (¶ 2*i*) and add an amount of volatile fatty acid stock standard mixture approximately equal to the largest amount of any single analyte expected in the sample. Process duplicate and fortified samples in the same way as other samples.

b. Calibration: Inject 1 μL of each of the volatile fatty acid calibration standard mixture concentrations (¶ 3*f*) into the GC for calibration. Preferably use the solvent flush technique for all standard and sample injections. Construct a calibration curve from the four calibration standard injections using the best fit through zero. The calibration curve may be used for up to 1 month if it is proven to be valid. Validate calibration curve daily by injection of a mid-point calibration curve validation standard. Inject a check standard (mid-point calibration curve standard) at end of each day.

c. Sample analysis: Use 1-μL injection volume for all analyses, including blank, duplicate, and laboratory-fortified samples. Analyze at least one blank sample (reagent water adjusted to pH 2 with phosphoric acid) for each set of 10 samples. Also analyze blank samples after high-level samples or standards to check system cleanliness.

5. Calculations

a. Data collection: Collect and process all data with chromatographic data system software.

b. Calibration curve: Use area and concentration of each fatty acid component in each of the calibration standards to construct calibration curve by the external standard method. Use first-order linear regression through zero and compute calibration factor. The correlation coefficient should be > 0.995. Recalculate each calibration point by comparison to the curve. The calculated values should be $\pm 20\%$ of the true concentration. If the correlation coefficient is lower than 0.995 or any of the recalculated values are more than 20% from the true value, correct the problem before proceeding.

c. Continuing calibration and check standards: Validate calibration curve daily (¶ 4*b*). The curve is valid if the concentration of the calibration curve validation standard is within 20% of the expected value for all components. If the concentration of the calibration curve validation standard is outside of 20% of the expected value for any component, construct a new curve. Analyze at least one check standard on each day of instrument use. "Bracket" the samples with check standards, that is, analyze check standards before and after the samples. The analysis is valid if concentration of the check standard components is within 10% of the expected values. Correct problem and repeat analysis of any samples analyzed after last acceptable check standard, if a check standard is not within 10%.

d. Fatty acid concentration: Identify volatile fatty acids in the samples by comparing sample retention times with those of the

† Luerlok or equivalent.

standards. Determine concentration of each of the fatty acids in each sample by multiplying area of each sample component with the calibration factor for that component. The volume of phosphoric acid used to adjust the pH may need to be factored to correct the results if that volume is significant when compared to sample volume. Similarly, consider volume of fortifying solution when it is a significant portion of the sample volume.

6. Quality Control

a. Blank samples: For each sample set of 10 samples or less, analyze at least one blank sample. Also analyze a blank sample after high standard or sample injections to ensure the cleanliness of the system. An acceptable blank sample has no volatile fatty acids above the MDL.

b. Duplicate samples: Analyze at least one sample in duplicate for each set of 10 or fewer samples. Calculate percent difference; acceptable difference is less than 10% for major components.

c. Laboratory-fortified samples (LFS): Analyze at least one LFS for each set of 10 or fewer samples. Calculate percent recovery; acceptable value is ±20%.

d. Minimum quantitation level: Establish method detection level (MDL)[1] either in matrix or in reagent water. The MDL is defined as three times the standard deviation of the replicate measurements. The minimum quantitation level (MQL) is defined as four times the MDL. MQL must be at or above lowest standard on calibration curve.

e. Reporting protocol: For sample analyses to be reportable, ensure that all quality assurance samples (including blank, duplicate, LFS, and check standards), as well as the calibration curve or calibration curve validation standard, are acceptable, and that the sample response lies between the highest and lowest points on the calibration curve. Annotate results below the MQL but greater than the MDL as "less than the MQL." Report results less than the MDL as "less than nominal value of the MDL." Report results with response higher than the highest point on the calibration curve as "greater than *x* mg/L," where *x* = concentration corresponding to the highest point on the calibration curve. Report results to two significant figures with units of mg/L. Refer to Section 1020B and 6020B for more information.

TABLE 5560:I. SINGLE-LABORATORY LABORATORY-FORTIFIED SAMPLE RECOVERY AND PRECISION *

Component	Concentration Added *mg/L*	Mean Recovery %	Relative Standard Deviation %
Acetic acid	120	95.2	6.8
Propionic acid	148	93.6	6.4
Isobutyric acid	176	90.3	6.5
Butyric acid	176	89.8	6.6
Isovaleric acid	204	88.9	6.4
Valeric acid	204	87.5	6.4
Isocaproic acid	232	83.2	6.0
Caproic acid	232	81.1	5.9
Heptanoic acid	260	63.9	6.2

* $n = 34$
Sample source is thermophilic and mesophilic digester sludge from the Los Angeles County Sanitation Districts, Joint Water Pollution Control Plant, Carson, CA.

TABLE 5560:II. SINGLE-LABORATORY DUPLICATE SAMPLE PRECISION*

Component	Mean Percent Difference %	Relative Standard Deviation %
Acetic acid	4.7	5.8
Propionic acid	3.6	4.6

* $n = 30$ for acetic acid and 25 for propionic acid.
Sample source is thermophilic and mesophilic digester sludge from the Los Angeles County Sanitation Districts, Joint Water Pollution Control Plant, Carson, CA.

7. Precision and Bias

The single-laboratory LFS recovery and precision data in Table 5560:I were generated by adding a fortifying solution to digester sludge. The sample recovery was over 87% and relative standard deviation less than 7% for acids from acetic through valeric. Data for caproic and heptanoic acids were added to illustrate how the recovery values worsen as the molecular weight of the fatty acids increases. The single-laboratory duplicate sample precision is presented in Table 5560:II. Data for only acetic and propionic acids are included because reportable data for other sample components are limited.

To check agreement between this gas chromatographic method and the distillation method, 18 thermophilic digester sludge samples were analyzed by Methods C and D. The results of the gas chromatographic method were converted to acetic acid equivalents for the purpose of comparison. The gas chromatographic method ranged from 118 to 593 mg volatile acids as acetic acid/L and the distillation method ranged from 128 to 610 mg volatile acids as acetic acid/L. The distillation method results ranged from 92% to 123% of the gas chromatographic method results, with a mean of 106% and a standard deviation of 7.7%.

8. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of the method detection limit. 40 CFR Part 136, Appendix B. *Federal Register* 49, No. 209.

9. Bibliography

SANS, C., J. MATA-ALVAREZ, F. CECCHI & P. PAVAN. 1995. Modelling of plug flow pilot reactor producing VFA by anaerobic fermentation of municipal solid waste. *Water Sci. Technol.* 30 (12):125.

GRIFFIN, M. E. 1996. Use of Molecular Tools to Enhance the Evaluation of Anaerobic Co-digestion of the Organic Fraction of Municipal Solid Waste and Sewage Sludge. M.Sc. thesis, Univ. of Illinois, Urbana-Champaign.

CHU, A., D. S. MAVINIC, W. D. RAMEY & H. G. KELLY. 1996. A biochemical model describing volatile fatty acid metabolism in thermophilic aerobic digestion of wastewater sludge. *Water Res.* 30:1759.

SIGMA-ALDRICH CO. 1998. Analyzing fatty acids by capillary gas chromatography. Supelco Bull. 855B, Bellefonte, Pa.

PAVAN, P., P. BATTISTONI, F. CECCHI & J. MATA-ALVAREZ. 2000. Two-phase anaerobic digestion of source sorted OFMSW (organic fraction of municipal solid waste): Performance and kinetic study. *Water Sci. Technol.* 41(3):111.

5710 FORMATION OF TRIHALOMETHANES AND OTHER DISINFECTION BY-PRODUCTS*

5710 A. Introduction

Trihalomethanes (THMs) are produced during chlorination of water. Only four THM compounds normally are found: chloroform ($CHCl_3$), bromodichloromethane ($CHBrCl_2$), dibromochloromethane ($CHBr_2Cl$), and bromoform ($CHBr_3$). Additional chlorination by-products can be formed (including haloacetic acids and halonitriles; for example, see 5710D) during the relatively slow organic reactions that occur between free chlorine and naturally occurring organic precursors such as humic and fulvic acids. The formation potentials of these additional by-products also can be determined, but different quenching agents and different analytical procedures may be needed. Predictive models for estimating/calculating THM formation exist, but because eventual THM concentrations cannot be calculated precisely from conventional analyses, methods to determine the potential for forming THMs are useful in evaluating water treatment processes or water sources or for predicting THM concentrations in a distribution system.

