PART 4000

INORGANIC NONMETALLIC CONSTITUENTS

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4120 Segmented Continuous Flow Analysis ..... Theresa M. Wright
4130 Inorganic Nonmetals by Flow Injection Analysis ..... Scott Stieg
4140 Inorganic Anions by Capillary Ion Electrophoresis ..... Roy-Keith Smith
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4500-CN^- Cyanide ..... Scott Stieg, Roy-Keith Smith
4500-Cl^- Chloride ..... Scott Stieg
4500-ClO_2 Chlorine Dioxide ..... Robert P. Fisher
4500-F^- Fluoride ..... Scott Stieg
4500-I Iodine ..... George T.F. Wong
4500-I^- Iodide ..... George T.F. Wong
4500-IO_3^- Iodate ..... George T.F. Wong
4500-N Nitrogen ..... Scott Stieg, Thomas R. Holm
4500-NH_3 Nitrogen (Ammonia) ..... Scott Stieg
4500-NO_3^- Nitrogen (Nitrate) ..... Scott Stieg, Thomas R. Holm
4500-N_{org} Nitrogen (Organic) ..... Scott Stieg
4500-O_3 Ozone (Residual) ..... Kerwin Rakness
4500-P Phosphorus ..... Scott Stieg, William Nivens
4500-$KMnO_4$ Potassium Permanganate ..... Philip A. Vella
4500-SiO_2 Silica ..... Scott Stieg
4500-S^{2-} Sulfide ..... Scott Stieg, Thomas R. Holm
4500-SO_4^{2-} Sulfate ..... Scott Stieg

Summary of Major Changes since 1998

Determination of Anions by Ion Chromatography (4110) contains a new method using single-column ion chromatography with direct conductivity detection, a new ion chromatographic method for the determination of oxyhalides and bromide, and new material on minimum detectable concentrations and limitations of the method. Nitrogen (4500-N) includes a new conductimetric method for inorganic nitrogen. A new method added to Nitrogen (Nitrate) (4500-NO_3^-) uses the second derivative of a sample spectrum and eliminates the background contribution from natural organic matter. A new persulfate method permitting the simultaneous determination of total nitrogen as well as total phosphorus appears in Phosphorus (4500-P). Sulfide (4500-S^{2-}) includes a new method for acid-volatile sulfide, as well as revisions to the determination of un-ionized hydrogen sulfide, and new precision and bias data in the methylene blue flow injection analysis.

Revisions to Cyanide (4500-CN^-) include new techniques for preservation of samples and the designation of magnesium chloride as optional in the distillation procedure.

4010 INTRODUCTION

The analytical methods included in this part make use of classical wet chemical techniques and their automated variations and such modern instrumental techniques as ion chromatography. Methods that measure various forms of chlorine, nitrogen, and phosphorus are presented. The procedures are intended for use in the assessment and control of receiving water quality, the treatment and supply of potable water, and the measurement of operation and process efficiency in wastewater treatment. The methods also are appropriate and applicable in evaluation of environmental water-quality concerns. The introduction to each procedure contains reference to special field sampling conditions, appropriate sample containers, proper procedures for sampling and storage, and the applicability of the method.

4020 QUALITY ASSURANCE/QUALITY CONTROL

4020 A. Introduction

Without quality control results there is no confidence in analytical results reported from tests. As described in Part 1000 and 4020B, essential quality control measurements include: method calibration, standardization of reagents, assessment of individual capability to perform the analysis, performance of blind check samples, determination of the sensitivity of the test procedure (method detection level), and daily evaluation of bias, precision, and the presence of laboratory contamination or other analytical interference. Details of these procedures, expected ranges of results, and frequency of performance should be formalized in a written Quality Assurance Manual and Standard Operating Procedures.

For some of the procedures contained in Part 4000, the traditional determination of bias, using a known addition to either a sample or a blank, is not possible. Examples of these procedures include pH, dissolved oxygen, residual chlorine, and carbon dioxide. The inability to perform a reliable known addition does not relieve the analyst of the responsibility for evaluating test bias. Analysts are encouraged to purchase certified ready-made solutions of known levels of these constituents as a means of measuring bias. In any situation, evaluate precision through analysis of sample duplicates.

Participate in a regular program (at a minimum, annually, and preferably semi-annually) of proficiency testing (PT)/performance evaluation (PE) studies. The information and analytical confidence gained in the routine performance of the studies more than offset any costs associated with these studies. An unacceptable result on a PT study sample is often the first indication that a test protocol is not being followed successfully. Investigate circumstances fully to find the cause. Within many jurisdictions, participation in PT studies is a required part of laboratory certification.

Many of the methods contained in Part 4000 include specific quality-control procedures. These are considered to be the minimum quality controls necessary to successful performance of the method. Additional quality control procedures can and should be used. Note that some regulatory programs may require additional QC or have alternative acceptance limits. Section 4020B describes a number of QC procedures that are applicable to many of the methods.

4020 B. Quality Control Practices

1. Initial Quality Control

a. Initial demonstration of capability: Verify analyst capability before analyzing any samples and repeat periodically to demonstrate proficiency with the analytical method. Verify that the method being used provides sufficient sensitivity for the purpose of the measurement. Test analyst capability by analyzing at least four reagent water portions containing known additions of the analyte of interest. Confirm proficiency by generating analytical results that demonstrate precision and bias within acceptable limits representative of the analytical method.

b. Method detection level (MDL): Before samples are analyzed, determine the MDL for each analyte by the procedures of Section 1030, or other applicable procedure.[1] Determine MDL at least annually for each method and major matrix category. Verify MDL for a new analyst or whenever instrument hardware or method operating conditions are modified. Analyze samples for MDL determinations over a 3- to 5-d period to generate a realistic value. Preferably use pooled data from several analysts rather than data from a single analyst.

c. Dynamic range (DR): Before using a new method, determine the dynamic range, i.e., the concentration range over which a method has an increasing response (linear or second-order), for each analyte by analyzing several standard solutions that bracket the range of interest. Each standard measurement should be within 10% of the true value for acceptance into the DR determination. Take measurements at both the low and high end of the calibration range to determine method suitability. Analytical instrumentation with curve-fitting features may allow utilization

of nonlinear instrument response. Not all methods permit the use of non-linear responses.

2. Calibration

a. Initial calibration: Calibrate initially with a minimum of a blank and three calibration standards of the analyte(s) of interest. Select calibration standards that bracket the expected concentration of the sample and that are within the method's dynamic range. The number of calibration points depends on the width of the dynamic range and the shape of the calibration curve. One calibration standard should be at or below the reporting limit for the method. As a general rule, differences between calibration standard concentrations should not be greater than one order of magnitude (i.e., 1, 10, 100, 1000). Most methods for inorganic nonmetals do not have wide dynamic ranges. Standards for initial calibration therefore should be spaced more closely than one order of magnitude under these circumstances. Apply linear or polynomial curve-fitting statistics, as appropriate, for analysis of the concentration-instrument response relationship. The appropriate linear or nonlinear correlation coefficient for standard concentration to instrument response should be ≥ 0.995. Use initial calibration for quantitation of analyte concentration in samples. Use calibration verification, ¶ *b* below, only for checks on the initial calibration and not for sample quantitation. Repeat initial calibration daily and whenever calibration verification acceptance criteria are not satisfied.

b. Calibration verification: Calibration verification is the periodic confirmation that instrument response has not changed significantly from the initial calibration. Verify calibration by analyzing a midpoint or lower calibration standard (check standard) and calibration blank at the beginning and end of a sample run, periodically during a run (normally after each set of ten samples). A check standard determination outside 90 to 110% of the expected concentration indicates a potential problem. If a check standard determination is outside 90 to 110% of the expected concentration, immediately cease sample analyses and initiate corrective action. Some methods may have wider or narrower limits specified. Repeat initial calibration and sample determinations since the last acceptable calibration verification. Alternatively, verify calibration with two standards, one near the low end and one near the high end, if the blank is used to zero the instrument, and use 90 to 110% or, if available, method-specified limits. Use calculated control limits (Section 1020B) to provide better indications of system performance and to provide tighter limits.

c. Quality control sample: Analyze an externally generated quality control sample of known concentration at least quarterly and whenever new calibration stock solutions are prepared. Obtain this sample from a source external to the laboratory or prepare it from a source different from those used to prepare working standards. Use to validate the laboratory's working standards both qualitatively and quantitatively.

3. Batch Quality Control

a. Method blank (MB): A method blank (also known as reagent blank) is a portion of reagent water treated exactly as a sample, including exposure to all equipment, glassware, procedures, and reagents. The MB is used to assess whether analytes or interference are present within the analytical process or system. No analyte of interest should be present in the MB at a warning level based on the end user's requirements. Undertake immediate corrective action for MB measurements above the MDL. Include a minimum of one MB with each set of 20 or fewer samples.

b. Laboratory-fortified blank (LFB): The laboratory-fortified blank (also known as blank spike) is a method blank that has been fortified with a known concentration of analyte. It is used to evaluate ongoing laboratory performance and analyte recovery in a clean matrix. Prepare fortified concentrations approximating the midpoint of the calibration curve or lower with stock solutions prepared from a source different from those used to develop working standards. Preferably alternate concentrations for the LFB to cover different parts of the calibration curve. Calculate percent recovery, plot control charts, and determine control limits (Section 1020B) for these measurements. Ensure that the LFB meets performance criteria for the method when such criteria are specified. Establish corrective actions to be taken in the event that LFB does not satisfy acceptance criteria. Include a minimum of one LFB with each set of 20 or fewer samples.

c. Duplicates: Use duplicate samples of measurable concentration to measure precision of the analytical process. Randomly select routine samples to be analyzed twice. Process duplicate sample independently through entire sample preparation and analytical process. Include a minimum of one duplicate for each matrix type with each set of 20 or fewer samples. Calculate control limits for duplicates when method-specific limits are not provided.

d. Laboratory-fortified matrix (LFM)/Laboratory-fortified matrix duplicate: Use LFM (also known as matrix spike) and LFM duplicate to evaluate the bias and precision, respectively, of the method as influenced by a specific matrix. Prepare by adding a known concentration of analytes to a randomly selected routine sample. Prepare addition concentrations to approximately double the concentration present in the original sample. If necessary, dilute sample to bring the measurement within the established calibration curve. Limit addition volume to 5% or less of sample volume. Calculate percent recovery and relative percent difference, plot control charts, and determine control limits (Section 1020B). Ensure the performance criteria for the method are satisfied. Process fortified samples independently through entire sample preparation and analytical process. Include a minimum of one LFM/LFM duplicate with each set of 20 or fewer samples. Preferably rotate concentrations over a range to verify performance at different levels.

4. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1995. Definition and procedure for the determination of the method detection limit, revision 1.11. 40 CFR Part 136, Appendix B. *Federal Register* 5:23703.

4110 DETERMINATION OF ANIONS BY ION CHROMATOGRAPHY*

4110 A. Introduction

Determination of the common anions such as bromide, chloride, fluoride, nitrate, nitrite, phosphate, and sulfate often is desirable to characterize a water and/or to assess the need for specific treatment. More recently, the need to measure the concentration of the disinfection by-products chlorite, chlorate, and bromate has arisen. Although conventional colorimetric, electrometric, or titrimetric methods are available for determining individual anions, ion chromatography provides a single instrumental technique that may be used for their rapid, sequential measurement. Ion chromatography eliminates the need to use hazardous reagents and it effectively distinguishes among the halides (Br^-, Cl^-, and F^-) and the oxyhalides (ClO_2^-, ClO_3^-, and BrO_3^-), and the oxy-ions (PO_4^{3-}, SO_4^{2-}, NO_2^-, and NO_3^-).

Methods 4110B and 4110C are applicable, after filtration to remove particles larger than 0.45 μm, to surface, ground, and wastewaters as well as drinking water. Some industrial process waters, such as boiler water and cooling water, also may be analyzed by this method. Method 4110D is applicable to untreated and finished drinking water as well as drinking water at various stages of treatment.

* Approved by Standard Methods Committee, 2000.
Joint Task Group: Richard Mosher (chair), Bruce A. Hale, Daniel P. Hautman, Peter E. Jackson, S. V. Karmarkar, James Krol, James W. O'Dell, Roy-Keith Smith.

4110 B. Ion Chromatography with Chemical Suppression of Eluent Conductivity

1. General Discussion

a. Principle: A water sample is injected into a stream of eluent and passed through a series of ion exchangers. The anions of interest are separated on the basis of their relative affinities for a low-capacity, strongly basic anion exchanger (guard and analytical columns). The separated anions are directed through a suppressor device that provides continuous suppression of eluent conductivity and enhances analyte response. In the suppressor the separated anions are converted to their highly conductive acid forms while the conductivity of the eluent is greatly decreased. The separated anions in their acid forms are measured by conductivity. They are identified on the basis of retention time as compared to standards. Quantitation is by measurement of peak area or peak height.

b. Interferences: Any substance that has a retention time coinciding with that of any anion to be determined and produces a detector response will interfere. Low-molecular-weight organic acids, bromate, and chlorite may interfere with the determination of chloride and fluoride. A high concentration of any one ion also interferes with the resolution, and sometimes retention, of others. Sample dilution or gradient elution overcomes many interferences. To resolve uncertainties of identification or quantitation use the method of known additions. Spurious peaks may result from contaminants in reagent water, glassware, or sample processing apparatus. Modifications such as preconcentration of samples, gradient elution, or reinjection of portions of the eluted sample may alleviate some interferences but require individual validation for precision and bias and are beyond the scope of this method.

c. Method detection level: The detection level of an anion is a function of sample size. Table 4110:I presents detection levels obtained for reagent water with a 25-μL sample loop. Detection levels in natural waters may be substantially higher because of the presence of high levels of some of the anions.

TABLE 4110:I. DETECTION LEVEL FOR ANIONS IN REAGENT WATER.*

Anion	MDL μg/L
Fluoride	2.0
Chloride	4.0
Nitrite-N	3.7
Bromide	14
Nitrate-N	2.7
Orthophosphate-P	14
Sulfate	18

* See Figure 4110:1 for experimental conditions.

d. Limitations: Exercise caution if this method is used to determine F^- in unknown matrices. Two problems are commonly encountered: first, with some column/eluent combinations the fluoride peak elutes very close to the baseline depression caused by the elution of water, the so-called "water dip". This may cause difficulty in quantitating samples with low fluoride concentrations; second, the simple organic acids (formic, acetate) elute close to fluoride and may interfere. Determine precision and bias before analyzing samples. If fluoride is to be determined, preferably select a column/eluent combination that resolves water, fluoride, and simple organic acids.

Because of the utilization of nitrate, nitrite, and phosphate as nutrients by some species of bacteria, store samples at 4°C and analyze within 48 h. Disinfected samples to be analyzed for nitrate may be held for up to 14 d because all nitrite will already be converted to nitrate. Store samples to be analyzed for sulfate

at 4°C and analyze within 28 d. The other analytes do not require cold storage. Complete analysis within 28 d.

2. Apparatus

a. Ion chromatograph, including an injection valve, a sample loop, guard column, analytical column, suppressor device, a temperature-compensated small-volume conductivity cell and detector (6 μL or less), and an electronic peak integrator or chromatography data acquisition system. Use an ion chromatograph capable of delivering 2 to 5 mL eluent/min at a pressure of 5600 to 28 000 kPa (800 to 4000 psi).

b. Analytical column: Any commercially available anion-exchange column capable of resolving fluoride, bromide, chloride, nitrate, nitrite, phosphate, and sulfate is acceptable.*

c. Guard column, identical to separator column† to protect analytical column from fouling by particulates or organics.

d. Suppressor device:‡ Place this ion-exchange-based device between column and detector to reduce background conductivity of the eluent and enhance conductivity of the target analytes. Several such devices with different operational principles are available commercially; any that provides the required sensitivity and baseline stability may be used.

3. Reagents

a. Reagent water free from interferences at the method detection level of each constituent with 18 megohm resistivity and containing no particles larger than 0.2 μm. See Section 1080.

b. Eluent solution, appropriate to column used to resolve target anions. The eluent used to produce the chromatogram in Figure 4110:1 was sodium bicarbonate-sodium carbonate, 1.7 m*M* $NaHCO_3^-$, 1.8 m*M* Na_2CO_3. Dissolve 0.5712 g $NaHCO_3$ and 0.7632 g Na_2CO_3 in water and dilute to 4 L. Degas eluent before use either by vacuum filtration to simultaneously remove particles greater than 0.45 μm or by purging with helium for 10 min.

c. Regenerant solution: Required with some types of suppressors. See manufacturer's recommendations.

d. Standard anion solutions, 1000 mg/L: Purchase stock standard solutions as certified solutions or prepare from ACS reagent-grade salts. Prepare standard anion solutions by weighing the indicated amount of salt (Table 4110:II), dried to a constant weight at 105°C, and diluting to 100 mL with distilled/deionized water. Store in plastic bottles at 4°C. These solutions are stable for at least 6 months except for the nitrite and phosphate standards, which should be discarded after 1 month. Prepare most dilute working standards monthly, nitrite and phosphate, daily.

4. Procedure

a. System equilibration: Turn on ion chromatograph and adjust eluent flow rate to manufacturer's recommendations for the column/eluent combination being used. A representative chromatogram is presented in Figure 4110:1. Adjust detector to desired setting (usually 10 to 30 μS) and let system come to equilibrium (15 to 20 min). A stable base line indicates equilibrium conditions. Adjust detector offset to zero out eluent conductivity. If regenerant is used with the suppressor, adjust flow rate to manufacturer's specifications.

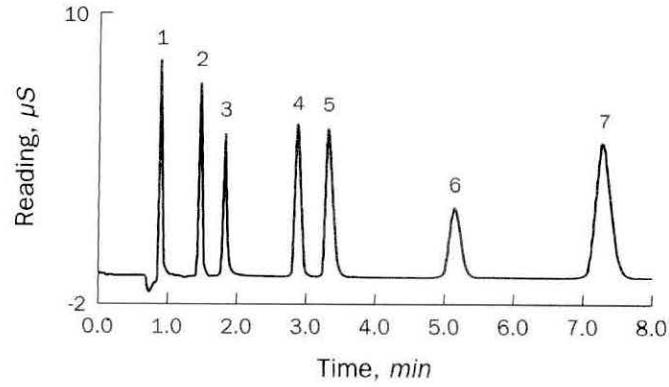

Figure 4110:1. Typical inorganic anion separation. Eluent: 1.7 m*M* $NaHCO_3$, 1.8 m*M* $NaCO_3$; sample loop: 25 μL; flow rate: 2.0 mL/min; column: Dionex AG4A-SC plus AS4A-SC.

Anion	Conc *mg/L*
1. Fluoride	2.0
2. Chloride	3.0
3. Nitrite	5.0
4. Bromide	10.0
5. Nitrate	10.0
6. Orthophosphate	15.0
7. Sulfate	15.0

b. Calibration: Inject standards containing a single anion or a mixture and determine approximate retention times. Observed times vary with conditions. If the analytical column and eluent mentioned in ¶s 2*b* and 3*b*, respectively, are used, retention always is in the order F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, and SO_4^{2-}. Inject at least three different concentrations for each anion to be measured. Use concentrations that will bracket the expected analyte concentrations in samples. Construct a calibration curve by plotting peak height or area versus concentration using appropriate software. Verify calibration curves with a mid-range check standard from a source independent of that of the calibration standards. Check validity of existing calibration curves daily with a mid-range calibration standard. Results should be within 10% of original curve at mid-range. Recalibrate

TABLE 4110:II. STOCK STANDARD PREPARATIONS

Anion	Salt	Amount *g/100 mL*
Fluoride	NaF	0.2210
Chloride	NaCl	0.1649
Bromide	NaBr	0.1288
Nitrate-N*	$NaNO_3$	0.6068
Nitrite-N*	$NaNO_2$	0.4926
Phosphate-P	KH_2PO_4	0.4394
Sulfate	K_2SO_4	0.1814

* Do not oven-dry. Dry to constant weight in a dessicator.

* Dionex P/N 37041, Lachat P/N 28084, Waters P/N 26765, or equivalent.
† Dionex P/N 37042, Lachat P/N 28085, or equivalent.
‡ Dionex P/N 46081, Lachat P/N 28097, Alltech P/N 535101, or equivalent.

TABLE 4110:III. SINGLE-LABORATORY PRECISION (ONE STANDARD DEVIATION) AND BIAS DATA FOR 30 SETS OF SAMPLES OVER A 2-MONTH-PERIOD

Element	LFB Concentration mg/L	LFB Recovery and Precision %	Known Addition Concentration mg/L	Known Addition Recovery and Precision %
Chloride	25	104 ± 4.5	25	107 ± 10
Nitrite as N	1	97 ± 4	1	103 ± 7
Bromide	0.02	101 ± 8	0.1 to 0.5	106 ± 10
Bromide	0.3	102 ± 3	—	—
Nitrate as N	2.5	106 ± 2.6	2.5	113 ± 5
Orthophospate-P	10	101 ± 4	10	102 ± 4
Sulfate	50	105 ± 4	50	111 ± 6

whenever the detector setting, eluent, or regenerant is changed. To minimize the effect of the "water dip"§ on F^- analysis, analyze standards that bracket the expected result or eliminate the water dip by diluting the sample with eluent or by adding concentrated eluent to the sample to give the same concentration as in the eluent. If sample adjustments are made, adjust standards and blanks identically.

If linearity is established ($r \geq 0.99$) over the calibration range, the average response factor is acceptable. Record peak height or area for calculation of the response factor, RF. HPO_4^{2-} is nonlinear below 1.0 mg/L.

c. Sample analysis: If sample is collected with an autosampler that does not automatically filter samples, remove particulates by filtering through a prewashed 0.45-μm (or smaller) pore membrane. With either manual or automated injection, flush loop with several volumes of sample. Take care to prevent carryover of analytes from samples of high concentration. After last peak has appeared and detector signal has returned to base line, another sample can be injected.

d. Solid matrices: Soluble forms of the target anions may be determined in solid matrices (soils, sludges) after extraction and filtration of the extract. A slurry of the solid to be extracted is prepared with either reagent water or eluent and is either shaken or sonicated. (A representative standardized method for such extractions is available.[1]) Document the precision of the extraction process and the analyte recovery achieved by analyzing duplicate laboratory-fortified matrices for each distinct matrix.

5. Calculations

Determine the concentration of each anion, in milligrams per liter, by referring to the appropriate calibration curve. Alternatively, when the response is shown to be linear, use the following equation:

$$C = H \times RF \times D$$

where:

C = mg anion/L,
H = peak height or area,
RF = response factor = concentration of standard/height (or area) of standard, and
D = dilution factor.

§ Water dip occurs because water conductivity in sample is less than eluent conductivity (eluent is diluted by water).

6. Quality Control

See Section 4020 for minimum QC guidelines. Preferably check recovery daily at reporting level using a reporting-level standard. Recovery should be between 75 and 125%. Alternate analysis of mid-range and high-range check standards after each 10 samples. Recovery should be between 90 and 110%. If the results are to be used for environmental compliance monitoring, document precision and accuracy of the method by the analysis of four replicates of a mid-range calibration standard and calculation of the average percent recovery, and the standard deviation of the recoveries, for each analyte. Additional QC may be required for regulatory purposes.

7. Precision and Bias

Multilaboratory data from a joint validation study with EPA and ASTM using older columns and hardware can be found in the 20th Edition of *Standard Methods*. Table 4110: III shows single-laboratory recoveries for laboratory-fortified blanks (LFB) and known additions to a variety of raw waters and finished drinking waters obtained with columns and equipment described herein.

8. Reference

1. AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1992. Method D3987. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing & Materials, Philadelphia, Pa.

9. Bibliography

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PFAFF, J.D., D.F. HAUTMAN & D.J. MUNCH. 1997. Determination of Inorganic Anions in Drinking Water by Ion Chromatography. Method 300.1, U.S. Environmental Protection Agency, National Exposure Research Lab., Off. Research & Development, Cincinnati, Ohio.

4110 C. Single-Column Ion Chromatography with Direct Conductivity Detection

1. General Discussion

a. Principle: An aqueous sample is injected into an ion chromatograph consisting of an injector port, analytical column, and conductivity detector. The sample merges with the eluent stream and is pumped through the analytical column where the anions are separated on the basis of their affinity for the active sites of the column packing material. Direct conductivity detection without chemical suppression is used to determine concentrations.

b. Interferences: See 4110B.1*b*. Note that HCO_3^- elutes between F^- and Cl^- with this method. See Figure 4110:2.

Because method sensitivity is high, take care to avoid contamination by reagent water and equipment.

c. Method detection level: The detection level of an anion is a function of sample volume injected and the signal-to-noise ratio of the detector electronics. Generally, minimum detectable concentrations are about 0.02 to 0.12 mg/L (20 to 120 μg/L) for the anions with an injection volume of 100 μL (Table 4110:IV). Larger injection volumes can reduce detection levels. However, coelution is a possible problem with large injection volumes. Determine method detection level for each anion of interest under the conditions used to produce reportable data.

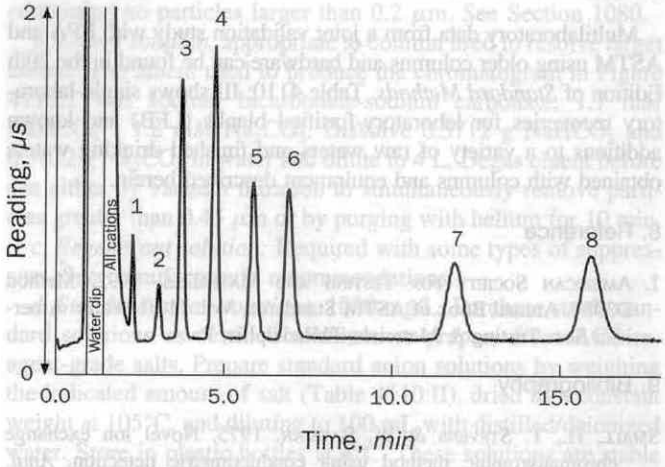

Figure 4110:2. Typical inorganic anion separation. Eluent: borate/gluconate containing 12% acetonitrile; flow rate: 1.0 mL/min; injection volume: 100 μL; column: Waters IC Pak A/HR; background conductivity: 240 μS.

Anion	Conc mg/L
1. Fluoride	1.0
2. Bicarbonate	—*
3. Chloride	2.0
4. Nitrite	4.0
5. Bromide	4.0
6. Nitrate	4.0
7. Orthophosphate	6.0
8. Sulfate	4.0

* Not quantified.

TABLE 4110:IV. DETECTION LEVEL FOR ANIONS IN REAGENT WATER

Anion	MDL μg/L
Fluoride	40
Chloride	20
Nitrite-N	15
Bromide	75
Nitrate-N	17
Orthophosphate-P	40
Sulfate	75

2. Apparatus

a. Ion chromatograph, complete with all required accessories including syringes, analytical columns, detector, and a data system. Required accessories are listed below.

b. Guard column, placed before analytical column to protect it from fouling by particulates or organic constituents.*

c. Analytical column, packed with low-capacity anion-exchange resin capable of resolving fluoride, chloride, nitrite, bromide, nitrate, orthophosphate, and sulfate.†

d. Conductivity detector, flow-through, with integral heat-exchange unit allowing direct temperature control and with separate working and reference electrodes.

e. Pump, constant flow between 0.5 and 5.0 mL/min at a pressure of 5600 to 28 000 kPa (800 to 4000 psi).

f. Data system, preferably computer with software capable of acquiring and processing chromatographic data. An integrator also may be used.

g. Sample injector: Either an automatic sample processor or a manual injector. The automatic device must be able to inject a minimum sample volume of 10 μL.

3. Reagents

a. Reagent water: See Section 4110B.3*a*.

b. Borate/gluconate concentrate: Place approximately 25 g of a strong cation-exchange resin‡ in the hydrogen form in a 60-mL plastic syringe fitted with a 0.45-μm filter. Wash resin with five 20-mL portions of deionized water. Dissolve 9.06 g sodium gluconate in 20 mL deionized water. Transfer this solution to the 60-mL syringe and slowly pass through the syringe into a 1-L volumetric flask. Wash resin with five 20-mL portions deionized water, adding washings to flask. Discard syringe and resin. Alternatively use commercially available 50% gluconic acid. This usually comes as a brown solution that when diluted gives a yellow tint to the eluent that may affect long-term performance. Remove color by passing the 50% gluconic acid through a C_{18} cartridge (5 mL/cartridge). Use 13.2 mL of the 50% gluconic acid for the eluent concentrate.

* Waters P/N WAT010551 or equivalent.
† Waters P/N WAT0026765 or equivalent.
‡ Bio-Rad AG-50W-X12 or equivalent.

Adjust volume in the 1-L volumetric flask to approximately 500 mL with deionized water and add a stirring bar. Add 7.2 g lithium hydroxide monohydrate and 25.5 g boric acid. Stir until all reagents are dissolved. Add 94 mL 95% glycerol and mix well. Remove stirring bar and dilute to 1 L. This may be stored at room temperature for 6 months or at 4°C for 1 year (warm to ambient before use). Discard if evidence of microbial growth appears.

c. Eluent solution, 8.25 m*M* borate, 0.83 m*M* gluconate, 12% (v/v) acetonitrile: Combine 20 mL borate/gluconate concentrate and 120 mL HPLC-grade acetonitrile, and dilute to 1 L with reagent water. Vacuum filter through a 0.45-μm- (or smaller) pore size membrane before use.

d. Stock standard solutions: See 4110B.3*d*.

4. Procedure

a. System equilibration: Set up ion chromatograph in accordance with the manufacturer's directions. Install guard and separator columns and begin pumping eluent until a stable base line is achieved. The background conductivity of the eluent solution is 240 ± 20 μS.

b. Calibration: Determine retention time for each anion by injecting a standard solution containing only the anion of interest and noting the time required for a peak to appear. Retention times may vary with operating conditions and with anion concentration. The order of elution is shown in Figure 4110:2.

Construct a calibration curve by injecting prepared standards including each anion of interest. Use at least three concentrations plus a blank. Bracket the range of concentrations expected for samples. Construct calibration curve by plotting either peak height or peak area versus concentration. If a data system is being used, make a hard copy of the calibration curve available.

After generating calibration curves, verify them with a mid-range check standard from a source independent of that of the calibration standards. Verify working calibration curves daily by injecting a mid-range standard. Agreement should be within 10%. Also verify curves with each batch of eluent. If linearity is established over the calibration range, the average response factor is acceptable. Record peak height or area for calculation of the response factor, RF.

c. Sample analysis: Inject sufficient sample (about two to three times the loop volume) to insure that sample loop is properly flushed. Let all peaks elute before injecting another sample.

TABLE 4110:V. SINGLE-COLUMN CHROMATOGRAPHY SINGLE-OPERATOR PRECISION AND BIAS*

Anion	Sample Type†	Amount Added *mg/L*	Mean Recovery *%*	SD *mg/L*
Cl^-	RW	16	105	1.6
	DW	16	98	1.9
NO_2^--N	RW	4	101	0.10
	DW	4	101	0.43
Br^-	RW	16	104	0.75
	DW	16	98	2.3
NO_3^--N	RW	8	103	0.75
	DW	8	87	1.9
PO_4^{3-}-P	RW	16	113	0.92
	DW	16	110	1.6
SO_4^{2-}	RW	32	101	0.42
	DW	32	94	4.8

* Data provided by EPA/EMSL (NERL), Cincinnati, OH 45268. Seven replicates were analyzed for each anion and sample type.
† RW=reagent water; DW=drinking water.

Compare response in peak height or peak area and retention time to values obtained in calibration.

5. Calculation

See Section 4110B.5.

6. Quality Control

See Section 4110B.6.

7. Precision and Bias

Precision and bias data are given in Table 4110:V. These data were produced in 1988. NOTE: The columns and other hardware discussed herein were not available when the study was conducted. The advances in technology that have occurred since that time should be considered when using these data.

8. Reference

1.. GLASER, J., D. FOERST, G. MCKEE, S. QUAVE & W. BUDDE. 1981. Trace analyses for wastewater. *Environ. Sci. Technol.* 15:1426.