To obtain reproducible and meaningful results, control such variables as temperature, reaction time, chlorine dose and residual, and pH. THM formation is enhanced by elevated temperatures and alkaline pH and by increasing concentrations of free chlorine residuals, although THM formation tends to level off at free chlorine residuals of 3 mg/L and above; a longer reaction time generally increases THM formation.[1,2]

Low concentrations of bromide exist in most natural waters and are responsible for the formation of brominated organic compounds. Figure 5710:1 shows that an oxidant ratio of about 40 times more chlorine than bromine (on a molar basis, = 40 on the x axis) is required to form equimolar amounts of substituted organic chloride and bromide (= 1 on the y axis); small amounts of bromide also can increase the molar yield of THMs.[3]

The possible addition of organic precursors contained in reagent solutions cannot be accounted for accurately without a great deal of extra work; therefore, sample dilutions resulting from reagent additions (approximately 2%) are ignored in the final calculations. However, sample dilution may need to be taken into account if other volumes are used. Sample dilution also changes the concentrations of bromide and organic matter, potentially leading to speciation changes.

* Approved by Standard Methods Committee, 2000.
Joint Task Group: 20th Edition—Leown A. Moore (chair), James K. Edzwald, Robert C. Hoehn, Bart Koch, Neil M. Ram, James M. Symons.

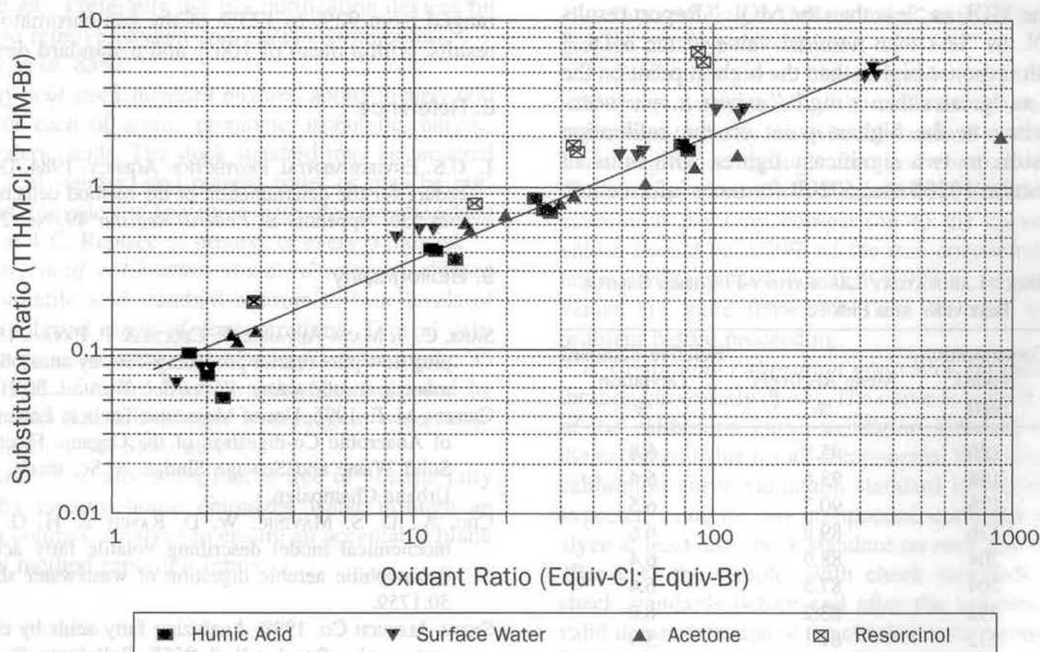

Figure 5710:1. Effect of changing molar oxidant ratios of free chlorine: free bromine on molar ratios of substituted organic chloride: organic bromide, using four different precursor substrates. Reaction times varied between 1 and 7 d. Standard conditions were used at pH=7.0, except that the free chlorine residual after 7 d storage for the surface water was 17 mg/L instead of the 3 to 5 mg/L range for the other three substrates.

1. Definition of Terms

See Figures 5710:2a and b for the relationship among the following definitions.

Total trihalomethane ($TTHM_T$) is the sum of all four THM compound concentrations (see Section 5710B.5) produced at any time *T* (usually days). $TTHM_0$ is the total THM concentration at the time of sampling. $TTHM_0$ concentrations can range from nondetectable, which usually means the sample has not been chlorinated, to several hundred micrograms per liter if the sample has been chlorinated. $TTHM_7$ is the sum of all four THM compound concentrations produced during reactions of sample precursors with excess free chlorine over a 7-d reaction time.

Standard reaction conditions (see Section 5710B) are as follows: free chlorine residual at least 3 mg/L and not more than 5 mg/L at the end of a 7-d reaction (incubation) period, with sample incubation temperature of 25 ± 2°C, and pH controlled at 7.0 ± 0.2 with phosphate buffer. Standard conditions are not intended to simulate water treatment processes but are most useful for estimating the concentration of THM precursors, as well as for measuring the effectiveness of water-treatment options for reducing levels of THM precursors in the raw water.

Special applications permit different test conditions, but they must be stated explicitly when reporting results.

Trihalomethane formation potential (*THMFP or Δ THMFP*) is the difference between the final $TTHM_T$ concentration and the initial $TTHM_0$ concentration. If sample does not contain chlorine at the time of collection, $TTHM_0$ will be close to zero and the term THMFP may be used. If sample does contain chlorine at the time of collection, because of formation of THMs, use the term Δ THMFP (the increase of THM concentration during storage) when reporting the difference between TTHM concentrations.

The term "THMFP" often has been equated to the final TTHM concentration, even if the sample had contained chlorine when collected. To use this definition, explicitly define the term when reporting data.

Simulated distribution system trihalomethane (*SDS-THM*), Section 5710C, is the concentration of TTHMs in a sample that has been disinfected comparably to finished drinking water and under the same conditions and time as in a water distribution system. It includes pre-existing THMs plus those produced during storage. This method can be used in conjunction with laboratory, pilot, or full-scale studies of treatment processes to estimate expected concentration of THMs in a distribution system. Do not use SDS-THM to estimate the precursor removal efficiency of a treatment process, because THM yields are highly variable at low chlorine residual concentrations. For SDS-type testing, low chlorine residuals (< 1 mg Cl_2/L) are often encountered, thereby resulting in lower THM formation than would be obtained for higher chlorine residuals. THM yields at higher chlorine concentrations (> 3 mg/L) tend to level off and become relatively independent of variations in free chlorine residuals.

2. Sampling and Storage

Collect samples in 1-L glass bottles sealed with TFE-lined screw caps. If multiple tests will be performed for each sample, or if many different analyses will be performed for each sample (see Section 5710D), collect 4 L instead. Further, if multiple reaction time periods will be used to study reaction rates, process

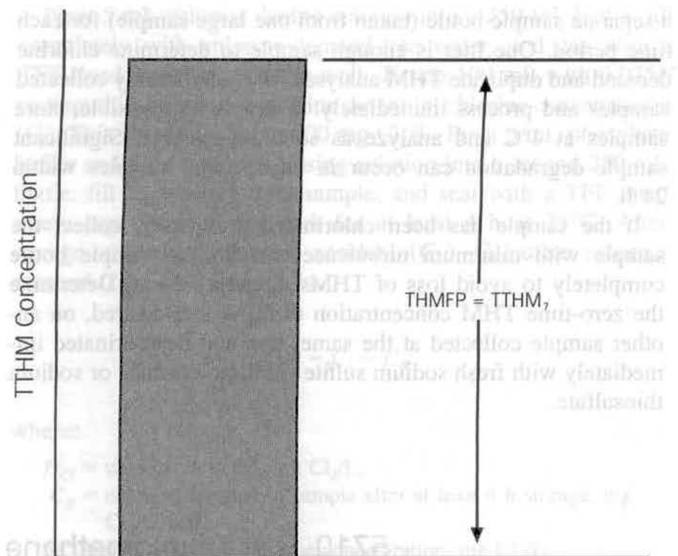

Figure 5710:2a. Relationships between definitions used in the formation potential test, for a sample that did not contain free chlorine at the time of sampling. Total THM concentration at the time of sampling ($TTHM_0$) was very close to or equal to zero; therefore, the THM formation potential for the 7-d reaction time (THMFP, with a free chlorine residual of at least 3 mg/L) was essentially equal to the total THM concentration in the sample at the end of the reaction storage time ($TTHM_7$).