4110 D. Ion Chromatographic Determination of Oxyhalides and Bromide

1. General Discussion

a. Principle: See Section 4110B.1.

b. Interferences: The need to quantitate low levels of disinfection by-products and their precursors in the presence of much higher levels of the common anions poses an analytical problem. Any ionic material that coelutes with a target analyte will interfere with the determination of that analyte. Bromate has been shown to be subject to positive interferences in some matrices. The interference is noticeable usually as a flattened peak. It often can be eliminated by passing the sample through an H^+ cartridge.* This problem may be addressed by selection of a different column/eluent combination or by dilution of the eluent, which will increase retention times and spread the chromato-

* Dionex PN 039596.

TABLE 4110:VI. DETECTION LEVEL FOR ANIONS IN REAGENT WATER*

Anion	MDL μg/L
Bromide	0.98
Bromate	1.32
Chlorate	2.55
Chlorite	1.44

* Determined under the following operating conditions: Eluent: 9 m*M* Na_2CO_3; flow rate: 1.25 mL/min; columns: Dionex AG9-HC and AS9-HC, 4 mm; sample loop: 200 μL; suppressor: Dionex ASRS-I, 300 mA in external water mode; detector: Dionex CD20.
Source: PFAFF, J.D., D.F. HAUTMAN & D.J. MUNCH. 1997. Determination of Inorganic Ions in Drinking Water by Ion Chromatography. EPA Method 300.1, U.S. Environmental Protection Agency, National Exposure Research Lab., Off. Research & Development, Cincinnati, Ohio.

gram. Additionally, chloride or a non-target analyte present in unusually high concentration may overlap with a target analyte sufficiently to cause problems in quantitation or may cause retention-time shifts. Dilution of the sample may resolve this problem. Care must be exercised to avoid carryover from a sample of high concentration to the subsequent sample. Method interferences also may be caused by contamination of reagents, reagent water, glassware, syringes, and other equipment used to process samples.

c. Method detection level: The detection levels for bromide, bromate, chlorate, and chlorite in reagent water free of interfering anions are shown in Table 4110:VI.

d. Sample collection and storage: Collect samples to be analyzed for chlorite in opaque containers and store them at 4°C. All of the oxyhalides, including chlorite, require preservation with 50 mg/L ethylenediamine (EDA).

Residual chlorine dioxide present in the sample will result in the formation of additional chlorite between the times of sampling and analysis. If chlorine dioxide is suspected to be present, purge sample with an inert gas such as nitrogen, helium, or argon for approximately 4 min at time of collection, before adding ethylenediamine preservative.

2. Apparatus

a. Ion chromatograph: See Section 4110B.2*a*.

b. Analytical column: Any commercially available column† capable of resolving the oxyhalides chlorite, chlorate, and bromate, from the other anions that may be present in drinking water, including fluoride, chloride, nitrite, nitrate, phosphate, and sulfate, is acceptable.

c. Guard column: This is a short column‡ packed with the same stationary phase as the analytical column, used to protect the analytical column from particulates and organics.

d. Pretreatment cartridge for removal of interferences such as cations and carbonates.

e. Suppressor device: See Section 4110B.2*d*.

† Dionex P/N 51786, or equivalent.
‡ Dionex P/N 51791, or equivalent.

3. Reagents

a. Reagent water: See Section 4110B.3*a*.

b. Eluent solution, appropriate to the column used to resolve the target anions. Figure 4110:3 was obtained by using 9 m*M* sodium carbonate prepared as follows: Dissolve 1.91 g sodium carbonate in reagent water and dilute to 2 L. Vacuum filter eluent through a 0.45-μm or smaller filter to remove particles that could ultimately plug the column. This also degasses the eluent.

c. Regenerant solution (required with some suppressors): See manufacturers' recommendations.

d. Standard anion solutions, 1000 mg/L: Purchase stock standard solutions as certified solutions or prepare from ACS reagent-grade potassium or sodium salts. See Table 4110:VII.

e. Ethylenediamine (EDA) preservation solution, 100 mg/mL: Dilute 2.8 mL EDA (99%) to 25 mL with reagent water. This solution is stable for 1 month.

4. Procedure

a. System equilibration: See Section 4110B.4*a*.

b. Calibration: Prepare a series of calibration standards by diluting the stock standards appropriately to bracket the expected

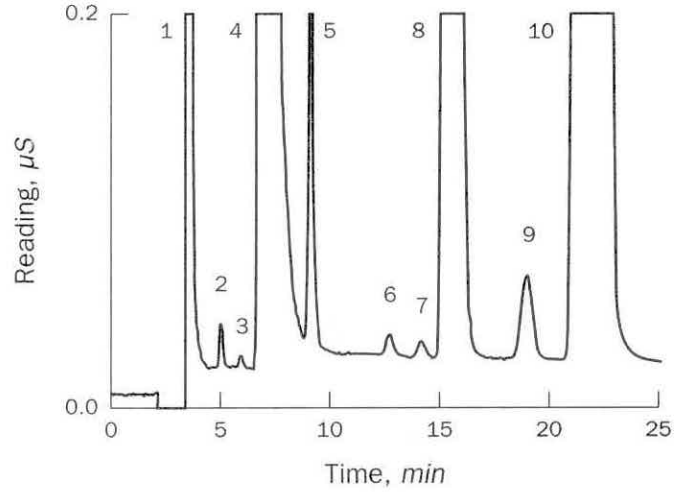

Figure 4110:3. Typical separation in a simulated drinking water sample. Eluent: 9 m*M* Na_2CO_3; flow rate: 1.0 mL/min; columns: Dionex AG9-HC and AS9-HC, 4 mm; sample loop: 200 μL; suppressor: Dionex ASRS, 100 mA in external water mode at 10 mL/min; detector: Dionex CD20 stabilized at 35°C.

Anion	Conc mg/L
1. Fluoride	1.0
2. Chlorite	0.01
3. Bromate	0.005
4. Chloride	50
5. Nitrite	0.1
6. Bromide	0.01
7. Chlorate	0.01
8. Nitrate	10
9. Phosphate	0.1
10. Sulfate	50

TABLE 4110:VII. STOCK STANDARD PREPARATION

Anion	Salt	Amount *g/100 mL*
Bromide	NaBr	0.1288
Bromate	$NaBrO_3$	0.1180
Chlorate	$NaClO_3$	0.1275
Chlorite*	$NaClO_2$	0.1676

* High-purity sodium chlorite is not commercially available. The value assumes the use of a salt that is exactly 80% pure. Preferably confirm concentration of the chlorite stock standard by iodometric titration (4500-ClO_2·C). Also demonstrate that this stock is free of the other target anions before preparing mixed working standards.

analyte concentrations in the samples. If the analytical column and eluent described in ¶s 2*b* and 3*b* are used, retention is in the order chlorite, bromate, bromide, and chlorate (See Figure 4110:3). Use at least three concentrations for each anion and construct calibration curves by plotting peak height or area (recommended) against concentration using appropriate software. If linearity is established ($r \geq 0.99$) the average response factor may be used in place of a calibration curve. Verify calibration curves with a mid-range standard from a source independent of that of the calibration standards. Check validity of existing calibration curves daily with a mid-range calibration standard. Agreement should be within 10% at mid-range. Also verify existing calibration curves whenever eluent or regenerant is changed. A change in detector setting usually will require recalibration. Samples preserved with EDA may show a bias attributable to the presence of this compound. If such a bias is suspected, add EDA to each calibration standard.

c. Sample analysis: See Section 4110B.4*c*.

5. Calculations

See Section 4110B.5.

6. Quality Control

See Section 4110B.6.

NOTE: If any sample is pretreated, also pretreat QC samples.

7. Precision and Bias

The data in Table 4110:VIII provide single-laboratory precision and bias information for a variety of matrices. The HIW matrix is designed to simulate a high-ionic-strength field sample. It was prepared from reagent water that was fortified with chloride at 100 mg/L, carbonate at 100 mg/L, nitrate at 10 mg/L as nitrogen, phosphate at 10 mg/L as phosphorus, and sulfate at 100 mg/L.

TABLE 4110:VIII. SINGLE-OPERATOR PRECISION AND ACCURACY FOR BROMIDE, CHLORATE, CHLORITE, AND BROMATE*

Analyte	Matrix†	Unfortified Conc µg/L‡	Fortified Conc µg/L	Mean Conc µg/L	Mean Recovery§ %R	SD(n−1) µg/L	RSD %
Bromide	RW	<DL	20.0	20.9	104	0.50	3.82
			100	107	107	0.60	0.56
	HIW	3.24	20.0	21.8	92.5	0.79	3.63
			100	105	102	1.05	1
	SW	31.0	20.0	51.3	—§	0.97	1.9
			100	140	109	1.88	1.35
	GW	151	20.0	172	—	0.78	0.45
			100	265	—	2.18	0.82
	ClW	16.3	20.0	39.3	115	0.64	1.62
			100	125	109	2.00	1.6
	CDW	11.5	20.0	34.4	115	0.76	2.22
			100	125	113	1.24	0.99
	O3W	39.8	20.0	65.4	—	3.67	5.61
			100	153	113	1.00	0.65
Chlorate	RW	<DL	100	98.3	98.3	0.80	0.82
			500	520	104	4.15	0.8
	HIW	<DL	100	86.1	86.1	1.47	1.7
			500	502	100	4.52	0.9
	SW	3.18	100	102	98.3	1.57	1.55
			500	513	102	7.11	1.39
	GW	<DL	100	93.5	93.5	2.00	2.14
			500	510	102	3.84	0.75
	ClW	34.4	100	136	102	1.01	0.74
			500	549	103	3.11	0.57
	CDW	121	100	223	—	3.20	1.44
			500	651	106	3.50	0.54
	O3W	6.15	100	106	100	1.20	1.13
			500	523	103	2.45	0.47
Chlorite	RW	<DL	100	96.2	96.2	0.95	0.99
			500	523	105	3.13	0.60
	HIW	<DL	100	102	102	2.19	2.15
			500	520	104	3.64	0.70
	SW	<DL	100	91.4	91.4	1.22	1.33
			500	495	99.0	7.54	1.52
	GW	<DL	100	92.9	92.9	1.65	1.77
			500	490	98.1	3.40	0.69
	ClW	<DL	100	87.4	87.4	0.59	0.68
			500	485	97.1	6.36	1.31
	CDW	292	100	396	—	1.64	0.41
			500	811	104	4.00	0.49
	O3W	<DL	100	84.4	84.4	0.46	0.54
			500	481	96.1	3.24	0.67
Bromate	RW	<DL	5.00	5.04	101	0.45	8.86
			25.0	26.5	106	1.71	6.47
	HIW	<DL	5.00	4.88	97.5	0.95	19.5
			25.0	25.6	102	1.37	5.37
	SW	<DL	5.00	4.46	89.2	0.58	13.0
			25.0	26.3	105	1.10	4.18
	GW	<DL	5.00	5.10	102	0.50	9.75
			25.0	22.2	88.9	1.29	5.81
	ClW	<DL	5.00	4.63	92.6	0.77	16.7
			25.0	25.1	100	1.64	6.55
	CDW	<DL	5.00	4.14	82.7	0.62	15.1
			25.0	25.1	101	1.28	5.09
	O3W	1.45	5.00	5.49	80.9	0.61	11.1
			25.0	24.1	90.6	1.13	4.69

* Nine replicates were analyzed for each matrix/concentration combination. Analytical conditions: Ion chromatograph — Dionex DX500; columns — Dionex AG9-HC/AS9-HC, 2 mm; detector — Dionex CD20; suppressor — ASRS-I, external water electrolytic mode, 100 mA current; eluent — 9.0 m*M* Na_2CO_3; eluent flow — 0.40 mL/min; sample loop − 50 µL.

† RW = reagent water
HIW = high-ionic-strength water
SW = surface water
GW = ground water
ClW = chlorinated drinking water
CDW = chlorine-dioxide-treated drinking water
O3W = ozonated drinking water

‡ <DL indicates less than minimum detection level.

§ Blank (—) indicates mean was not calculated since amount fortified was less than unfortified native matrix concentration.

Source: PFAFF, J.D., D.F. HAUTMAN & D.J. MUNCH. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1997. Determination of Inorganic Anions in Drinking Water by Ion Chromatography. Method 300.1, U.S. Environmental Protection Agency, National Exposure Research Lab, Off. Research & Development, Cincinnati, Ohio.

4120 SEGMENTED CONTINUOUS FLOW ANALYSIS*

4120 A. Introduction

1. Background and Applications

Air-segmented flow analysis (SFA) is a method that automates a large number of wet chemical analyses. An SFA analyzer can be thought of as a "conveyor belt" system for wet chemical analysis, in which reagents are added in a "production-line" manner. Applications have been developed to duplicate manual procedures precisely. SFA was first applied to analysis of sodium and potassium in human serum, with a flame photometer as the detection device, by removing protein interferences with a selectively porous membrane (dialyzer).

The advantages of segmented flow, compared to the manual method, include reduced sample and reagent consumption, improved repeatability, and minimal operator contact with hazardous materials. A typical SFA system can analyze 30 to 120 samples/h. Reproducibility is enhanced by the precise timing and repeatability of the system. Because of this, the chemical reactions do not need to go to 100% completion. Decreasing the number of manual sample/solution manipulations reduces labor costs, improves workplace safety, and improves analytical precision. Complex chemistries using dangerous chemicals can be carried out in sealed systems. Unstable reagents can be made up in situ. An SFA analyzer uses smaller volumes of reagents and samples than manual methods, producing less chemical waste needing disposal.

SFA is not limited to single-phase colorimetric determinations. Segmented-flow techniques often include analytical procedures such as mixing, dilution, distillation, digestion, dialysis, solvent extractions, and/or catalytic conversion. In-line distillation methods are used for the determinations of ammonia, fluoride, cyanide, phenols, and other volatile compounds. In-line digestion can be used for the determination of total phosphorous, total cyanide, and total nitrogen (kjeldahl + NO_2 + NO_3). Dialysis membranes are used to eliminate interferences such as proteins and color, and other types of membranes are available for various analytical needs. SFA also is well-suited for automated liquid/liquid extractions, such as in the determination of MBAS. Packed-bed ion exchange columns can be used to remove interferences and enhance sensitivity and selectivity of the detection.

Specific automated SFA methods are described in the sections for the analytes of interest.

2. Bibliography

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4120 B. Segmented Flow Analysis Method

1. General Discussion

a. Principle: A rudimentary system (Figure 4120:1) contains four basic components: a sampling device, a liquid transport device such as a peristaltic pump, the analytical cartridge where the chemistry takes place, and the detector to quantify the analyte.

In a generalized system, samples are loaded onto an automatic sampler. The sampler arm moves the sample pickup needle between the sample cup and a wash reservoir containing a solution closely matching the sample matrix and free of the analyte. The wash solution is pumped continuously through the reservoir to eliminate cross-contamination. The sample is pumped to the analytical cartridge as a discrete portion separated from the wash by an air-bubble created during the sampler arm's travel from wash reservoir to sample cup and back.

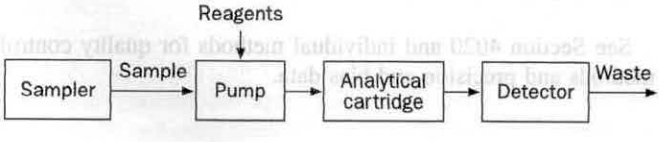

Figure 4120:1. Schematic of a segmented flow analyzer.

In the analytical cartridge, the system adds the sample to the reagent(s) and introduces proportionately identical air-bubbles to reagent or sample stream. Alternatively, another gas or immiscible fluid can be substituted for air. The analyzer then proportions the analyte sample into a number of analytical segments depending on sample time, wash time, and segmentation frequency. Relative flow and initial reagent concentration determine the amount and concentration of each reagent added. The micro-circulation pattern enhances mixing, as do mixing coils, which swirl the analytical system to utilize gravitational forces. Chemical reactions, solvent separation, catalytic reaction, dilution, distillation, heating, and/or special applications take place in their appropriate sections of the analytical cartridge as the segmented stream flows toward the detector.