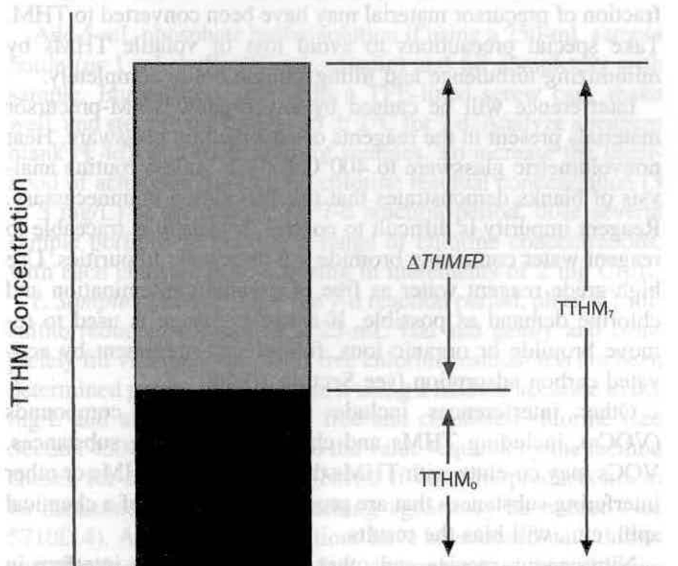

Figure 5710:2b. Relationships between definitions used in the formation potential test, for a sample that already contained free chlorine at the time of sampling. Total THM concentration at the time of sampling ($TTHM_0$) was a significant fraction of the final value obtained after 7-d storage ($TTHM_7$) with an excess of at least 3 mg/L free chlorine. Δ THMFP is the difference between these two values.

a separate sample bottle (taken from one large sample) for each time period. One liter is enough sample to determine chlorine demand and duplicate THM analyses. Use only freshly collected samples and process immediately. If this is not possible, store samples at 4°C and analyze as soon as possible. Significant sample degradation can occur in unpreserved samples within 24 h.

If the sample has been chlorinated previously, collect the sample with minimum turbulence and fill the sample bottle completely to avoid loss of THMs already present. Determine the zero-time THM concentration ($TTHM_0$), if desired, on another sample collected at the same time and dechlorinated immediately with fresh sodium sulfite solution, crystals, or sodium thiosulfate.

3. References

1. STEVENS, A.A. & J.M. SYMONS. 1977. Measurement of trihalomethanes and precursor concentration changes. *J. Amer. Water Works Assoc.* 69:546.
2. SYMONS, J.M., A.A. STEVENS, R.M. CLARK, E.E. GELDREICH, O.T. LOVE, JR. & J. DEMARCO. 1981. Treatment Techniques for Controlling Trihalomethanes in Drinking Water. EPA-600/2-81-56, U.S. Environmental Protection Agency, Cincinnati, Ohio.
3. SYMONS, J.M., S.W. KRASNER, L. SIMMS & M. SCLIMENTI. 1993. Measurement of trihalomethane and precursor concentrations revisited: The impact of bromide ion. *J. Amer. Water Works Assoc.* 85(1):51.

5710 B. Trihalomethane Formation Potential (THMFP)

1. General Discussion

a. Principle: Under standard conditions, samples are buffered at pH 7.0 ± 0.2, chlorinated with an excess of free chlorine, and stored at 25 ± 2°C for 7 d to allow the reaction to approach completion. As a minimum, pH is buffered at a defined value and a free chlorine residual of 3 to 5 mg Cl_2/L exists at the end of the reaction time. THM concentration is determined by using liquid-liquid extraction (see Section 6232B) or purge and trap (see Section 6200).

b. Interference: If the water was exposed to free chlorine before sample collection (e.g., in a water treatment plant), a fraction of precursor material may have been converted to THM. Take special precautions to avoid loss of volatile THMs by minimizing turbulence and filling sample bottle completely.

Interference will be caused by any organic THM-precursor materials present in the reagents or adsorbed on glassware. Heat nonvolumetric glassware to 400°C for 1 h, unless routine analysis of blanks demonstrates that this precaution is unnecessary. Reagent impurity is difficult to control. It usually is traceable to reagent water containing bromide ion or organic impurities. Use high-grade reagent water as free of organic contamination and chlorine demand as possible. If anion exchange is used to remove bromide or organic ions, follow such treatment by activated carbon adsorption (see Section 1080).

Other interferences include volatile organic compounds (VOCs), including THMs and chlorine-demanding substances. VOCs may co-elute with THMs during analysis. THMs or other interfering substances that are present as the result of a chemical spill, etc., will bias the results.

Nitrogenous species and other constituents may interfere in the determination of free residual chlorine. Add enough free chlorine to oxidize chorine-demanding substances and leave a free chlorine residual of at least 3 mg/L, but not more than 5 mg/L, at the end of the incubation period. A free chlorine residual of at least 3 mg/L decreases the likelihood that a combined residual will be mistaken for a free residual and assures that THM formation occurs under conditions that are reasonably independent of variations in chlorine residual concentrations.

c. Minimum detectable quantity: The sensitivity of the method is determined by the analytical procedure used for THM.

2. Apparatus

a. Incubator, to maintain temperature of 25 ± 2°C.

b. Bottles, glass, with TFE-lined screw caps to contain 245 to 255 mL, 1-L, 4-L.

c. Vials, glass, 25- or 40-mL with TFE-lined screw caps.

d. pH meter, accurate to within ± 0.1 unit.

3. Reagents

Prepare aqueous reagents in organic-free water (¶ 3*e* below) unless chlorine-demand-free water (¶ 3*f* below) is specified.

a. Standardized stock hypochlorite solution: Dilute 1 mL, using a 1-mL volumetric pipet, 5% aqueous sodium hypochlorite (NaOCl, to be referred to as stock hypochlorite) solution to approximately 25 mL with chlorine-demand-free water (see ¶ 3*f* below), mix well, and titrate to a starch-iodide end point using 0.100*N* sodium thiosulfate titrant (see Section 4500-Cl.B). Calculate chlorine concentration of the stock hypochlorite solution as:

$$\text{Stock hypochlorite, mg Cl}_2\text{/mL} = \frac{N \times 35.45 \times \text{mL titrant}}{\text{mL stock hypochlorite added}}$$

where N is the normality of the titrant (= 0.100). Use at least 10 mL titrant; if less is required, standardize 2 mL stock hypochlorite solution. Measure chlorine concentration each time a dosing solution (¶ 3*b* below) is made; discard stock hypochlorite solution if its chlorine concentration is less than 20 mg Cl_2/mL.

b. Chlorine dosing solution, 5 mg Cl_2/mL: Calculate volume of stock hypochlorite solution required to produce a chlorine concentration of 5 mg Cl_2/mL:

$$\text{mL required} = \frac{1250}{\text{stock hypochlorite conc, mg } Cl_2/\text{mL}}$$

Dilute this volume of stock hypochlorite solution in a 250-mL volumetric flask to the mark with chlorine-demand-free water. Mix and transfer to an amber bottle, seal with a TFE-lined screw cap, and refrigerate. Keep away from sunlight. Discard if the chlorine concentration drops below 4.7 mg Cl_2/mL; this will occur if the "initial chlorine concentration" (as determined in ¶ 4*a* below) drops below 94 mg Cl_2/L.

c. Phosphate buffer: Dissolve 68.1 g potassium dihydrogen phosphate (anhydrous), KH_2PO_4, and 11.7 g sodium hydroxide, NaOH, in 1 L water. Refrigerate when not in use. If a precipitate develops, filter through a glass fiber filter. After buffer is added to a sample, a pH of 7.0 should result. Check before use with a sample portion that can be discarded.

d. Sodium sulfite solution: Dissolve 10 g sodium sulfite, Na_2SO_3, in 100 mL water. Use for dechlorination: 0.1 mL will destroy about 5 mg residual chlorine. Make fresh every 2 weeks. NOTE: More dilute solutions oxidize readily.

e. Organic-free water: Pass distilled or deionized water through granular-activated-carbon columns. A commercial system may be used.* Special techniques such as preoxidation, activated carbon adsorption (perhaps accompanied by acidification and subsequent reneutralization), or purging with an inert gas to remove THMs may be necessary.

f. Chlorine-demand-free water: Follow the procedure outlined in 4500-Cl.C.3*m*, starting with organic-free water. After residual chlorine has been destroyed completely, purge by passing a clean, inert gas through the water until all THMs have been removed.

g. DHBA solution: Dissolve 0.078 g anhydrous 3,5-dihydroxy-benzoic acid (DHBA) in 2 L chlorine-demand-free water. This solution is not stable; make fresh before each use.

h. Nitric or hydrochloric acid, HNO_3 or HCl, concentrations of 1:1, 1.0*N*, and 0.1*N*.

i. Sodium hydroxide, NaOH, 1.0*N* and 0.1*N*.

j. Borate buffer (optional): Dissolve 30.9 g anhydrous boric acid, H_3BO_3, and 10.8 g sodium hydroxide, NaOH, in 1 L water. Filter any precipitate that may form with a glass fiber filter. This solution will keep sample pH at 9.2; check before using. NOTE: Waters containing significant amounts of calcium may precipitate calcium phosphate (or carbonate) at higher pH values.

k. Mixed buffer (optional): Mix equal amounts of phosphate and borate buffer solutions, then adjust pH as desired before using with samples. Determine the amount of acid or base needed on a separate sample that can be discarded. This mixed buffer is reasonably effective in the pH range of 6 to 11.