A typical SFA detector is a spectrophotometer that measures the color development at a specific wavelength. Other detectors, such as flame photometers and ion-selective electrodes, can be used. SFA detectors utilize flow-through cells, and typically send their output to a computerized data-collection system and/or a chart-recorder. The baseline is the reading when only the reagents and wash water

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* Approved by Standard Methods Committee, 1997.
Joint Task Group: 20th Edition—Theresa M. Wright (chair), Johan Menting, Lang Allen Reeves.

are flowing through the system. Because gas bubbles are compressible, highly reflective, and electrically nonconductive, they severely distort the signal in the detector; therefore, many systems remove the bubbles before the optical light path. However, if the system removes the bubbles at any point within the system, the segregated liquids will be able to interact and pool. This interaction can cause cross-contamination or loss of wash, and decreases the rate at which samples can be processed. Real-time analog or digital data reconstruction techniques known as curve regeneration can remove the effect of pooling at the flow-cell debubbler and/or any other unsegmented zones of the system. "Bubble-gating" is a technique that does not remove the bubbles, but instead uses analog or digital processing to remove the distortion caused by the bubbles. Bubble-gating requires a sufficiently fast detector response time and requires that the volume of the measurement cell be smaller than the volume of the individual liquid segment.

b. Sample dispersion and interferences: Theoretically, the output of the detector is square-wave. Several carryover processes can deform the output exponentially. The first process, longitudinal dispersion, occurs as a result of laminar flow. Segmentation of the flow with air bubbles minimizes the dispersion and mixing between segments. The second process is axial or lag-phase dispersion. It arises from stagnant liquid film that wets the inner surfaces of the transmission tubing. Segmented streams depend on wet surfaces for hydraulic stability. The back-pressure within non-wet tubing increases in direct proportion to the number of bubbles it contains and causes surging and bubble breakup. Corrective measures include adding specific wetting agents (surfactants) to reagents and minimizing the length of transmission tubing.

Loose or leaking connections are another cause of carryover and can cause poor reproducibility. Wrap TFE tape around leaking screw fittings. When necessary, slightly flange the ends of types of tubing that require it for a tight connection. For other connections, sleeve one size of tubing over another size. Use a noninterfering lubricant for other tubing connections. Blockages in the tubing can cause back-pressure and leaks. Clean out or replace any blocked tubing or connection. A good indicator for problems is the bubble pattern; visually inspect the system for any abnormal bubble pattern that may indicate problems with flow.

For each analysis, check individual method for compounds that can interfere with color development and/or color reading. Other possible interferences include turbidity, color, and salinity. Turbid and/or colored samples may require filtration. In another interference-elimination technique, known as matrix correction, the solution is measured at two separate wavelengths, and the result at the interference wavelength is subtracted from that at the analytical wavelength.

2. Apparatus

a. Tubing and connections: Use mini- or micro-bore tubing on analytical cartridges. Replace flexible tubing that becomes discolored, develops a "sticky" texture, or loses ability to spring back into shape immediately after compression. Also see manufacturer's manual and specific methods.

b. Electrical equipment and connections: Make electrical connections with screw terminals or plug-and-socket connections. Use shielded electrical cables. Use conditioned power or a universal power supply if electrical current is subject to fluctuations. See manufacturer's manual for additional information.

c. Automated analytical equipment: Dedicate a chemistry manifold and tubing to each specific chemistry. See specific methods and manufacturer's manual for additional information.

d. Water baths: When necessary, use a thermostatically controlled heating/cooling bath to decrease analysis time and/or improve sensitivity. Several types of baths are available; the most common are coils heated or cooled by water or oil. Temperature-controlled laboratories reduce drift in temperature-sensitive chemistries if water baths are not used.

3. Reagents

Prepare reagents according to specific methods and manufacturer's instructions. If required, filter or degas a reagent. Use reagent water (see Section 1080) if available; if not, use a grade of water that is free of the analyte and interfering substances. Run blanks to demonstrate purity of the water used to prepare reagents and wash SFA system. Minimize exposure of reagents to air, and refrigerate if necessary. If reagents are made in large quantities, preferably decant a volume sufficient for one analytical run into a smaller container. If using a wetting agent, add it to the reagent just before the start of the run. Reagents and wetting agents have a limited shelf-life. Old reagents or wetting agents can produce poor reproducibility and distorted peaks. Do not change reagent solutions or add reagent to any reagent reservoirs during analysis. Always start with a sufficient quantity to last through the analytical run.

4. Procedure

For specific operating instructions, consult manufacturer's directions and methods for analytes of interest. At startup of a system, pump reagents and wash water through system until system has reached equilibrium (bubble pattern smooth and consistent) and base line is stable. Meanwhile, load samples and standards into sample cups or tubes and type corresponding tags into computer table. When ready, command computer to begin run. Most systems will run the highest standard to trigger the beginning of the run, followed by a blank to check return to base line, and then a set of standards covering the analytical range (sampling from lowest to highest concentration). Construct a curve plotting concentration against absorbance or detector reading and extrapolate results (many systems will do this automatically). Run a new curve daily immediately before use. Calculation and interpretation of results depend on individual chemistry and are analogous to the manual method. Insert blanks and standards periodically to check and correct for any drift of base line and/or sensitivity. Some systems will run a specific standard periodically as a "drift," and automatically will adjust sample results. At end of a run, let system flush according to manufacturer's recommendations.

5. Quality Control

See Section 4020 and individual methods for quality control methods and precision and bias data.

4130 INORGANIC NONMETALS BY FLOW INJECTION ANALYSIS*

4130 A. Introduction

1. Principle

Flow injection analysis (FIA) is an automated method of introducing a precisely measured portion of liquid sample into a continuously flowing carrier stream. The sample portion usually is injected into the carrier stream by either an injection valve with a fixed-volume sample loop or an injection valve in which a fixed time period determines injected sample volume. As the sample portion leaves the injection valve, it disperses into the carrier stream and forms an asymmetric Gaussian gradient in analyte concentration. This concentration gradient is detected continuously by either a color reaction or another analyte-specific detector through which the carrier and gradient flow.

When a color reaction is used as the detector, the color reaction reagents also flow continuously into the carrier stream. Each color reagent merges with the carrier stream and is added to the analyte gradient in the carrier in a proportion equal to the relative flow rates of the carrier stream and merging color reagent. The color reagent becomes part of the carrier after it is injected and has the effect of modifying or derivatizing the analyte in the gradient. Each subsequent color reagent has a similar effect, finally resulting in a color gradient proportional to the analyte gradient. When the color gradient passes through a flow cell placed in a flow-through absorbance detector, an absorbance peak is formed. The area of this peak is proportional to the analyte concentration in the injected sample. A series of calibration standards is injected to generate detector response data used to produce a calibration curve. It is important that the FIA flow rates, injected sample portion volume, temperature, and time the sample is flowing through the system ("residence time") be the same for calibration standards and unknowns. Careful selection of flow rate, injected sample volume, frequency of sample injection, reagent flow rates, and residence time determines the precise dilution of the sample's original analyte concentration into the useful concentration range of the color reaction. All of these parameters ultimately determine the sample throughput, dynamic range of the method, reaction time of the color reaction discrimination against slow interference reactions, signal-to-noise ratio, and method detection level (MDL).

2. Applications

FIA enjoys the advantages of all continuous-flow methods: There is a constantly measured reagent blank, the "base line" against which all samples are measured; high sample throughput encourages frequent use of quality control samples; large numbers of samples can be analyzed in batches; sample volume measurement, reagent addition, reaction time, and detection occur reproducibly without the need for discrete measurement and transfer vessels such as cuvettes, pipets, and volumetric flasks; and all samples share a single reaction manifold or vessel consisting of inert flow tubing.

Specific FIA methods are presented as Sections 4500-Br^-.D, 4500-Cl^-.G, 4500-CN^-.N and O, 4500-F^-.G, 4500-NH_3.H, 4500-NO_3^-.I, 4500-N.B, 4500-N_{org}.D, 4500-P.G, H, and I, 4500-SiO_2.F, 4500-SO_4^{2-}.G, and 4500-S^{2-}.I.

*Approved by Standard Methods Committee, 1997.
Joint Task Group: 20th Edition—Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

4130 B. Quality Control

When FIA methods are used, follow a formal laboratory quality control program. The minimum requirements consist of an initial demonstration of laboratory capability and periodic analysis of laboratory reagent blanks, fortified blanks, and other laboratory solutions as a continuing check on performance. Maintain performance records that define the quality of the data generated.

See Section 1020, Quality Assurance, and Section 4020 for the elements of such a quality control program.

4140 INORGANIC ANIONS BY CAPILLARY ION ELECTROPHORESIS*

4140 A. Introduction

Determination of common inorganic anions such as fluoride, chloride, bromide, nitrite, nitrate, orthophosphate, and sulfate is a significant component of water quality analysis. Instrumental techniques that can determine multiple analytes in a single analysis, i.e., ion chromatography (Section 4110) and capillary ion electrophoresis, offer significant time and operating cost savings over traditional single-analyte wet chemical analysis.

Capillary ion electrophoresis is rapid (complete analysis in less than 5 min) and provides additional anion information, i.e., organic acids, not available with isocratic ion chromatography (IC). Operating costs are significantly less than those of ion chromatography. Capillary ion electrophoresis can detect all anions present in the sample matrix, providing an anionic "fingerprint."

Anion selectivity of capillary ion electrophoresis is different from that of IC and eliminates many of the difficulties present in the early portion of an IC chromatogram. For example, sample matrix neutral organics, water, and cations do not interfere with anion analysis, and fluoride is well resolved from monovalent organic acids. Sample preparation typically is dilution with reagent water and removal of suspended solids by filtration. If necessary, hydrophobic sample components such as oil and grease can be removed with the use of HPLC solid-phase extraction cartridges without biasing anion concentrations.

* Approved by Standard Methods Committee, 1997.
Joint Task Group: 20th Edition—Roy-Keith Smith (chair), James Krol, Yuefeng Xie.

4140 B. Capillary Ion Electrophoresis with Indirect UV Detection

1. General Discussion

a. Principle: A buffered aqueous electrolyte solution containing a UV-absorbing anion salt (sodium chromate) and an electroosmotic flow modifier (OFM) is used to fill a 75-μm-ID silica capillary. An electric field is generated by applying 15 kV of applied voltage using a negative power supply; this defines the detector end of the capillary as the anode. Sample is introduced at the cathodic end of the capillary and anions are separated on the basis of their differences in mobility in the electric field as they migrate through the capillary. Cations migrate in the opposite direction and are not detected. Water and neutral organics are not attracted towards the anode; they migrate after the anions and thus do not interfere with anion analysis. Anions are detected as they displace charge-for-charge the UV-absorbing electrolyte anion (chromate), causing a net decrease in UV absorbance in the analyte anion zone compared to the background electrolyte. Detector polarity is reversed to provide positive mv response to the data system (Figure 4140:1). As in chromatography, the analytes are identified by their migration time and quantitated by using time-corrected peak area relative to standards. After the analytes of interest are detected, the capillary is purged with fresh electrolyte, eliminating the remainder of the sample matrix before the next analysis.

b. Interferences: Any anion that has a migration time similar to the analytes of interest can be considered an interference. This method has been designed to minimize potential interference typically found in environmental waters, groundwater, drinking water, and wastewater.

Formate is a common potential interference with fluoride; it is a common impurity in reagent water, has a migration time similar to that of fluoride, and is an indicator of loss of water purification system performance and TOC greater than 0.1 mg/L. The addition of 5 mg formate/L in the mixed working anion standard, and to sample where identification of fluoride is in question, aids in the correct identification of fluoride.

Generally, a high concentration of any one ion may interfere with resolution of analyte anions in close proximity. Dilution in reagent water usually is helpful. Modifications in the electrolyte formulation can overcome resolution problems but require individual validation for precision and bias. This method is capable of interference-free resolution of a 1:100 differential of Br^- to Cl^-, and NO_2^- and NO_3^- to SO_4^{2-}, and 1:1000 differential of Cl^- and SO_4^{2-}.

Dissolved ferric iron in the mg/L range gives a low bias for PO_4. However, transition metals do not precipitate with chromate because of the alkaline electrolyte pH.

c. Minimum detectable concentrations: The minimum detectable concentration for an anion is a function of sample size. Generally, for a 30-s sampling time, the minimum detectable concentrations are 0.1 mg/L (Figure 4140:2). According to the method for calculating MDL given in Section 1030, the calculated detection levels are below 0.1 mg/L. These detection levels can be compromised by analyte impurities in the electrolyte.

d. Limitations: Samples with high ionic strength may show a decrease in analyte migration time. This variable is addressed by using normalized migration time with respect to a reference peak, chloride, for identification, and using time-corrected area for quantitation. With electrophoresis, published data indicate that analyte peak area is a function of migration time. At high analyte anion concentrations, peak shape becomes asymmetrical; this phenomenon is typical and is different from that observed in ion chromatography.

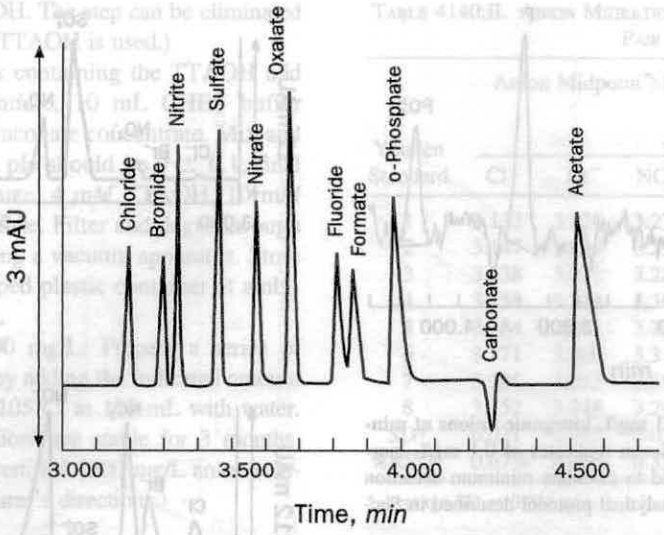

Figure 4140:1. Electropherogram of the inorganic anions and typically found organic acids using capillary ion electrophoresis and chromate electrolyte. Electrolyte: 4.7 m*N* Na_2CrO_4/4.0 m*M* TTAOH/10 m*M* CHES/0.1 m*M* calcium gluconate; capillary: 75-μm-ID × 375-μm-OD × 60-cm length, uncoated silica; voltage: 15 kV using a negative power supply; current: 14 ± 1 μA; sampling: hydrostatic at 10 cm for 30 s; detection: indirect UV with Hg lamp and 254-nm filter.

Anion	Conc *mg/L*	Migration Time *min*	Migration Time Ratio to Cl	Peak Area	Time-Corrected Peak Area
Chloride	2.0	3.200	1.000	1204	376.04
Bromide	4.0	3.296	1.030	1147	348.05
Nitrite	4.0	3.343	1.045	2012	601.72
Sulfate	4.0	3.465	1.083	1948	562.05
Nitrate	4.0	3.583	1.120	1805	503.69
Oxalate	5.0	3.684	1.151	3102	842.14
Fluoride	1.0	2.823	1.195	1708	446.65
Formate	5.0	3.873	1.210	1420	366.61
o-Phosphate	4.0	4.004	1.251	2924	730.25
Carbonate & bicarbonate		4.281	1.338		
Acetate	5.0	4.560	1.425	3958	868.01

2. Apparatus

*a. Capillary ion electrophoresis (CIE) system:** Various commercial instruments are available that integrate a negative high-voltage power supply, electrolyte reservoirs, covered sample carousel, hydrostatic sampling mechanism, capillary purge mechanism, self-aligning capillary holder, and UV detector capable of 254-nm detection in a single temperature-controlled compartment at 25°C. Optimal detection limits are attained with a fixed-wavelength UV detector with Hg lamp and 254-nm filter.

b. Capillary: 75-μm-ID × 375-μm-OD × 60-cm-long fused silica capillary with a portion of its outer coating removed to act as the UV detector window. Capillaries can be purchased premade* or on a spool and prepared as needed.

*c. Data system:** HPLC-based integrator or computer. Optimum performance is attained with a computer data system and electrophoresis-specific data processing including data acquisition at 20 points/s, migration times determined at midpoint of peak width, identification based on normalized migration times with respect to a reference peak, and time-corrected peak area.