4. Procedure

a. Chlorine demand determination: Determine or accurately estimate the 7-d sample chlorine demand. A high chlorine dose is specified below to drive the reaction close to completion quickly. The following procedure yields only a rough estimate of chlorine demand; other techniques may be used.

Pipet 5 mL chlorine dosing solution into a 250-mL bottle, fill completely with chlorine-demand-free water, and cap with a TFE-lined screw cap. Shake well. Titrate 100 mL with 0.025*N* sodium thiosulfate to determine the initial chlorine concentration (C_I). This should be about 100 mg Cl_2/L. Pipet 5 mL phosphate buffer and 5 mL chlorine dosing solution into a second 250-mL bottle, fill completely with sample, and seal with a TFE-lined screw cap. Store in the dark for at least 4 h at 25°C. After storage, determine chlorine residual (C_R). Calculate chlorine demand (D_{Cl}) as follows:

$$D_{Cl} = C_I - C_R$$

where:

D_{Cl} = chlorine demand, mg Cl_2/L,
C_R = chlorine residual of sample after at least 4 h storage, mg Cl_2/L, and
C_I = initial (dosed) chlorine concentration, mg Cl_2/L.

b. Sample chlorination: If sample contains more than 200 mg/L alkalinity or acidity, adjust pH to 7.0 ± 0.2 using 0.1 or 1.0*N* HNO_3, HCl, or NaOH and a pH meter. With a graduated pipet, transfer appropriate volume of the 5 mg Cl_2/mL chlorine dosing solution, V_D, into sample bottle:

$$V_D = \frac{D_{Cl} + 3}{5} \times \frac{V_S}{1000}$$

where:

V_S = volume of sample bottle, mL, and
V_D = volume of dosing solution required, mL.

Add 5 mL phosphate buffer solution if using a 250-mL sample bottle (or 1 mL buffer/50 mL sample) and fill completely with sample. Immediately seal with a TFE-lined screw cap, shake well, and store in the dark at 25 ± 2°C for 7 d. Analyze a reagent blank (¶ 4*d*) with each batch of samples. To increase the likelihood of achieving the desired chlorine residual concentration (3 to 5 mg/L) at the end of the 7-d reaction period, dose several sample portions to provide a range of chlorine concentrations, with each chlorine dose differing in increments of 2 mg Cl_2/L.

c. Sample analysis: After the 7-d reaction period, place 0.1 mL sulfite reducing solution in a 25-mL vial and gently and completely fill vial with sample. If free chlorine residual has not been determined previously, measure it using a method accurate to 0.1 mg/L and able to distinguish free and combined chlorine (see Section 4500-Cl). Adjust pH to the value required by the method chosen for chlorine analysis. [NOTE: If other by-products are to be measured, a different quenching agent may be needed (see 5710D.4). Also, if sample portions have been dosed with different chlorine concentrations, first determine the free chlorine residual and select only that portion having the required chlorine residual concentration of 3 to 5 mg/L for further processing.] If THMs will not be analyzed immediately, lower the pH to <2 by adding 1 or 2 drops of 1:1 HCl to the reduced sample in the vial. Seal vial with TFE-lined screw cap. Store samples at 4°C until ready for THM analysis (preferably no longer than 7 d). Let sample reach room temperature before beginning analysis.

* Milli-Q, Millipore Corp., or equivalent.

d. Reagent blank: Add 1 mL chlorine dosing solution to 50 mL phosphate buffer, mix, and completely fill a 25-mL vial, seal with a TFE-lined screw cap, and store with samples. (NOTE: This reagent blank is for quality control of reagent solutions only and is not a true blank, because the reagent concentrations in this blank are considerably higher than those in samples. THM concentrations in the reagent blank will be biased high and cannot be subtracted from sample values. Make no further dilutions before the reaction because the reagent water itself might contribute to THM formation.) After reaction for 7 d, pipet 1 mL sulfite reducing solution into a 250-mL bottle and add, without stirring, 5.0 mL reacted reagent mixture. Immediately fill bottle with organic-free water that has been purged free of THMs and seal with a TFE-lined screw cap. Mix. Analyze a portion of this reagent blank for THMs using the same method used for samples. The sum of all THM compounds in the reagent blank should be less than 5 μg TTHM as $CHCl_3$/L.

The reagent blank is a rough measure of THMs contributed by reagents added to the samples, but it cannot be used as a correction factor. If the reagent blank is greater than 5% of the sample value or greater than 5 μg TTHM/L, whichever is larger, additional treatment for reagent water is necessary. See Section 1080. It also may be necessary to obtain reagents of higher purity. Analyze a reagent blank each time samples are analyzed and each time fresh reagents are prepared.

5. Calculation

Report concentration of each of the four common THM compounds separately because it is desirable to know their relative concentrations. Larger amounts of bromine-substituted compounds, relative to chlorine-substituted compounds, indicate a higher concentration of dissolved bromide in the water (see Figure 5710:1). Also report free chlorine concentration at end of reaction time along with the incubation time, temperature, and pH.

THM concentrations may be reported as a single value as micrograms $CHCl_3$ per liter (μg $CHCl_3$/L), or micromoles per liter (μM). Do not use the simple sum of mass units micrograms per liter except when required for regulatory reporting. Compute TTHM concentration using one of the following equations:

To report TTHM in units of μg $CHCl_3$/L:

$$TTHM = A + 0.728B + 0.574C + 0.472D$$

where:

$A = \mu g\ CHCl_3/L$,
$B = \mu g\ CHBrCl_2/L$,
$C = \mu g\ CHBr_2Cl/L$, and
$D = \mu g\ CHBr_3/L$.

To report TTHM in units of μM as $CHCl_3$:

$$TTHM = \frac{TTHM,\ \mu g\ CHCl_3/L}{119}$$

To report TTHM on a weight basis as μg/L (not used except for regulatory purposes):

$$TTHM = A + B + C + D$$

To report a change of TTHM concentration over 7 d:

$$\Delta\ THMFP = TTHM_7 - TTHM_0$$

Finally, if $TTHM_0 = 0$, then:

$$THMFP = TTHM_7 = \Delta\ THMFP$$

Do not make blank correction or a correction for sample dilution resulting from addition of reagents. If conditions differ from pH of 7, 25°C, 7-d reaction time, and 3 to 5 mg/L chlorine residual, report these nonstandard test conditions with the results. Nonstandard test conditions may mimic water quality conditions in a specific distribution system or may be relevant to other investigations (see Section 5710C).

6. Quality Control

a. Use dihydroxy-benzoic acid solution (DHBA) as a quality-control check, especially for the presence of interfering bromides in reagents or reagent water.

Dilute 1.0 mL chlorine dosing solution to 1000 mL with chlorine-demand-free water (diluted chlorine dosing solution). Pipet 5 mL phosphate buffer solution (pH = 7.0) into each of two 250-mL bottles; add 1.00 mL DHBA solution to one bottle and fill both bottles completely with diluted chlorine dosing solution; seal with TFE-lined screw caps. Store in the dark for 7 d at 25 ± 2°C, and analyze as directed in ¶ 4*c*.

b. The THM concentration of the solution containing the added DHBA minus the THM concentration of the blank (i.e., the bottle that does not contain added DHBA, which is a true blank for this application only and differs from the reagent blank discussed in ¶ 4*b* above) should be about 119 μg/L THM as $CHCl_3$, with essentially no contribution from bromide-containing THMs. If there is a significant contribution from brominated THMs, 10% or more of the total THM, it may be necessary to remove bromide from the reagent water or to obtain higher-purity reagents containing less bromide. Determine source of bromide and correct the problem. If the THM concentration of the water blank exceeds 20 μg/L, treat reagent water to reduce contamination.

7. Precision and Bias

The precision of this method is determined by the analytical precision and bias of the method used for measuring THM as well as the control of variables such as pH, chlorine residual, temperature, sample homogeneity, etc. Method bias can be determined only for synthetic solutions (e.g., the DHBA solution), because THM formation potential is not an intrinsic property of the sample but rather a quantity defined by this method.

Table 5710:I presents single-operator precision and bias data for samples processed under standard conditions. The values were obtained by analyzing DHBA solutions and blanks. The expected value for the samples listed is 116 μg/L TTHM (as $CHCl_3$), rather than 119 μg/L, because the DHBA reagent used was only 97% pure. Percent recovery was calculated by the formula:

$$\%\ recovery = \frac{\text{DHBA sample} - \text{average blank}}{116} \times 100$$

TABLE 5710:I. SINGLE-OPERATOR PRECISION AND BIAS DATA FOR THMFP*

Sample	THM μg/L				THMFP μg/L as $CHCl_3$	Recovery %
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$		
Blank 1	0.8	—	—	—	0.8	—
Blank 2	1.9	—	—	—	1.9	—
Blank 3	0.1	0.1	—	—	0.2	—
Blank 4	0.7	—	—	—	0.7	—
Blank 5	0.5	—	—	—	0.5	—
Blank 6	0.7	—	—	—	0.7	—
Average					0.8	—
Standard deviation					±0.6	—
DHBA 1	114.1	0.1	—	—	114.2	97.8
DHBA 2	113.2	—	—	—	113.2	96.9
DHBA 3	107.8	—	—	—	107.8	92.2
DHBA 4	108.3	—	—	—	108.3	92.7
DHBA 5	109.6	0.1	—	—	109.7	93.9
DHBA 6	111.8	0.1	—	—	111.9	95.8
DHBA 7	112.6	—	—	—	112.6	96.4
Average					111.1†	95.1
Standard deviation					±2.5	2.2

* Source: MOORE, L., Unpublished data. U.S. Environmental Protection Agency, Cincinnati, Ohio.
† Expected value = 116.

TABLE 5710:II. SINGLE-OPERATOR PRECISION AND BIAS DATA FOR TTHM (pH = 9.2)*

Sample	THM μg/L				TTHM μg $CHCl_3$/L	Recovery %
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$		
Blank 1	3.0	0.3	—	—	3.2	—
Blank 2	1.7	0.1	—	—	1.8	—
Blank 3	1.3	0.1	—	—	1.4	—
Blank 4	1.6	0.1	—	—	1.7	—
Blank 5	2.3	0.2	—	—	2.4	—
Blank 6	2.6	0.1	—	—	2.7	—
Blank 7	2.5	0.2	—	—	2.6	—
Average					2.3	—
Standard deviation (relative standard deviation)					± 0.6 (± 26.1%)	
DHBA 1	45.4	3.3	0.1	—	47.9	98.3
DHBA 2	51.0	3.9	0.1	—	53.9	111.2
DHBA 3	39.2	3.0	0.1	—	41.4	84.3
DHBA 4	48.3	3.6	0.1	—	51.0	105.0
DHBA 5	47.6	3.7	0.1	—	50.4	103.7
DHBA 6	43.4	3.2	0.1	—	45.8	93.8
DHBA 7	46.0	3.6	0.1	—	48.7	100.0
Average					48.4	99.5
Standard deviation (relative standard deviation)					± 3.7 (± 7.6%)	
RWS 1†	33.1	17.2	11.3	0.5	52.3	—
RWS 2	31.7	16.1	10.6	0.5	49.7	—
RWS 3	38.7	18.4	11.7	0.6	59.1	—
RWS 4	35.1	18.0	11.7	0.8	55.3	—
RWS 5	36.0	17.9	11.7	0.6	56.0	—
RWS 6	38.7	18.7	11.7	0.6	59.3	—
RWS 7	37.7	18.1	11.2	0.6	57.6	—
Average					55.6	
Standard deviation (relative standard deviation)					± 3.3 (± 5.9%)	

* Source: MOORE, L., Unpublished data. U.S. Environmental Protection Agency, Cincinnati, Ohio.
† RWS = raw water sample, filtered and diluted 1 part filtrate, 2 parts organic-free water.

Table 5710:II presents the same data set, except that the pH of samples and blanks was adjusted to 9.2 with borate buffer. Also included are results for single-operator precision with filtered river-water samples that had been diluted with 2 parts organic-free water to 1 part filtrate, again using borate-buffered samples at a pH of 9.2.

5710 C. Simulated Distribution System Trihalomethanes (SDS-THM)

1. General Discussion

a. Principle: The SDS-THM testing method uses bench-scale techniques to provide an estimate of the THMs formed in a distribution system after disinfection.[1] It may be used to estimate the THM concentration at any point in a distribution system or to evaluate the formation of other disinfectant by-products (5710D). However, to measure efficiency of any unit treatment process for precursor removal, see Section 5710B.

The term "disinfection," rather than "chlorination," is used because free chlorine residuals are not necessarily provided in all distribution systems. For example, monochloramine residuals may be used.

SDS-THM concentrations measured by this procedure generally will be lower than THM concentrations measured by procedures in 5710B because disinfectant concentrations used in SDS-type samples are intended to mimic conditions in a distribution system and are almost always lower than disinfectant concentrations used with standardized formation potential procedures.

Two types of SDS procedures may be used: (1) a simple storage method that requires only the addition, at the end of the desired storage time, of a quenching agent, sodium sulfite, to a sample collected from the entry to a distribution system; and (2) a comprehensive method that involves one or more steps described in Section 5710B, with appropriate modifications.

SDS procedures are not standard procedures in the traditional sense. Test variables are modified to mimic local distribution-system conditions with bench-top procedures. These conditions include temperature, pH, disinfectant dose and residual, bromide ion concentration, and reaction time (corresponding to the residence time of water within the distribution system). However, the method used to simulate a distribution system can be standardized according to specific needs.

2. Apparatus

See 5710B.2.

Temperature control: Appropriate equipment, such as a water bath or incubator, to control sample storage temperature, capable of a range of temperature adjustments required.

3. Reagents

See 5710B.3, and also 5710D.3, if applicable.

4. Procedure

a. Simple storage procedure: Measure and report both initial and final values for all variables, especially pH, temperature, $TTHM_0$ (if desired), and residual disinfectant concentration using a method accurate to 0.1 mg/L and able to distinguish among the various disinfectant forms—see Section 4500-Cl for chlorine analyses.

Collect treated, disinfected water from the clearwell of a treatment plant or other suitable location in either a 1-L or a 250-mL completely filled bottle. Seal with a TFE-lined screw cap and store at selected temperature for selected length of time. The storage time frequently chosen is the maximum residence time within the distribution system, and the temperature is representative of the distribution system.

A second sample for immediate quenching provides an initial THM concentration if desired ($TTHM_0$). Quench by adding 2 drops (0.1 mL) sodium sulfite solution to a 25-mL glass vial, and gently and completely fill vial with sample. [NOTE: If other by-products will be analyzed, a different quenching agent may be needed (see 5710D.4).]

At end of storage period, quench a portion of stored sample with sodium sulfite solution. Report all values (pH, temperature, and residual disinfectant concentration) together with THM results. Ideally, residual disinfectant concentration after storage equals residual disinfectant concentration found in the distribution system.

b. Bench-top procedure: Use any or all steps given in Section 5710B, except that all variables, such as disinfectant dose, residual concentrations, temperature, pH, and storage time are adjusted to simulate distribution system conditions. For better sample pH control, use a buffer such as the mixed-buffer solution (5710B.3*k*). If a buffer is used, adjust buffer pH to the appropriate value before adding to the sample. Add buffer to a sample portion to be discarded after titrating to the desired pH with either NaOH or HCl solution; add this determined amount of acid or base to the buffer solution before adding to actual samples. When measuring disinfectant residuals, adjust sample pH to that required by the analytical method for the residual disinfectant, because the buffer capacity of the sample may be greater than the amount of buffer required by the method. For example, DPD Methods 4500-Cl.F and 4500-Cl.G require control of the pH within the range from 6.2 to 6.5 for stable color development, but the sample pH might be buffered to a value of 8.3, requiring adjustment of the sample pH to the appropriate range by addition of mineral acid before color development with DPD reagents.

Also add disinfectant to sample if it does not already contain enough to provide the desired disinfectant residual for the chosen storage time and temperature. Use the chlorine demand procedure (5710B.4*a*) as a guide, or develop correlations between disinfectant use and TOC or other variables.

Process sample generally following procedures in 5710B. Measure and report both initial and final values of all test

variables, especially temperature, pH, and residual disinfectant concentration, as well as all THM results.

5. Calculation

See 5710B.5. Report $TTHM_T$ values for SDS-type samples in any appropriate units, except that the concept of "formation potential" is not applicable. Use prefix "SDS-" to distinguish between "SDS-TTHM" and "THMFP" results. Also report each compound concentration (e.g., "μg/L SDS_T-$CHCl_3$").

6. Quality Control

See 5710B.6 for check on reagent purity or as a check on analytical precision and control by using a reaction with a pure, organic compound under more stable, standardized conditions. (This applies only to the formation of THM, using free chlorine.)

7. Precision and Bias

No data are available. More variability of results is expected for SDS-type testing than for samples tested with the standard conditions of 5710B. A larger number of replicates will be required for SDS procedures, as opposed to standard conditions, to obtain reliable estimates of effects of treatment changes and techniques.

8. Reference

1. KOCH, B., S.W. KRASNER, M.J. SCLIMENTI & W.K. SCHIMPFF. 1991. Predicting the formation of DBPs by the simulated distribution system. *J. Amer. Water Works Assoc.* 83 (10):62.