* Waters Corp. or equivalent.

3. Reagents

a. Reagent water: See Section 1080. Ensure that water is analyte-free. The concentration of dissolved organic material will influence overall performance; preferably use reagent water with <50 μg TOC/L.

b. Chromate electrolyte solution: Prepare as directed from individual reagents, or purchase electrolyte preformulated.

1) *Sodium chromate concentrate,* 100 m*M:* In a 1-L volumetric flask dissolve 23.41 g sodium chromate tetrahydrate, $Na_2CrO_4 \cdot 4H_2O$, in 500 mL water and dilute to 1 L with water. Store in a capped glass or plastic container at ambient temperature; this reagent is stable for 1 year.

2) *Electroosmotic flow modifier concentrate,* 100 m*M:* In a 100-mL volumetric flask dissolve 3.365 g tetradecyltrimethyl ammonium bromide (TTAB), mol wt 336.4, in 50 mL water and dilute to 100 mL. Store in a capped glass or plastic container at ambient temperature; this reagent is stable for 1 year.

3) *Buffer concentrate,* 100 m*M:* In a 1-L volumetric flask dissolve 20.73 g 2-[*N*-cyclohexylamino]-ethane sulfonate (CHES), mol wt 207.29, in 500 mL water and dilute to 1 L. Store

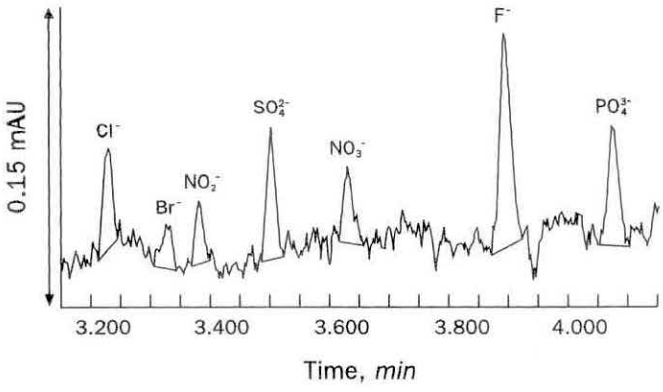

Figure 4140:2. Electropherogram of 0.1 mg/L inorganic anions at minimum detection level. Seven replicates of 0.1 mg/L inorganic anion standard used to calculate minimum detection levels, as mg/L, using analytical protocol described in Section 1030E.

Chloride = 0.046	Nitrite = 0.072
Nitrate = 0.084	Phosphate = 0.041
Bromide = 0.090	Sulfate = 0.032
Fluoride = 0.020	

in a capped glass or plastic container at ambient temperature; this reagent is stable for 1 year.

4) *Calcium gluconate concentrate,* 1 m*M:* In a 1-L volumetric flask dissolve 0.43 g calcium gluconate, mol wt 430.38, in 500 mL water and dilute to 1 L. Store in a capped glass or plastic container at ambient temperature; this reagent is stable for 1 year.

5) *Sodium hydroxide solution,* NaOH, 100 m*M*: In a 1-L plastic volumetric flask dissolve 4 g sodium hydroxide, NaOH, in 500 mL water and dilute to 1 L. Store in a capped plastic container at ambient temperature; this reagent is stable for 1 month.

6) *Chromate electrolyte solution:* Prerinse an anion exchange cartridge in the hydroxide form with 10 mL 100-m*M* NaOH followed by 10 mL water; discard the washings. Slowly pass 4 mL 100-m*M* TTAB concentrate through the cartridge into a 100-mL volumetric flask. Rinse cartridge with 10 mL water and add to flask. (NOTE: This step is needed to convert the TTAB from the bromide

TABLE 4140:I. COLLABORATIVE DESIGN AS FOUR YOUDEN PAIR SETS*

	Anion Concentration in Individual Youden Pair Standards *mg/L*							
Anion	1	2	3	4	5	6	7	8
Cl^-	0.7	2.0	3.0	15.0	40.0	20.0	50.0	0.5
Br^-	2.0	3.0	15.0	40.0	20.0	50.0	0.7	0.5
NO_2^-	3.0	40.0	20.0	15.0	50.0	0.5	2.0	0.7
SO_4^{2-}	40.0	50.0	0.5	0.7	2.0	3.0	15.0	20.0
NO_3^-	15.0	20.0	40.0	50.0	0.5	0.7	2.0	3.0
F^-	2.0	0.7	0.5	3.0	10.0	7.0	20.0	25.0
PO_4^{3-}	50.0	40.0	20.0	0.5	3.0	2.0	0.7	15.0

* The collaborative design is intended to demonstrate performance between 0.1 and 50 mg anion/L, except for fluoride between 0.1 and 25 mg/L. The concentrations among anions are varied so as not to have any one standard at all low or all high anion concentrations.

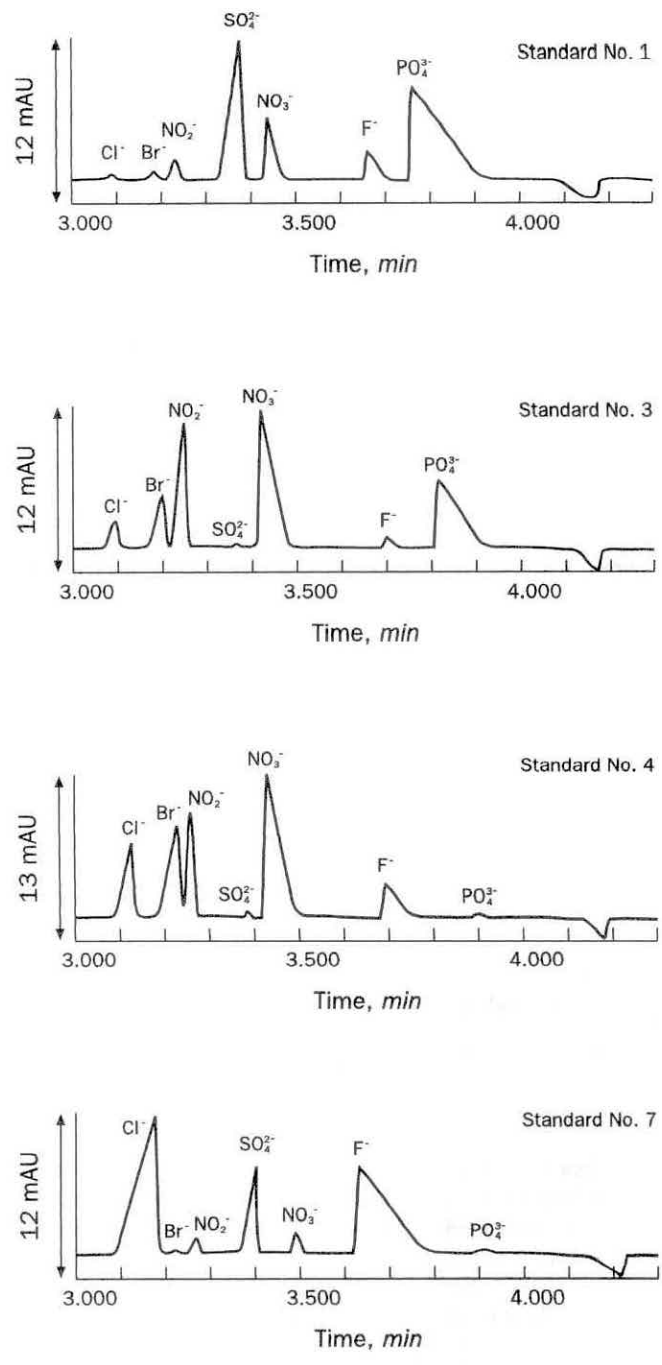

Figure 4140:3. Representative electropherograms of Youden anion standards. For composition of standards, see Table 4140:I.

form into the hydroxide form TTAOH. The step can be eliminated if commercially available 100 m*M* TTAOH is used.)

To the 100-mL volumetric flask containing the TTAOH add 4.7 mL sodium chromate concentrate, 10 mL CHES buffer concentrate, and 10 mL calcium gluconate concentrate. Mix and dilute to 100 mL with water. The pH should be 9 ± 0.1; final solution is 4.7 m*M* sodium chromate, 4 m*M* TTAOH, 10 m*M* CHES, and 0.1 m*M* calcium gluconate. Filter and degas through a 0.45-μm aqueous membrane, using a vacuum apparatus. Store any remaining electrolyte in a capped plastic container at ambient temperature for up to 1 month.

c. Standard anion solution, 1000 mg/L*:* Prepare a series of individual standard anion solutions by adding the indicated amount of salt, dried to constant weight at 105°C, to 100 mL with water. Store in plastic bottles; these solutions are stable for 3 months. (Alternatively, purchase individual certified 1000-mg/L anion standards and store following manufacturer's directions.)

Anion	Salt	Amount *g/100mL*
Chloride	NaCl	0.1649
Bromide	NaBr	0.1288
Formate	$NaCO_2H$	0.1510
Fluoride	NaF	0.2210
Nitrite	$NaNO_2$	0.1499* (1000 mg NO_2^-/L = 304.3 mg NO_2^- −N/L)
Nitrate	$NaNO_3$	0.1371 (1000 mg NO_3^-/L = 225.8 mg NO_3^-−N/L)
Phosphate	Na_2HPO_4†	0.1500 (1000 mg PO_4^{3-}/L = 326.1 mg PO_4^{3-}−P/L)
Sulfate	Na_2SO_4†	0.1480 (1000 mg SO_4^{2-}/L = 676.3 mg SO_4^{2-}−S/L)

* Do not oven-dry, but dry to constant weight in a desiccator over phosphorous pentoxide.

† Potassium salts can be used, but with corresponding modification of salt amounts.

d. Mixed working anion standard solutions: Prepare at least three different working anion standard solutions that bracket the expected sample range, from 0.1 to 50 mg/L. Add 5 mg formate/L to all standards. Use 0.1 mL standard anion solution/100 mL working anion solution (equal to 1 mg anion/L). (Above 50 mg/L each anion, chloride, bromide, nitrite, sulfate, and nitrate are no longer baseline-resolved. Analytes that are not baseline-resolved may give a low bias. If the analytes are baseline-resolved, quantitation is linear to 100 mg/L.) Store in plastic containers in the refrigerator; prepare fresh standards weekly. Figure 4140:3 shows representative electropherograms of anion standards and Table 4140:I gives the composition of the standards.

e. Calibration verification sample: Use a certified performance evaluation standard, or equivalent, within the range of the mixed working anion standard solutions analyzed as an unknown. Refer to Section 4020.

f. Analyte known-addition sample: To each sample matrix add a known amount of analyte, and use to evaluate analyte recovery.

4. Procedure

a. Capillary conditioning: Set up CIE system according to manufacturer's instructions. Rinse capillary with 100 m*M* NaOH for 5 min. Place fresh degassed electrolyte into both reservoirs and purge capillary with electrolyte for 3 min to remove all previous solutions and air bubbles. Apply voltage of 15 kV and note the current; if the expected 14 ± 1 μA is observed, the CIE system is ready for use. Zero UV detector to 0.000 absorbance.

TABLE 4140:II. ANION MIGRATION TIME REPRODUCIBILITY FROM YOUDEN PAIR STANDARDS

Youden Standard	Anion Midpoint Migration Time, Average of Triplicate Samplings, *min*						
	Cl^-	Br^-	NO_2^-	SO_4^{2-}	NO_3^-	F^-	PO_4^{3-}
1	3.132	3.226	3.275	3.405	3.502	3.761	3.906
2	3.147	3.239	3.298	3.431	3.517	3.779	3.931
3	3.138	3.231	3.283	3.411	3.497	3.771	3.925
4	3.158	3.244	3.307	3.434	3.510	3.781	3.963
5	3.184	3.271	3.331	3.435	3.551	3.787	3.981
6	3.171	3.260	3.312	3.418	3.537	3.776	3.964
7	3.191	3.272	3.315	3.437	3.544	3.773	3.978
8	3.152	3.248	3.294	3.418	3.526	3.739	3.954
SD*	0.021	0.015	0.018	0.012	0.20	0.015	0.027
%RSD*	0.67%	0.46%	0.55%	0.36%	0.56%	0.40%	0.68%

* Average SD = 0.018 min = 1.1 s; average %RSD = 0.53%.

b. Analysis conditions: Program CE system to apply constant current of 14 μA for the run time. Use 30 s hydrostatic sampling time for all standard and sample introduction. Analysis time is 5 min.

c. Analyte migration time calibration: Determine migration time of each analyte daily using the midrange mixed working anion standard. Perform duplicate analysis to insure migration time stability. Use the midpoint of peak width, defined as midpoint between the start and stop integration marks, as the migration time for each analyte; this accounts for the observed nonsymmetrical peak shapes. (Use of peak apex may result in analyte misidentification.) The migration order is always Cl^-, Br^-, NO_2^-, SO_4^{2-}, NO_3^-, F^-, and PO_4^{3-}. Dissolved HCO_3^- is the last peak in the standard (see Figure 4140:1). Set analyte migration time window as 2% of the migration time determined above, except for Cl^-, which is set at 10%. Chloride is always the first peak and is used as the reference peak for analyte qualitative identification; identify anions on the basis of normalized migration times with respect to the reference peak, or migration time ratio. (See Figure 4140:1 and Table 4140:II.)

d. Analyte response calibration: Analyze all three mixed working anion standards in duplicate. Plot time-corrected peak area for each analyte versus concentration using a linear regression through zero. (In capillary electrophoresis peak area is a function of analyte migration time, which may change during analyses. Time-corrected peak area is a well-documented CE normalization routine, i.e., peak area divided by migration time. (NOTE: *Do not use analyte peak height.*) Calibration is accepted as linear if regression coefficient of variation, R^2, is greater than 0.995. Linearity calibration curves for anions are shown in Figures 4140:4 through 6.

e. Sample analysis: After initial calibration run samples in the following order: calibration verification sample, reagent blank, 10 unknown samples, calibration verification sample, reagent blank, etc. Filter samples containing high concentrations of

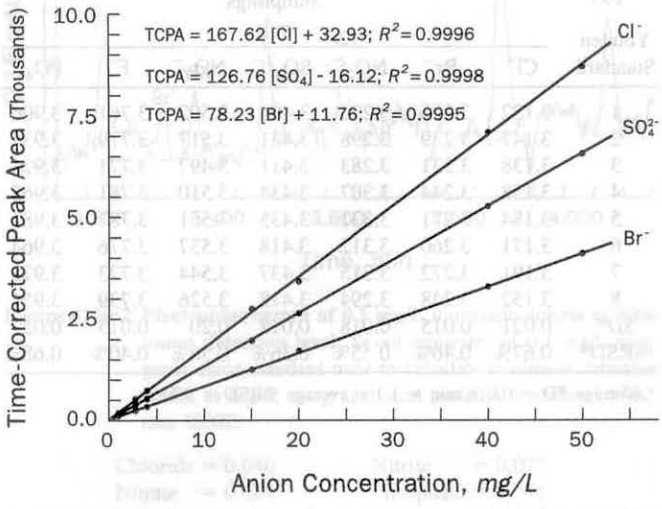

Figure 4140:4. Linearity calibration curve for chloride, bromide, and sulfate. Three data points were used per concentration; based on Youden pair design.

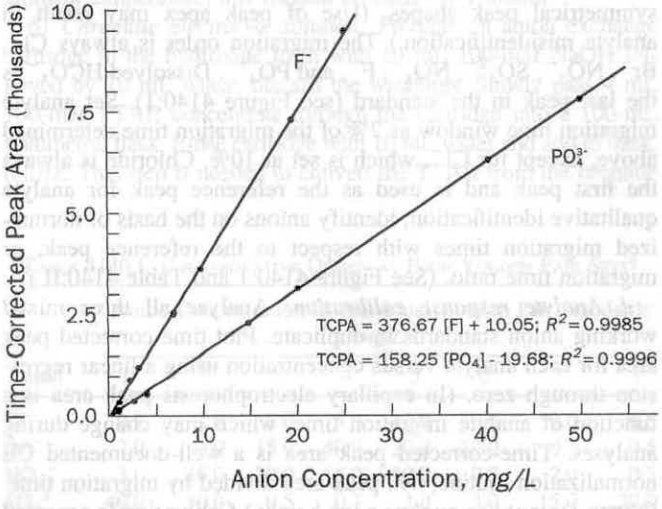

Figure 4140:5. Linearity calibration curve for fluoride and *o*-phosphate. Three data points were used per concentration; based on Youden pair design.

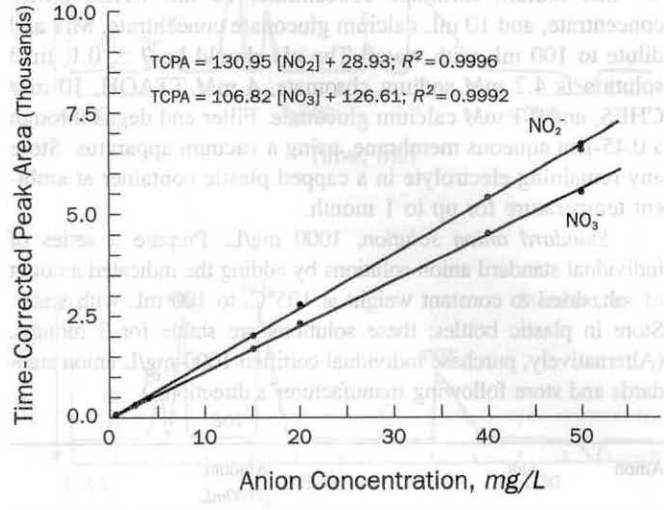

Figure 4140:6. Linearity calibration curve for nitrite and nitrate. Three data points were used per concentration; based on Youden pair design.

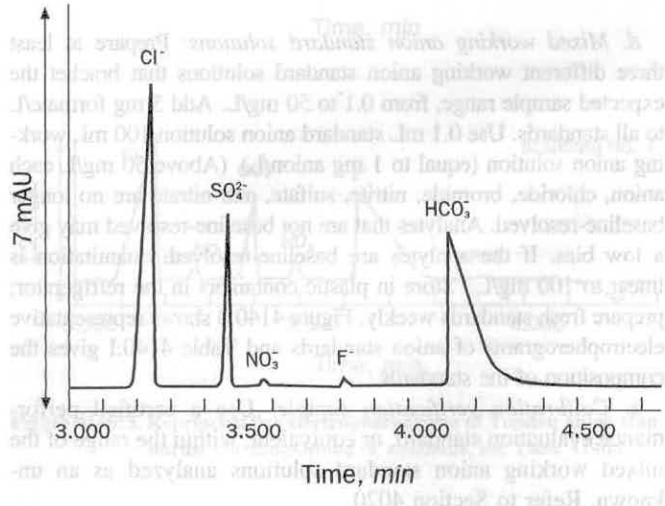

Figure 4140:7. Electropherogram of typical drinking water.

Chloride = 24.72 mg/L	Fluoride < 0.10 mg/L
Sulfate = 7.99 mg/L	Carbonate &
Nitrate = 0.36 mg/L	bicarbonate = natural

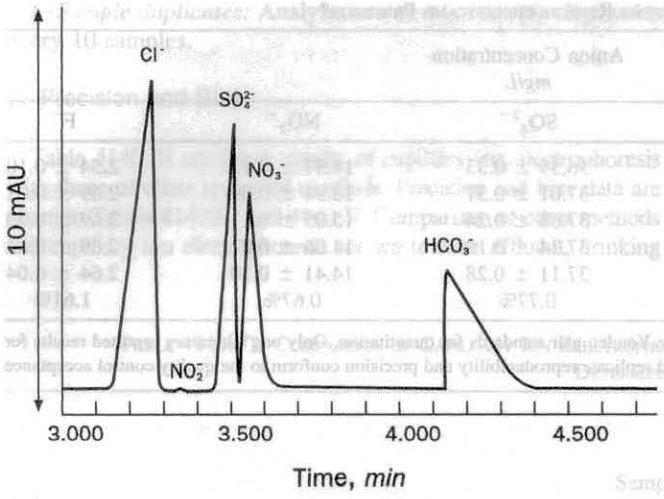

Figure 4140:8. Electropherogram of typical municipal wastewater discharge, undiluted.

Chloride = 93.3 mg/L Nitrate < 40.8 mg/L
Nitrate = 0.46 mg/L Carbonate &
Sulfate = 60.3 mg/L bicarbonate = natural

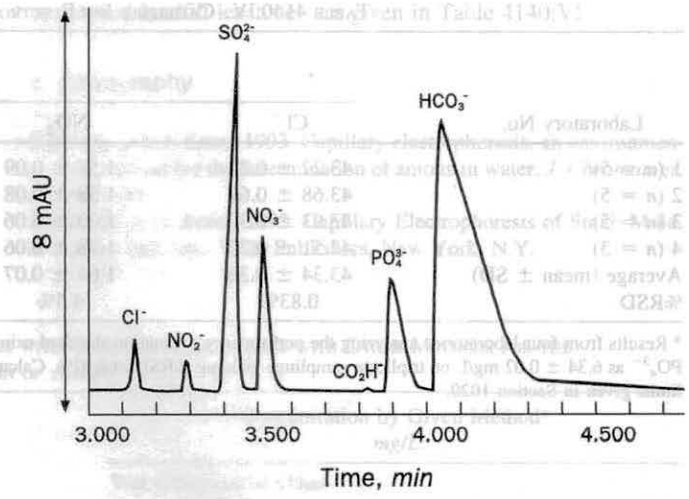

Figure 4140:9. Electropherogram of typical industrial wastewater discharge, undiluted.

Chloride = 2.0 mg/L Formate < 0.05 mg/L
Nitrite = 1.6 mg/L Phosphate = 12.3 mg/L
Sulfate = 34.7 mg/L Carbonate &
Nitrate = 16.5 mg/L bicarbonate = natural

suspended solids. If peaks are not baseline-resolved, dilute sample 1:5 with water and repeat analysis for unresolved analyte quantitation. Resolved analytes in the undiluted sample are considered correct quantitation. Electropherograms of typical samples are shown in Figures 4140:7 through 9.

5. Calculation

Relate the time-corrected peak area for each sample analyte with the calibration curve to determine concentration of analyte. If the sample was diluted, multiply anion concentration by the dilution factor to obtain original sample concentration, as follows:

$$C = A \times F$$

where:

C = analyte concentration in original sample, mg/L,
A = analyte concentration from calibration curve, mg/L, and
F = scale factor or dilution factor. (For a 1:5 sample dilution, F = 5.)

TABLE 4140:III. COMPARISON OF CAPILLARY ION ELECTROPHORESIS AND OTHER METHODS

Source	Statistic	Value for Given Anion, mg/L					
		Cl^-	NO_2^-	SO_4^{2-}	NO_3^-	F^-	PO_4^{3-}
Performance evaluation standard*	True value	43.00	1.77	37.20	15.37	2.69	6.29
Wet chemical and ion chromatography methods†	Measured mean	43.30	1.77	37.00	15.42	2.75	6.38
	Measured SD	3.09	0.07	2.24	1.15	0.26	0.21
CIE using chromate electrolyte‡	Average (n = 18)	43.34	1.64	37.11	14.41	2.64	6.34
	CIE/mean	1.003	0.927	1.003	0.935	0.959	0.993
	CIE/true value	1.008	0.927	0.996	0.938	0.981	1.008

* Purchased from APG Laboratories, June 1996; diluted 1:100 with deionized water.
† Measured result is the average from numerous laboratories using approved *Standard Methods* and EPA wet chemistry and ion chromatography methods.
‡ CIE results determined in July 1996 with proposed EPA and ASTM method, operationally identical to 4140; they are the average from four laboratories using the Youden pair standards for quantitation. These data can be considered known addition of the performance evaluation standard in reagent water; they conform to quality control acceptance limits given in Section 1020.

TABLE 4140:IV. CAPILLARY ION ELECTROPHORESIS REPRODUCIBILITY AND PRECISION*

Laboratory No.	Anion Concentration mg/L				
	Cl^-	NO_2^-	SO_4^{2-}	NO_3^-	F^-
1 (n = 5)	43.22 ± 0.22	1.58 ± 0.09	36.39 ± 0.33	14.57 ± 0.12	2.54 ± 0.10
2 (n = 5)	43.68 ± 0.61	1.58 ± 0.08	37.01 ± 0.37	13.94 ± 0.09	2.69 ± 0.02
3 (n = 5)	43.93 ± 0.39	1.60 ± 0.06	37.68 ± 0.24	15.05 ± 0.11	2.69 ± 0.03
4 (n = 3)	42.51 ± 0.22	1.78 ± 0.06	37.34 ± 0.19	14.06 ± 0.07	2.69 ± 0.02
Average (mean ± SD)	43.34 ± 0.36	1.64 ± 0.07	37.11 ± 0.28	14.41 ± 0.10	2.64 ± 0.04
%RSD	0.83%	4.5%	0.77%	0.67%	1.61%

* Results from four laboratories analyzing the performance evaluation standard using the Youden pair standards for quantitation. Only one laboratory reported results for PO_4^{3-} as 6.34 ± 0.02 mg/L on triplicate samplings yielding %RSD of 0.07%. Calculated replicate reproducibility and precision conform to the quality control acceptance limits given in Section 1020.

6. Quality Control

a. Analytical performance check: Unless analyst has already demonstrated ability to generate data with acceptable precision and bias by this method, proceed as follows: Analyze seven replicates of a certified performance evaluation standard containing the analytes of interest. Calculate mean and standard deviation of these data. The mean must be within the performance evaluation standard's 95% confidence interval. Calculate percent relative standard deviation (RSD) for these data as (SD × 100) / mean; % RSD should conform to acceptance limit given in Section 1020B.

b. Calibration verification: Analyze an independent, certified performance evaluation standard at the beginning and end of the analyses, or if many samples are analyzed, after every 10 samples. The determined analyte concentration should be within ±10% of the true value, and the migration time of the Cl^- reference peak should be within 5% of the calibrated migration time. If the Cl^- reference peak differs by more than 5% of the calibrated migration time, repeat capillary conditioning and recalibrate before proceeding.

c. Water blank analysis: At the beginning of every set of analyses run a water blank to demonstrate that the water is free of analyte anions. Dissolved bicarbonate will always be observed as a positive or negative peak having a migration time greater than PO_4^{3-} and does not interfere with the analysis. Any negative peak indicates the presence of an anion impurity in the electrolyte; a positive peak indicates the presence of an impurity in the reagent water. If this is noted, discard electrolyte and prepare electrolyte and sample dilutions again with water from a different source.

d. Analyte recovery verification: For each sample matrix analyzed, e.g., drinking water, surface water, groundwater, or wastewater, analyze duplicate known-addition samples (¶ 3*f*). Analyte recoveries should conform to acceptance limits given in Section 1020B.

e. Blind check sample: Analyze an unknown certified performance evaluation check sample at least once every 6 months to verify method accuracy.

TABLE 4140:V. CAPILLARY ION ELECTROPHORESIS KNOWN-ADDITION RECOVERY AND PRECISION OF PERFORMANCE EVALUATION STANDARD WITH DRINKING WATER

Variable	Value for Given Anion					
	Cl^-	NO_2^-	SO_4^{2-}	NO_3^-	F^-	PO_4^{3-}
Milford drinking water (MDW) n = 3, concentration as mg/L	24.72 ± 0.18	Not detected	7.99 ± 0.07	0.36 ± 0.05	Not detected	Not detected
%RSD	0.73	—	0.91	13.3	—	—
Performance evaluation standard (PES), concentration as mg/L	43.00	1.77	37.20	15.37	2.69	6.29
MDW + PES,* n = 3, concentration as mg/L	66.57 ± 0.34	1.74 ± 0.03	45.19 ± 0.17	15.42 ± 0.12	2.62 ± 0.07	5.55 ± 0.31
%RSD	0.51%	1.85%	0.38%	0.79%	2.69%	5.52%
% Recovery	97.9%	98.3%	100.2%	98.1%	97.4%	88.2%

* Performance evaluation standard diluted 1:100 with Milford drinking water. Calculated analyte recovery and precision conform to the quality control acceptance limits given in Section 1020.

f. Sample duplicates: Analyze one or more sample duplicates every 10 samples.

7. Precision and Bias

Table 4140:III compares results of capillary ion electrophoresis with those of other approved methods. Precision and bias data are given in Tables 4140:IV and 4140:V. Comparison of other methods and capillary ion electrophoresis for wastewater effluent, drinking water, and landfill leachates are given in Table 4140:VI.

8. Bibliography

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TABLE 4140:VI. COMPARISON OF CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE WITH OTHER METHODS FOR THE DETERMINATION OF ANIONS

Anion	Matrix	Sample No.	Concentration by Given Method* *mg/L*		
			Wet Chemical or Other Method†	IC‡	CIE
Chloride	Effluent	1	—	149	147
		2	—	162	161
		3	—	152	151
		4	—	139	139
		5	—	111	110
		6	—	109	107
		7	—	3.6	3.5
	Drinking water	1	5.5	5.1	5.0
		2	5.5	5.0	5.0
		3	5.3	5.2	5.2
		4	5.5	5.1	5.1
		5	5.3	5.0	5.1
		6	5.3	4.9	4.9
		7	5.5	4.9	4.9
	Landfill leachate	1	0.1	<0.1	ND
		2	230	245	240
Fluoride	Effluent	1	1.7	1.2	1.5
		2	0.9	0.6	0.6
		3	0.8	0.5	0.6
		4	0.8	0.4	0.7
		5	0.9	0.5	0.8
		6	0.9	0.5	0.7
		7	<0.1	ND	<0.1
	Drinking water	1	1.2	0.9	0.9
		2	1.3	0.9	0.9
		3	1.3	0.9	0.9
		4	1.3	0.9	0.9
		5	1.3	0.9	0.9
		6	0.9	0.6	0.6
		7	1.3	0.9	0.9
	Landfill leachate	1	<0.2	ND	ND
		2	16	10.6	10.9

TABLE 4140:VI. CONT.

Anion	Matrix	Sample No.	Concentration by Given Method* mg/L: Wet Chemical or Other Method†	IC‡	CIE
Sulfate	Effluent	1	98	87.5	86.4
		2	110	95.3	95.9
		3	130	118	115
		4	130	139	136
		5	110	113	110
		6	100	107	106
		7	6	5.6	5.8
	Drinking water	1	6	5.8	6.0
		2	6	5.8	6.0
		3	6	5.9	6.1
		4	6	5.9	6.1
		5	5	5.8	6.2
		6	4	3.0	3.4
		7	5	5.8	6.1
	Landfill leachate	1	<1	ND	ND
		2	190	211	201
Nitrite & nitrate§ (as N)	Effluent	1	0.3	ND	ND
		2	—	ND	ND
		3	—	ND	ND
		4	—	ND	0.5
		5	—	2.1	2.4
		6	2.4	1.9	2.2
		7	0.7	0.3	0.4
	Drinking water	1	0.6	0.3	0.4
		2	0.6	0.3	0.4
		3	0.4	0.3	0.4
		4	0.6	0.3	0.3
		5	0.6	0.3	0.4
		6	0.3	0.1	0.1
		7	0.5	0.3	0.4
	Landfill leachate	1	—	ND	ND
		2	—	ND	ND
Orthophosphate (as P)	Effluent	1	3.4	ND	2.8
		2	4.9	ND	4.4
		3	4.7	ND	4.5
		4	5.3	ND	4.2
		5	3.0	ND	3.0
		6	2.9	ND	2.3
		7	<0.1	ND	0.04
	Drinking water	1	<0.1	ND	ND
		2	<0.1	ND	ND
		3	—	ND	ND
		4	<0.1	ND	ND
		5	<0.1	ND	ND
		6	—	ND	ND
		7	—	ND	ND
	Landfill leachate	1	<0.1	ND	0.1
		2	2.2	1.6	1.4

* — = test not performed; ND = not detected.

† Methods used were: chloride—iodometric method (4500-Cl^-.C); fluoride—ion selective electrode method (4500-F^-.C); sulfate—turbidimetric method (4500-SO_4^{2-}.E); nitrite + nitrate (total)—cadmium reduction method (4500-NO_3^-.E); orthophosphate—ascorbic acid method (4500-P.E).