5710 D. Formation of Other Disinfection By-Products (DBPs)

1. General Discussion

a. Principle: The techniques and principles discussed in 5710A through C may be applied to other disinfection by-products (DBPs) and total/dissolved organic halogen (TOX/DOX) as well as for trihalomethanes (THMs). Although all the DBPs listed in this method may result from chlorination reactions, some may be formed by disinfectants other than free chlorine.

This method may be extended to cover formation potentials (5710B) and distribution system simulations (5710C) for additional by-products formed by reactions between other disinfectants (ozone, chlorine dioxide, chloramines, etc.) with dissolved organic matter. Some of the commonly found DBPs are listed in this method, but many cannot be determined because well-defined analytical methods are not yet available.

The procedures by which formation potentials and behavior in distribution systems of other DBPs can be measured are exactly as described in 5710A through C, except that different quenching agents may be required for different compounds.

In general, by-product concentrations increase with reaction time, but exceptions exist and different variables may produce different results. For example, at high pH values, THM concentrations increase with time, but if the pH is high enough, trichloroacetic acid will not form at all; at high pH, however, the concentrations of dihaloacetonitriles (DHANs) quickly reach a maximum value in a relatively short time and then decrease because of hydrolysis reactions. Further, some compounds, such as brominated haloacetic acids, are not stable and can degrade during storage—either during a long reaction time (7 d may be too long for some compounds) or after the reaction has been quenched (even if stored at 4°C).

Small concentrations of bromide ion may have significant effects. If a compound contains more than one halogen atom in its molecular formula, compounds containing all the possible combinations of mixed chloro-/bromo- formulas might also be formed.

The most common other disinfection by-products are: trihaloacetic acids (THAAs), including trichloroacetic acid (TCAA), bromodichloroacetic acid (BDCAA), dibromochloroacetic acid (DBCAA), and tribromoacetic acid (TBAA); dihaloacetic acids (DHAAs), including dichloroacetic acid (DCAA), bromochloroacetic acid (BCAA), and dibromoacetic acid (DBAA); monohaloacetic acids (MHAAs), including monochloroacetic acid (MCAA) and monobromoacetic acid (MBAA); chloral hydrate (CH); dihaloacetonitriles (DHANs), including dichloroacetonitrile (DCAN), bromochloroacetonitrile (BCAN), and dibromoacetonitrile (DBAN); 1,1,1-trichloropropanone (111-TCP); chloropicrin (CP), which may be produced either with free or combined chlorine; cyanogen chloride, formed either with free chlorine or monochloramine (and is more stable in solutions containing monochloramine); and dissolved organic halide (DOX) (see Section 5320). Total organic halide (TOX) also may be determined if the sample is not filtered.

The present method differs from those described in 5710B and C, in the means by which reactions are quenched: the different by-products formed require different quenching agents to stop reactions. Sodium sulfite is used to quench the THM reaction, but it may react with and degrade other compounds formed, such as DHANs. The procedure below lists the recommended quenching steps. Several portions of the same sample may require different quenching agents, depending upon the by-products to be determined. Use appropriate analytical methods to determine the different types of by-products formed.

2. Apparatus

See 5710B.2.

Vials: 40-mL glass vials with TFE-lined screw caps.

3. Reagents

In addition to the reagents listed in 5710B.3, the following reagents also may be needed, depending upon the by-products to be measured:

a. Ammonium chloride solution: Weigh 5 g NH_4Cl and dissolve in 100 mL organic-free water.

b. Nitric acid solution (approx. 3.5*N*): Dilute 80 mL conc HNO_3 (CAUTION: *strong oxidant*) to 250 mL with organic-free water.

c. Methyl tert-*butyl ether* (*MtBE*), highest purity.

d. Other reagents: Those required by analytical methods for determination of specific by-product concentrations.

4. Procedure

Procedures for the formation of by-products during reactions between disinfectants and dissolved organic matter have been described in 5710A through C, but the quenching agents needed to stop these reactions depend on the specific compounds to be analyzed. For THMs, the quenching agent is sodium sulfite, and its use has already been described (5710B.4*c*). For the other by-products listed above, substitute as described below. Store all samples headspace-free and sealed with TFE-lined screw caps.

a. Chloral hydrate (CH): This compound may be analyzed with THMs. Use sulfite reducing solution to quench. Adjust sample pH 6 to 7 with (1.0*N* or 0.1*N*) HCl. Determine amount of acid to be added with a separate portion containing the same amount of sulfite reducing solution added to sample. Analyze by liquid-liquid extraction, capillary column, GC/electron capture analysis.[1]

b. DHANs, CP, and 111-TCP: Add 4 drops (0.2 mL) ammonium chloride solution to a 40-mL vial and nearly fill with sample. Add a predetermined amount of HCl that will adjust sample pH to 6 to 7 (see ¶ *a* above) and fill completely. (Add 3 drops of phosphate buffer solution for more control of sample pH, if desired, before determining amount of acid solution needed for pH adjustment.) NH_4Cl quenches the reaction by converting free chlorine to monochloramine. Analyze by liquid-liquid extraction, capillary column, GC/electron capture analysis.[1]

c. Haloacetic acids (HAAs), mono-, di-, and trihaloacetic acids: Add 4 drops (0.2 mL) NH_4Cl solution to a 250-mL bottle, and fill bottle completely with sample. Before acidifying and extracting sample, add 1 mL sodium sulfite solution to the 250-mL sample, mix well, and analyze by liquid-liquid extraction, capillary column, GC/electron capture analysis (see Section 6251 or equivalent methods[2]). Sodium sulfite can slowly degrade some of the brominated haloacetic acids during storage; do not add until just before acidification. If a GC/MS method is used, remove water in the sample by drying with sodium sulfate crystals before methylation.

d. Cyanogen chloride: Analyze by GC/MS purge and trap method,[3] which uses ascorbic acid to dechlorinate samples. Also see Section 4500-CN.J. Hydrolysis of cyanogen chloride to cyanate occurs rapidly in the pH range of 8.5 to 9.0 (within 30 min), but the reaction is much slower at pH values of 7.0 and below.

e. DOX: Add 1 mL sodium sulfite solution to a 250-mL bottle and nearly fill with sample. Add sufficient 3.5*N* HNO_3 to lower pH to 2.0 (approximately 12 drops, or 0.6 mL) and fill completely. Analyze according to Section 5320. Total organic halogen (TOX) also may be determined by the same method if the sample is not filtered.

f. Other disinfection by-products not mentioned above: Disinfectants, such as chlorine, ozone, monochloramine, chlorine dioxide, etc., may form other disinfection by-products. Formation potentials or SDS-type data also can be determined for these additional compounds.

5. Calculation

Report concentrations of each compound separately in μg/L. Compare concentrations of different compounds on a micromolar basis (micromoles/L, or μM):

$$\text{Compound, } \mu M = \frac{\text{compound concentration, } \mu\text{g/L}}{MW}$$

where:

MW = molecular weight of the compound.

Concentrations of a series of compounds that can be grouped together may sometimes be reported as one value. For example, the dihaloacetic acid group (DHAAs) includes DCAA, BCAA and DBAA and may be reported in terms of a group concentration, obtained by adding the molar concentrations of the separate compounds within the class:*

$$\text{DHAA, } \mu M = \frac{\text{DCAA, } \mu\text{g/L}}{129} + \frac{\text{BCAA, } \mu\text{g/L}}{173} + \frac{\text{DBAA, } \mu\text{g/L}}{219}$$

Alternatively, report in terms of μg/L as DCAA by multiplying the molar concentration by the molecular weight of DCAA:

$$\text{DHAA, } \mu\text{g/L as DCAA} = \text{DHAA, } \mu M \times 129$$

or, for SDS testing:

$$\text{SDS-DHAA, } \mu\text{g/L as DCAA} = \text{SDS-DHAA, } \mu M \times 129$$

The definitions given in 5710A through C also are valid. For example, if the initial concentration of disinfectant by-products (DHAAs, for instance) is zero or insignificant, then:

$$\text{DHAAFP} = \text{DHAA}_7.$$

Alternatively, if there is a significant concentration of initial by-product, then:

$$\Delta\text{DHAAFP} = \text{DHAA}_7 - \text{DHAA}_0.$$

6. Quality Control

See 5710B.6 for check on reagent purity or as a check on analytical precision and control by using a reaction with a pure, organic compound under more stable, standardized conditions. The test detailed in Section 5710B.6 applies only to THM formation using excess free chlorination conditions.

* Federal regulations may require a simple sum in terms of mass units/L.

7. Precision and Bias

Precision and bias measurements depend, in part, on the analytical procedure used to measure each specific disinfectant by-product concentration. These measurements also depend upon compound properties such as stability toward oxidation and biodegradation. In general, however, formation potential reactions should be reproducible to the extent indicated in 5710B for chlorination reactions. SDS-type reactions (5710C) would not, however, be expected to be as accurate or as precise, although such reactions should predict distribution system concentrations reasonably well.