‡ Single-column ion chromatography with direct conductivity detection (4110C).

§ Each technique gave separate nitrite and nitrate values; because of their interconvertability, results were added for comparison purposes.

4500-B BORON*

4500-B A. Introduction

1. Occurrence and Significance

Boron (B) is the first element in Group IIIA of the periodic table; it has an atomic number of 5, an atomic weight of 10.81, and a valence of 3. The average abundance of B in the earth's crust is 9 ppm; in soils it is 18 to 63 ppm; in streams it is 10 μg/L; and in groundwaters it is 0.01 to 10 mg/L. The most important mineral is borax, which is used in the preparation of heat-resistant glasses, detergents, porcelain enamels, fertilizers, and fiberglass.

The most common form of boron in natural waters is H_3BO_3. Although boron is an element essential for plant growth, in excess of 2.0 mg/L in irrigation water, it is deleterious to certain plants and some plants may be affected adversely by concentrations as low as 1.0 mg/L (or even less in commercial greenhouses). Drinking waters rarely contain more than 1 mg B/L and generally less than 0.1 mg/L, concentrations considered innocuous for human consumption. Seawater contains approximately 5 mg B/L and this element is found in saline estuaries in association with other seawater salts.

The ingestion of large amounts of boron can affect the central nervous system. Protracted ingestion may result in a clinical syndrome known as borism.

2. Selection of Method

Preferably, perform analyses by the inductively coupled plasma method (3120). The inductively coupled plasma/mass spectrometric method (3125) also may be applied successfully in most cases (with lower detection limits), even though boron is not specifically listed as an analyte in the method.

The curcumin method (B) is applicable in the 0.10- to 1.0-mg/L range, while the carmine method (C) is suitable for the determination of boron concentration in the 1- to 10-mg/L range. The range of these methods can be extended by dilution or concentration of the sample.

3. Sampling and Storage

Store samples in polyethylene bottles or alkali-resistant, boron-free glassware.

* Approved by Standard Methods Committee, 2000.

4500-B B. Curcumin Method

1. General Discussion

a. Principle: When a sample of water containing boron is acidified and evaporated in the presence of curcumin, a red-colored product called rosocyanine is formed. The rosocyanine is taken up in a suitable solvent and the red color is compared with standards visually or photometrically.

b. Interference: NO_3^--N concentrations above 20 mg/L interfere. Significantly high results are possible when the total of calcium and magnesium hardness exceeds 100 mg/L as calcium carbonate ($CaCO_3$). Moderate hardness levels also can cause a considerable percentage error in the low boron range. This interference springs from the insolubility of the hardness salts in 95% ethanol and consequent turbidity in the final solution. Filter the final solution or pass the original sample through a column of strongly acidic cation-exchange resin in the hydrogen form to remove interfering cations. The latter procedure permits application of the method to samples of high hardness or solids content. Phosphate does not interfere.

c. Minimum detectable quantity: 0.2 μg B.

2. Apparatus

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

1) *Spectrophotometer,* for use at 540 nm, with a minimum light path of 1 cm.

2) *Filter photometer,* equipped with a green filter having a maximum transmittance near 540 nm, with a minimum light path of 1 cm.

b. Evaporating dishes, 100- to 150-mL capacity, of high-silica glass,* platinum, or other suitable material.

c. Water bath, set at 55 ± 2°C.

d. Glass-stoppered volumetric flasks, 25- and 50-mL capacity.

e. Ion-exchange column, 50 cm long by 1.3 cm in diameter.

3. Reagents

Store all reagents in polyethylene or boron-free containers.

a. Stock boron solution: Dissolve 571.6 mg anhydrous boric acid, H_3BO_3, in distilled water and dilute to 1000 mL; 1.00 mL = 100 μg B. Because H_3BO_3 loses weight on drying at 105°C, use a reagent meeting ACS specifications and keep the bottle tightly stoppered to prevent entrance of atmospheric moisture.

b. Standard boron solution: Dilute 10.00 mL stock boron solution to 1000 mL with distilled water; 1.00 mL = 1.00 μg B.

* Vycor, manufactured by Corning Glass Works, or equivalent.

c. Curcumin reagent: Dissolve 40 mg finely ground curcumin† and 5.0 g oxalic acid in 80 mL 95% ethyl alcohol. Add 4.2 mL conc HCl, make up to 100 mL with ethyl alcohol in a 100-mL volumetric flask, and filter if reagent is turbid (isopropyl alcohol, 95%, may be used in place of ethyl alcohol). This reagent is stable for several days if stored in a refrigerator.

d. Ethyl or isopropyl alcohol, 95%.

e. Reagents for removal of high hardness and cation interference:

1) *Strongly acidic cation-exchange resin.*

2) *Hydrochloric acid,* HCl, 1 + 5.

4. Procedure

a. Precautions: Closely control such variables as volumes and concentrations of reagents, as well as time and temperature of drying. Use evaporating dishes identical in shape, size, and composition to insure equal evaporation time because increasing the time increases intensity of the resulting color.

b. Preparation of calibration curve: Pipet 0 (blank), 0.25, 0.50, 0.75, and 1.00 μg boron into evaporating dishes of the same type, shape, and size. Add distilled water to each standard to bring total volume to 1.0 mL. Add 4.0 mL curcumin reagent to each and swirl gently to mix contents thoroughly. Float dishes on a water bath set at 55 ± 2°C and let them remain for 80 min, which is usually sufficient for complete drying and removal of HCl. Keep drying time constant for standards and samples. After dishes cool to room temperature, add 10 mL 95% ethyl alcohol to each dish and stir gently with a polyethylene rod to insure complete dissolution of the red-colored product.

Wash contents of dish into a 25-mL volumetric flask, using 95% ethyl alcohol. Make up to mark with 95% ethyl alcohol and mix thoroughly by inverting. Read absorbance of standards and samples at a wavelength of 540 nm after setting reagent blank at zero absorbance. The calibration curve is linear from 0 to 1.00 μg boron. Make photometric readings within 1 h of drying samples.

c. Sample treatment: For waters containing 0.10 to 1.00 mg B/L, use 1.00 mL sample. For waters containing more than 1.00 mg B/L, make an appropriate dilution with boron-free distilled water, so that a 1.00-mL portion contains approximately 0.50 μg boron.

Pipet 1.00 mL sample or dilution into an evaporating dish. Unless the calibration curve is being determined at the same time, prepare a blank and a standard containing 0.50 μg boron and run in conjunction with the sample. Proceed as in ¶ 4*b*, beginning with "Add 4.0 mL curcumin reagent. . . ." If the final solution is turbid, filter through filter paper‡ before reading absorbance. Calculate boron content from calibration curve.

d. Visual comparison: The photometric method may be adapted to visual estimation of low boron concentrations, from 50 to 200 μg/L, as follows: Dilute the standard boron solution 1 + 3 with distilled water; 1.00 mL = 0.20 μg B. Pipet 0, 0.05, 0.10, 0.15, and 0.20 μg boron into evaporating dishes as indicated in ¶ 4*b*. At the same time add an appropriate volume of sample (1.00 mL or portion diluted to 1.00 mL) to an identical evaporating dish. The total boron should be between 0.05 and 0.20 μg. Proceed as in ¶ 4*b*, beginning with "Add 4.0 mL curcumin reagent. . . ." Compare color of samples with standards within 1 h of drying samples.

e. Removal of high hardness and cation interference: Prepare an ion-exchange column of approximately 20 cm × 1.3 cm diam. Charge column with a strongly acidic cation-exchange resin. Backwash column with distilled water to remove entrained air bubbles. Keep the resin covered with liquid at all times. Pass 50 mL 1 + 5 HCl through column at a rate of 0.2 mL acid/mL resin in column/min and wash column free of acid with distilled water.

Pipet 25 mL sample, or a smaller sample of known high boron content diluted to 25 mL, onto the resin column. Adjust rate of flow to about 2 drops/s and collect effluent in a 50-mL volumetric flask. Wash column with small portions of distilled water until flask is filled to mark. Mix and transfer 2.00 mL into evaporating dish. Add 4.0 mL curcumin reagent and complete the analysis as described in ¶ 4*b* preceding.

5. Calculation

Use the following equation to calculate boron concentration from absorbance readings:

$$\text{mg B/L} = \frac{A_2 \times C}{A_1 \times S}$$

where:

A_1 = absorbance of standard,
A_2 = absorbance of sample,
C = μg B in standard taken, and
S = mL sample.

6. Precision and Bias

A synthetic sample containing 240 μg B/L, 40 μg As/L, 250 μg Be/L, 20 μg Se/L, and 6 μg V/L in distilled water was analyzed in 30 laboratories by the curcumin method with a relative standard deviation of 22.8% and a relative error of 0%.

7. Bibliography

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BUNTON, N.G. & B.H. TAIT. 1969. Determination of boron in waters and effluents using curcumin. *J. Amer. Water Works Assoc.* 61:357.

† Eastman No. 1179 or equivalent.
‡ Whatman No. 30 or equivalent.

4500-B C. Carmine Method

1. General Discussion

a. Principle: In the presence of boron, a solution of carmine or carminic acid in concentrated sulfuric acid changes from a bright red to a bluish red or blue, depending on the concentration of boron present.

b. Interference: The ions commonly found in water and wastewater do not interfere.

c. Minimum detectable quantity: 2 μg B.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 585 nm, with a minimum light path of 1 cm.

b. Filter photometer, equipped with an orange filter having a maximum transmittance near 585 nm, with a minimum light path of 1 cm.

3. Reagents

Store all reagents in polyethylene or boron-free containers.

a. Standard boron solution: Prepare as directed in Method B, ¶ 3*b*.

b. Hydrochloric acid, HCl, conc and 1 + 11.

c. Sulfuric acid, H_2SO_4, conc.

d. Carmine reagent: Dissolve 920 mg carmine N.F. 40, or carminic acid, in 1 L conc H_2SO_4. (If unable to zero spectrophotometer, dilute carmine 1 + 1 with conc H_2SO_4 to replace above reagent.)

4. Procedure

a. Preliminary sample treatment: If sample contains less than 1 mg B/L, pipet a portion containing 2 to 20 μg B into a platinum dish, make alkaline with 1*N* NaOH plus a slight excess, and evaporate to dryness on a steam or hot water bath. If necessary, destroy any organic material by ignition at 500 to 550°C. Acidify cooled residue (ignited or not) with 2.5 mL 1 + 11 HCl and triturate with a rubber policeman to dissolve. Centrifuge if necessary to obtain a clear solution. Pipet 2.00 mL clear concentrate into a small flask or 30-mL test tube. Treat reagent blank identically.

b. Color development: Prepare a series of boron standard solutions (100, 250, 500, 750, and 1000 μg) in 100 mL with distilled water. Pipet 2.00 mL of each standard solution into a small flask or 30-mL test tube.

Treat blank and calibration standards exactly as the sample. Add 2 drops (0.1 mL) conc HCl, carefully introduce 10.0 mL conc H_2SO_4, mix, and let cool to room temperature. Add 10.0 mL carmine reagent, mix well, and after 45 to 60 min measure absorbance at 585 nm in a cell of 1-cm or longer light path, using the blank as reference.

To avoid error, make sure that no bubbles are present in the optical cell while photometric readings are being made. Bubbles may appear as a result of incomplete mixing of reagents. Because carmine reagent deteriorates, check calibration curve daily.

5. Calculation

$$\text{mg B/L} = \frac{\mu\text{g B (in approx. 22 mL final volume)}}{\text{mL sample}} \times 1.25$$

6. Precision and Bias

A synthetic sample containing 180 μg B/L, 50 μg As/L, 400 μg Be/L, and 50 μg Se/L in distilled water was analyzed in nine laboratories by the carmine method with a relative standard deviation of 35.5% and a relative error of 0.6%.

7. Bibliography

HATCHER, J.T. & L.V. WILCOX. 1950. Colorimetric determination of boron using carmine. *Anal. Chem.* 22:567.

4500-Br⁻ BROMIDE*

4500-Br⁻ A. Introduction

1. Occurrence

Bromide occurs in varying amounts in ground and surface waters in coastal areas as a result of seawater intrusion and sea-spray-affected precipitation. The bromide content of ground waters and stream baseflows also can be affected by connate water. Industrial and oil-field brine discharges can contribute to the bromide in water sources. Under normal circumstances, the bromide content of most drinking waters is small, seldom exceeding 1 mg/L. Even levels of <100 μg/L can lead to formation of bromate or brominated by-products in disinfected waters.

2. Selection of Method

Described here are a colorimetric procedure suitable for the determination of bromide in most drinking waters and a flow injection analysis method. Bromide preferably is determined by the ion chromatography method (4110) or by capillary ion electrophoresis (4140).

* Approved by Standard Methods Committee, 1997.
Joint Task Group: 20th Edition (4500-Br⁻.D)—Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

4500-Br^- B. Phenol Red Colorimetric Method

1. General Discussion

a. Principle: When a sample containing bromide ions (Br^-) is treated with a dilute solution of chloramine-T in the presence of phenol red, the oxidation of bromide and subsequent bromination of the phenol red occur readily. If the reaction is buffered to pH 4.5 to 4.7, the color of the brominated compound will range from reddish to violet, depending on the bromide concentration. Thus, a sharp differentiation can be made among various concentrations of bromide. The concentration of chloramine-T and timing of the reaction before dechlorination are critical.

b. Interference: Most materials present in ordinary tap water do not interfere, but oxidizing and reducing agents and higher concentrations of chloride and bicarbonate can interfere. Free chlorine in samples should be destroyed as directed in Section 5210B.4*e*2); analyze bromide in a portion of dechlorinated sample. Addition of substantial chloride to the pH buffer solution (see ¶ 3*a* below) can eliminate chloride interference for waters with very low bromide/chloride ratios, such as those affected by dissolved road salt. Small amounts of dissolved iodide do not interfere, but small concentrations of ammonium ion interfere substantially. Sample dilution may reduce interferences to acceptable levels for some saline and waste waters. However, if two dilutions differing by a factor of at least five do not give comparable values, the method is inapplicable. Bromide concentration in diluted samples must be within the range of the method (0.1 to 1 mg/L).

c. Minimum detectable concentration: 0.1 mg Br^-/L.

2. Apparatus

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

1) *Spectrophotometer,* for use at 590 nm, providing a light path of at least 2 cm.

2) *Filter photometer,* providing a light path of at least 2 cm and equipped with an orange filter having a maximum transmittance near 590 nm.

3) *Nessler tubes,* matched, 100-mL, tall form.

b. Acid-washed glassware: Wash all glassware with 1 + 6 HNO_3 and rinse with distilled water to remove all trace of adsorbed bromide.

3. Reagents

a. Acetate buffer solution: Dissolve 90 g NaCl and 68 g sodium acetate trihydrate, $NaC_2H_3O_2 \cdot 3H_2O$, in distilled water. Add 30 mL conc (glacial) acetic acid and make up to 1 L. The pH should be 4.6 to 4.7.

b. Phenol red indicator solution: Dissolve 21 mg phenolsulfonephthalein sodium salt and dilute to 100 mL with distilled water.

c. Chloramine-T solution: Dissolve 500 mg chloramine-T, sodium *p*-toluenesulfonchloramide, and dilute to 100 mL with distilled water. Store in a dark bottle and refrigerate.

d. Sodium thiosulfate, 2M: Dissolve 49.6 g $Na_2S_2O_3 \cdot 5H_2O$ or 31.6 g $Na_2S_2O_3$ and dilute to 100 mL with distilled water.

e. Stock bromide solution: Dissolve 744.6 mg anhydrous KBr in distilled water and make up to 1000 mL; 1.00 mL = 500 μg Br^-.

f. Standard bromide solution: Dilute 10.00 mL stock bromide solution to 1000 mL with distilled water; 1.00 mL = 5.00 μg Br^-.