8. References

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1990. Method 551. Methods for the Determination of Organic Compounds in Drinking Water, Supplement I. Off. Research & Development, Washington, D.C.
2. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1990. Methods 552 and 552.1. Methods for the Determination of Organic Compounds in Drinking Water, Supplements I and II. Off. Research & Development, Washington, D.C.
3. FLESCH, J. & P. FAIR. 1988. The analysis of cyanogen chloride in drinking water. Proc. 16th Annu. AWWA Water Quality Technology Conf., Nov. 13–17, 1988, St. Louis, Mo., p. 465. American Water Works Assoc., Denver, Colo.

5910 UV-ABSORBING ORGANIC CONSTITUENTS*

5910 A. Introduction

1. Applications

Some organic compounds commonly found in water and wastewater, such as lignin, tannin, humic substances, and various aromatic compounds, strongly absorb ultraviolet (UV) radiation. UV absorption is a useful surrogate measure of selected organic constituents in fresh waters,[1–3] salt waters,[4–6] and wastewater.[7,8] Strong correlations may exist between UV absorption and organic carbon content, color, and precursors of trihalomethanes (THMs) and other disinfection by-products.[9,10] UV absorption also has been used to monitor industrial wastewater effluents[11] and to evaluate organic removal by coagulation,[10] carbon adsorption,[12,13,14] and other water treatment processes.[10] Specific absorption, the ratio of UV absorption to organic carbon concentration, has been used to characterize natural organic matter.[10,15,16]

Although UV absorption can be used to detect certain individual organic contaminants after separation (e.g., by HPLC), as described in Part 6000, the method described here is not suitable for detection of trace concentrations of individual chemicals. It is intended to be used to provide an *indication* of the aggregate concentration of UV-absorbing organic constituents.

2. References

1. DOBBS, R.A., R.H. WISE & R.B. DEAN. 1972. The use of ultra-violet absorbance for monitoring the total organic carbon of water and wastewater. *Water Res.* 6:1173.
2. WILSON, A.L. 1959. Determination of fulvic acids in water. *J. Appl. Chem.* 9:501.
3. COOPER, W.J. & J.C. YOUNG. 1984. Chemical non-specific organic analysis. *In* R.A. Minear & L. Keith, eds. Water Analysis, Vol. 3. Academic Press, New York, N.Y.
4. OGURA, N. & T. HANYA. 1968. Ultra-violet absorbance of the sea-water, in relation to organic and inorganic matters. *Int. J. Oceanol. Limnol.* 1:91.
5. OGURA, N. & T. HANYA. 1968. Ultra-violet absorbance as an index of pollution of seawater. *J. Water Pollut. Control Fed.* 40:464.
6. FOSTER, P. & A.W. MORRIS. 1971. The use of ultra-violet absorption measurements for the estimation of organic pollution in inshore sea waters. *Water Res.* 5:19.
7. BUNCH, R.L., E.F. BARTH & M.B. ETTINGER. 1961. Organic materials in secondary effluents. *J. Water Pollut. Control Fed.* 33:122.
8. MRKVA, M. 1971. Use of ultra-violet spectrophotometry in the determination of organic impurities in sewage. *Wasserwirtsch. Wassertech.* 21:280.
9. SINGER, P.C., J.J. BARRY, III, G.M. PALEN & A.E. SCRIVNER. 1981. Trihalomethane formation in North Carolina drinking waters. *J. Amer. Water Works Assoc.* 73:392.
10. EDZWALD, J.K., W.C. BECKER & K.L. WATTIER. 1985. Surrogate parameters for monitoring organic matter and THM precursors. *J. Amer. Water Works Assoc.* 77(4):122.
11. BRAMER, H.C., M.J. WALSH & S.C. CARUSO. 1966. Instrument for monitoring trace organic compounds in water. *Water Sewage Works* 113:275.
12. BISHOP, D.F., L.S. MARSHALL, T.P. O'FARRELL, R.B. DEAN, B. O'CONNOR, R.A. DOBBS, S.H. GRIGGS & R.V. VILLIERS. 1967. Studies on activated carbon treatment. *J. Water Pollut. Control Fed.* 39:188.
13. SONTHEIMER, H.N., E. HEILKER, M.R. JEKEL, H. NOLTE & F.H. VOLLMER. 1978. The Mülheim process. *J. Amer. Water Works Assoc.* 70(7):393.
14. SUMMERS, R.S., L. CUMMINGS, J. DEMARCO, D.J. HARTMAN, D. METZ, E.W. HOWE, B. MACLEOD & M. SIMPSON. 1992. Standardized Protocol for the Evaluation of GAC. No. 90615, AWWA Research Foundation & American Water Works Assoc., Denver, Colo.
15. THURMAN, E.M. 1985. Organic Geochemistry of Natural Waters. Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, Netherlands.
16. OWEN, D.M., G.L. AMY & Z.K. CHOWDHURY. 1993. Characterization of Natural Organic Matter and Its Relationship to Treatability, No. 90631, AWWA Research Foundation & American Water Works Assoc., Denver, Colo.

* Approved by Standard Methods Committee, 2000.
Joint Task Group: 20th Edition — James P. Malley, Jr. (chair), James K. Edzwald, Roger A. Yorton.

5910 B. Ultraviolet Absorption Method

1. General Discussion

a. Principle: UV-absorbing organic constituents in a sample absorb UV light in proportion to their concentration. Samples are filtered to control variations in UV absorption caused by particles. Adjustment of pH before filtration is optional.

UV absorption is measured at 253.7 nm (often rounded off to 254 nm). The choice of wavelength is arbitrary. Historically, 253.7 nm has been used as the standard wavelength; however, experienced analysts may choose a wavelength that minimizes interferences from compounds other than those of interest while maximizing absorption by the compound(s) of interest. If a wavelength other than 253.7 nm is used, state that wavelength when reporting results.

b. Interferences: The primary interferences in UV-absorption measurements are from colloidal particles, UV-absorbing organics other than those of interest, and UV-absorbing inorganics, notably ferrous iron, nitrate, nitrite, and bromide. Certain oxidants and reducing agents, such as ozone, chlorate, chlorite, chloramines, and thiosulfate, also will absorb ultraviolet light at 253.7 nm. Many natural waters and waters processed in drinking water treatment plants have been shown to be free of these interferences.

Evaluate and correct for UV absorption contributed by specific interfering substances. If cumulative corrections exceed 10% of the total absorption, select an alternate wavelength and/or use another method. Because UV absorption by organic matter may vary at pH values below 4 or above 10, avoid these values.[1]

A UV absorption scan from 200 to 400 nm can be used to determine presence of interferences. Typical absorption scans of natural organic matter are featureless curves of increasing absorption with decreasing wavelength. Sharp peaks or irregularities in the absorption scan may be indicative of inorganic interferences or unexpected organic contaminants. Because many organic compounds in water and wastewater (e.g., carboxylic acids and carbohydrates) do not absorb significantly in the UV wavelengths, correlate UV absorption to dissolved organic carbon (DOC) or soluble chemical oxygen demand (COD). However, use such correlations with care because they may vary from water to water, seasonally on the same water, and between raw and treated waters. In addition, chemical oxidation (e.g., ozonation, chlorination) of the organic material may reduce UV absorption without removing the organics and thus may change correlations. Because UV absorption and correlations with UV absorption are site-specific, they may not be comparable from one water source to another.

c. Minimum detectable concentration: The minimum detectable concentration cannot be determined rigorously because this is a nonspecific measurement. For precise measurement, select cell path length to provide an absorbance of approximately 0.005 to 0.900. Alternatively, dilute high-strength samples. The minimum detectable concentration of a particular constituent depends on the relationship between UV absorption, the desired characteristic (e.g., trihalomethane formation potential or DOC), and any interfering substances.

Certain regulatory programs may limit holding time prior to analysis. Check applicable regulations before sample collection.

2. Apparatus

a. Spectrophotometer, for use between 200 and 400 nm with matched quartz cells providing a light path of 1 cm. For low-absorbance samples use a path length of 5 or 10 cm. A scanning spectrophotometer is useful.

b. Filter: Use a glass-filter* without organic binder. Other filters that neither sorb UV-absorbing organics of interest nor leach interfering substances (e.g., nitrate or organics) into the water may be used, especially if colloidal matter must be removed. Alternatively use filters of TFE, polycarbonate, or silver. Prerinse filter with sample of organic-free water to remove soluble impurities. If alternate separation techniques, filters or filter preparations are used, demonstrate that equivalent results are produced. Filter pore size will influence test results, especially in raw waters. NOTE: Certain regulatory usage of this method (e.g., EPA's D/DBP Rule) may stipulate a specific pore size. Check applicable regulation before selecting product.

c. Filter assembly, glass, TFE, or stainless steel, capable of holding the selected filters.

3. Reagents

a. Organic-free water: Reagent water (see Section 1080) or equivalent water containing less than 0.05 mg DOC/L.

b. Hydrochloric acid (optional), HCl, 0.1*N*.

c. Sodium hydroxide (optional), NaOH, 0.1*N*.

d. Phosphate buffer (optional): Dissolve 4.08 g dried anhydrous KH_2PO_4 and 2.84 g dried anhydrous Na_2HPO_4 in 800 mL organic-free water. Verify that pH is 7.0 and dilute to 1 L with organic-free water. Store in brown glass bottle at 4°C. Prepare fresh weekly or more frequently if microbial growth is observed.

4. Procedure

a. Sample volume: Select sample volume on basis of the cell path length or dilution required to produce a UV absorbance between 0.005 and 0.900. For most applications a 50-mL sample is adequate. Use 100 mL sample if a 10-cm cell path length is required.

b. Sample preparation: Wash filter and filter assembly by passing at least 50 mL organic-free water through the filter. For specific applications and correlations, sample pH may be adjusted with HCl or NaOH. In poorly buffered samples an appropriate non-UV-absorbing buffer system such as a phosphate buffer may be used. Take care to avoid precipitate formation during pH adjustment. UV absorbance of fulvic acid solutions apparently remains constant between pH 4 and 10.[1] Report sample pH value used with recorded absorbance. Once sample pH has been adjusted and/or measured, filter sample. Prepare an organic-free water blank and the sample in an identical manner.

c. Spectrophotometric measurement: Let spectrophotometer equilibrate according to manufacturer's instructions. Set wave-

* Whatman grade 934AH; Gelman type A/E; Millipore type AP40; ED Scientific Specialties grade 161; or other products that give demonstrably equivalent results. Practical filter diameters are 2.2 to 4.7 cm.

TABLE 5910:I. PRECISION OF UV ANALYSES AND CORRELATION TO KHP SAMPLES

Analysis	UV_{254} Result for Given KHP Sample Concentration* cm^{-1}							
	0.54	0.93	1.79	4.87	9.61	25.0	50.0	100.0
Laboratory 1	0.008	0.015	0.034	0.079	0.158	0.323	0.638	1.282
Laboratory 2	0.009	0.016	0.026	0.070	0.134	0.401	0.803	1.612
Laboratory 3	0.010	0.017	0.027	0.081	0.161	0.353	0.695	1.343
Laboratory 4	0.007	0.020	0.033	0.070	0.132	0.319	0.750	1.590
Laboratory 5	0.009	0.018	0.030	0.087	0.140	0.394	0.643	1.447
Mean	0.0086	0.0142	0.0300	0.0774	0.1450	0.3580	0.7058	1.4548
Standard deviation	0.0011	0.0019	0.0035	0.0074	0.0136	0.0384	0.0708	0.1461
% Relative standard deviation†	12.8	11.1	11.7	9.56	9.38	10.7	10.0	10.0

* KHP sample concentration mg/L as C, measured as in Section 5310C.
† The percent relative standard deviation is given by:

$$\% RSD = \left[\frac{\text{standard deviation } (S)}{\text{mean } (\bar{X})}\right] \times 100$$

length to 253.7 nm and adjust spectrophotometer to read zero absorbance with the organic-free water blank. Measure UV absorbance at 253.7 nm of at least two filtered portions of sample at room temperature.

5. Calculation

Report mean UV absorption in units of cm^{-1} using the following notation. To report units in m^{-1} multiply the equation by one hundred.

$$UV_{\lambda}^{pH} = \left[\frac{\bar{A}}{b}\right] D$$

where:

UV_{λ}^{pH} = mean UV absorption, cm^{-1} (subscript denotes wavelength used, nm, and superscript denotes pH used if other than 7.0),

b = cell path length, cm,

$\bar{A}$ = mean absorbance measured, and

D = dilution factor resulting from pH adjustment and/or dilution with organic-free water.

$$D = \frac{\text{final sample volume}}{\text{initial sample volume}}$$

Correct results for absorption contributed by known interfering substances. If UV absorption contributed by interfering substances exceeds 10% of the total UV absorption do not use UV absorption at 253.7 nm as an indicator of organics.

TABLE 5910:II. SINGLE-OPERATOR PRECISION FOR UV ABSORPTION MEASUREMENTS OF FULVIC ACID SOLUTIONS

Replicate No.	Result cm^{-1}		
	DOC = 2.5 mg/L	DOC = 4.9 mg/L	DOC = 10.0 mg/L
1	0.110	0.240	0.480
2	0.120	0.230	0.480
3	0.110	0.240	0.470
4	0.100	0.230	0.480
5	0.110	0.240	0.480
6	0.100	0.240	0.470
7	0.110	0.240	0.480
8	0.110	0.230	0.480
9	0.120	0.240	0.480
10	0.110	0.240	0.480
Mean	0.110	0.237	0.478
Standard deviation	0.00667	0.00483	0.00422
% Relative standard deviation	6.06	2.05	0.882

6. Quality Control

a. Replicate measurements: Use at least two portions of filtered sample.

b. Duplicate analyses: Analyze every tenth sample in duplicate (i.e., duplicating the entire procedure) to assess method precision.

c. Baseline absorbance: Check system baseline UV absorbance at least after every 10 samples by measuring the absorbance of an organic-free water blank. A non-zero absorbance reading for the blank may indicate need for cell cleaning, problems with the reference cell if a dual-beam instrument is being used, or variation in the spectrophotometer response caused by heating or power fluctuations over time.

d. Spectrophotometer check: Difficulties in comparing UV absorption data from different spectrophotometers have been reported. Potassium hydrogen phthalate (KHP), also known as potassium biphthalate, standards were prepared in pH 7, phosphate-buffered (3*d*) reagent water without acidification (see Section 5310B.3*c*) and analyzed in five laboratories. The results are shown in Table 5910:I; these data suggest acceptable precision. These data also are useful for checking spectrophotometer results with KHP standards commonly used for TOC and/or COD analysis. A correlation equation for this 40-sample data set is:

$$UV_{254} = 0.0144\,KHP + 0.0018$$

with correlation coefficient (r^2) = 0.987, UV_{254} expressed in cm^{-1}, and KHP expressed as mg/L as C.

This equation can assist in verifying spectrophotometer performance. For example, if a set of UV_{254} analyses is performed and the results are in the 0.010 range, prepare a KHP standard of 0.5 mg/L as C. The projected UV_{254} of this KHP standard would be 0.009 cm^{-1}. If the measured UV_{254} is outside 13% relative standard deviation (RSD) of 0.009 cm^{-1}, the spectrophotometer may be suspect and require maintenance. The correlation between UV_{254} and KHP standards is presented solely as a useful means of verifying spectrophotometer performance.

7. Precision and Bias

Table 5910:I shows interlaboratory precision data for 40 KHP samples. The percent relative standard deviations (% RSD) ranged from 9.38 to 12.8.

Single-operator precision data are presented in Table 5910:II for fulvic acid solutions.[2] The % RSD ranged from 0.9 to 6%. Because UV absorption is an aggregate measure of organic carbon, true standards are not available and bias cannot be determined.

The precision of analyses by this method was determined under the Information Collection Rule.[3] Precision was determined as relative percent difference for duplicate analyses and was calculated only when both analyses in the duplicate pair showed concentrations at, or greater than, the ICR minimum reporting level (MRL= 0.009 cm^{-1}). Results were as follows:[4]

Data Quality Variable	N	Percentile 10	25	50 (median)	75	90
Precision (RPD)	33 306*	0.0	0.0	0.0	2.2	4.9

* 2744 excluded — both samples less than MRL.
NOTE: Median sample result was 0.040 cm^{-1}.

8. References

1. EDZWALD, J.K., W.C. BECKER & K.L. WATTIER. 1985. Surrogate parameters for monitoring organic matter and THM precursors. *J. Amer. Water Works Assoc.* 77(4):122.
2. MALLEY, J.P., JR. 1988. A Fundamental Study of Dissolved Air Flotation for Treatment of Low Turbidity Waters Containing Natural Organic Matter. Ph.D. thesis (unpublished), Univ. Massachusetts, Amherst.
3. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1996. Monitoring requirements for Public Drinking Water Supplies: Cryptosporidium, Giardia, Viruses, Disinfection Byproducts, Water Treatment Plant Data and Other Information Requirements. Final Rule. *Federal Register.* 61(94):24354.
4. FAIR, P.S., R. K. SORRELL, M. KARAPONDO, B.J. BOHL, C.P. FREBIS & M.A. FEIGE. 2002. Quality of ICR monitoring data. *In* M. J. McGuire, J. McLain & A. Oblensky, eds. Information Collection Rule Data Analysis. American Water Works Assoc. Research Foundation, Denver, Colo.