4. Procedure

a. Preparation of bromide standards: Prepare at least six standards, 0, 0.20, 0.40, 0.60, 0.80 and 1.00 mg Br^-/L, by diluting 0.0, 2.00, 4.00, 6.00, 8.00, and 10.00 mL standard bromide solution to 50.00 mL with distilled water. Treat standards the same as samples in ¶ 4*b*.

b. Treatment of sample: Add 2 mL buffer solution, 2 mL phenol red solution, and 0.5 mL chloramine-T solution to 50.0 mL sample or two separate sample dilutions (see 1*b* above) such that the final bromide concentration is in the range of 0.1 to 1.0 mg Br^-/L. Mix thoroughly immediately after each addition. Exactly 20 min after adding chloramine-T, dechlorinate by adding, with mixing, 0.5 mL $Na_2S_2O_3$ solution. Compare visually in nessler tubes against bromide standards prepared simultaneously, or preferably read in a photometer at 590 nm against a reagent blank. Determine the bromide values from a calibration curve of mg Br^-/L (in 55 mL final volume) against absorbance. A 2.54-cm light path yields an absorbance value of approximately 0.36 for 1 mg Br^-/L.

5. Calculation

mg Br^-/L = mg Br^-/L (from calibration curve) × dilution factor (if any). Results are based on 55 mL final volume for samples and standards.

6.Bibliography

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4500-Br⁻ C. (Reserved)

4500-Br⁻ D. Flow Injection Analysis

1. General Discussion

a. Principle: Bromide is oxidized to bromine by chloramine-T, followed by substitution of bromine on phenol red to produce bromphenol blue. The absorbance measured at 590 nm is proportional to the concentration of bromide in the sample. Sodium thiosulfate is added to reduce interference from chloride.

This method is suitable for the determination of bromide in waters containing up to 20 000 mg Cl^-/L, including drinking, ground, and surface waters, and domestic and industrial wastes. The method determines total bromide, or, if the sample is filtered through a 0.45-μm-pore-size filter, the result is called "dissolved bromide." The difference between total bromide and dissolved bromide is called "insoluble bromide."

Also see Section 4500-Br⁻.A and 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Chloride interference is reduced by the addition of sodium thiosulfate. Chloramine-T dissociates in aqueous solution to form hypochlorous acid, which can then react with chloride, causing substitution of chloride at positions ortho to the hydroxy groups on phenol red, just as in bromination. Sodium thiosulfate reacts with chlorine to reduce this interferent to a selectivity (ratio of analyte to interferent concentration) of >28 000.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold with flow cell (Figure 4500-Br⁻:1). Relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*

d. Absorbance detector, 590 nm, 10-nm bandpass.

e. Valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. Chloramine-T: To a tared 1-L container add 0.40 g chloramine-T hydrate (mol wt 227.65) and 999 g water. Cap and invert container to dissolve. Discard after 1 week.

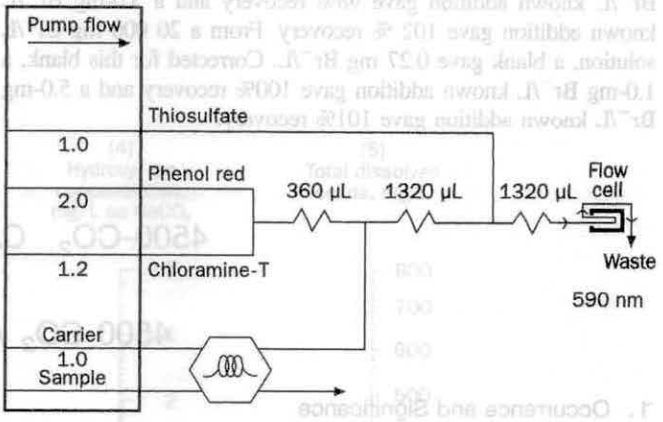

Figure 4500-Br⁻:1. FIA bromide manifold.

b. Phenol red: To a tared 1-L container add 929 g water and 30.0 g glacial acetic acid. Swirl contents of container. Add 41.0 g sodium acetate and swirl container until it is dissolved. Add 0.040 g phenol red. Mix with a magnetic stirrer. Discard after 1 week.

c. Thiosulfate: To a tared 1-L container, add 724 g water and 500 g sodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5H_2O$. Dissolve by adding the solid slowly while stirring. The solid should be completely dissolved within 30 min. Gentle heating may be required. Discard after 1 week.

d. Stock bromide standard, 100.0 mg Br^-/L: To a 1-L volumetric flask add 0.129 g sodium bromide, NaBr. Dissolve in sufficient water, dilute to mark, and invert to mix.

e. Stock bromide standard, 10.0 mg Br^-/L: To a 500-mL volumetric flask add 50 mL stock standard (¶ 3*d*). Dilute to mark and invert to mix. Prepare fresh monthly.

f. Standard bromide solutions: Prepare bromide standards for the calibration curve in the desired concentration range, using the stock standard (¶ *e*), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-Br⁻:1 and follow method supplied by manufacturer, or laboratory standard operating procedure for this method. Follow quality control guidelines outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold vs. bromide concentration. The calibration curve gives a good fit to a second-order polynomial.

* Teflon or equivalent.

6. Precision and Bias

a. Precision: With a 300-μL sample loop, ten replicates of a 5.0-mg Br^-/L standard gave a mean of 5.10 mg Br^-/L and a relative standard deviation of 0.73%.

b. Bias: With a 300-μL sample loop, solutions of sodium chloride were fortified in triplicate with bromide and mean blanks and recoveries were measured. From a 10 000-mg Cl^-/L solution, a blank gave 0.13 mg Br^-/L. Corrected for this blank, a 1.0-mg Br^-/L known addition gave 98% recovery and a 5.0-mg Br^-/L known addition gave 102 % recovery. From a 20 000 mg Cl^-/L solution, a blank gave 0.27 mg Br^-/L. Corrected for this blank, a 1.0-mg Br^-/L known addition gave 100% recovery and a 5.0-mg Br^-/L known addition gave 101% recovery.

c. MDL: Using a published MDL method[1] and a 300-μL sample loop, analysts ran 21 replicates of a 0.5-mg Br^-/L standard. These gave a mean of 0.468 mg Br^-/L, a standard deviation of 0.030 mg Br^-/L, and an MDL of 0.07 mg Br^-/L. A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagents flow rate.

7. References

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and procedure for the determination of method detection limits. Appendix B to CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.
2. ANAGNOSTOPOULOU, P. & M. KOUPPARIS. 1986. Automated FIA phenol red method for determination of bromide. *Anal. Chem.* 58:322.

4500-CO_2 CARBON DIOXIDE*

4500-CO_2 A. Introduction

1. Occurrence and Significance

Surface waters normally contain less than 10 mg free carbon dioxide (CO_2) per liter while some groundwaters may easily exceed that concentration. The CO_2 content of a water may contribute significantly to corrosion. Recarbonation of a supply during the last stages of water softening is a recognized treatment process. The subject of saturation with respect to calcium carbonate is discussed in Section 2330.

2. Selection of Method

A nomographic and a titrimetric method are described for the estimation of free CO_2 in drinking water. The titration may be performed potentiometrically or with phenolphthalein indicator. Properly conducted, the more rapid, simple indicator method is satisfactory for field tests and for control and routine applications if it is understood that the method gives, at best, only an approximation.

The nomographic method (B) usually gives a closer estimation of the total free CO_2 when the pH and alkalinity determinations are made immediately and correctly at the time of sampling. The pH measurement preferably should be made with an electrometric pH meter, properly calibrated with standard buffer solutions in the pH range of 7 to 8. The error resulting from inaccurate pH measurements grows with an increase in total alkalinity. For example, an inaccuracy of 0.1 in the pH determination causes a CO_2 error of 2 to 4 mg/L in the pH range of 7.0 to 7.3 and a total alkalinity of 100 mg $CaCO_3$/L. In the same pH range, the error approaches 10 to 15 mg/L when the total alkalinity is 400 mg as $CaCO_3$/L.

Under favorable conditions, agreement between the titrimetric and nomographic methods is reasonably good. When agreement is not precise and the CO_2 determination is of particular importance, state the method used.

The calculation of the total CO_2, free and combined, is given in Method D.

* Approved by Standard Methods Committee, 1997.

4500-CO_2 B. Nomographic Determination of Free Carbon Dioxide and the Three Forms of Alkalinity*

1. General Discussion

Diagrams and nomographs enable the rapid calculation of the CO_2, bicarbonate, carbonate, and hydroxide content of natural and treated waters. These graphical presentations are based on equations relating the ionization equilibria of the carbonates and water. If pH, total alkalinity, temperature, and total mineral content are known, any or all of the alkalinity forms and CO_2 can be determined nomographically.

A set of charts, Figures 4500-CO_2:1–4,† is presented for use where their accuracy for the individual water supply is confirmed. The nomographs and the equations on which they are

* See also Alkalinity, Section 2320.

† Copies of the nomographs in Figures 4500-CO_2:1–4, enlarged to several times the size shown here, may be obtained as a set from Standard Methods Manager, The American Water Works Association, 6666 West Quincy Ave., Denver, CO 80235.

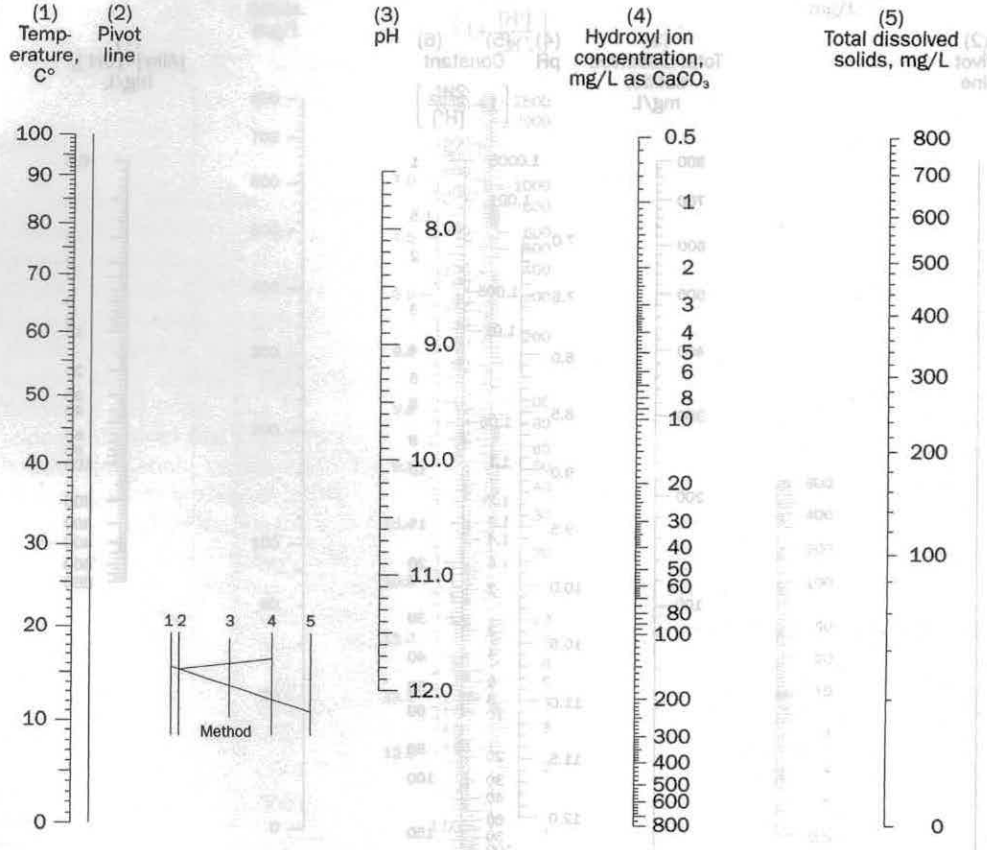

Figure 4500-CO_2:1. Nomograph for evaluation of hydroxide ion concentration. To use: Align temperature (Scale 1) and total dissolved solids (Scale 5); pivot on Line 2 to proper pH (Scale 3); read hydroxide ion concentration, as mg $CaCO_3$/L, on Scale 4. (Example: For 13°C temperature, 240 mg total dissolved solids/L, pH 9.8, the hydroxide ion concentration is found to be 1.4 mg as $CaCO_3$/L.)

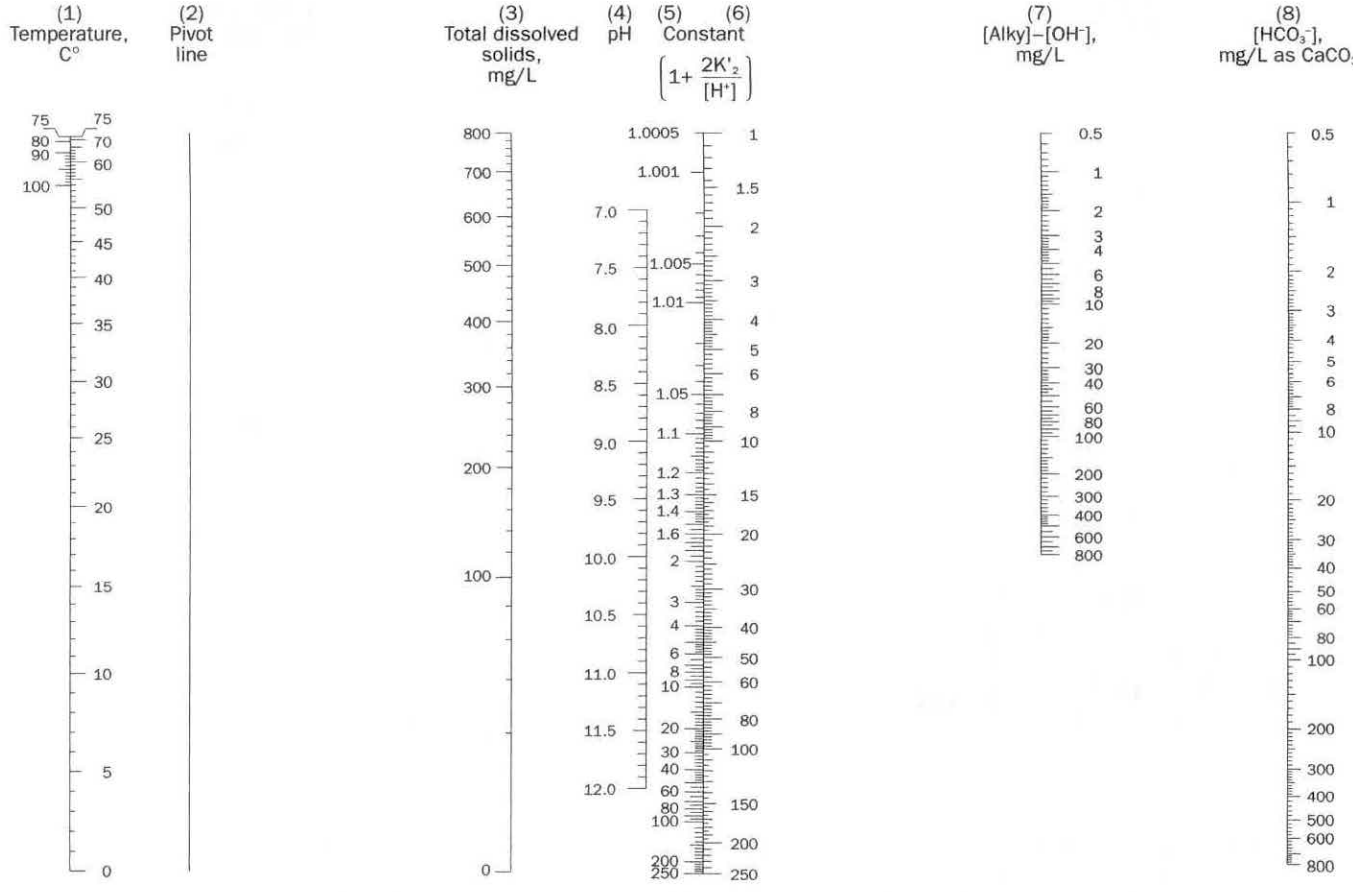

Figure 4500-CO_2:2. Nomograph for evaluation of bicarbonate alkalinity. To use: Align temperature (Scale 1) and total dissolved solids (Scale 3); pivot on Line 2 to proper pH (Scale 4) and read constant on Scale 5; locate constant on Scale 6 and align with nonhydroxide alkalinity (found with aid of Figure 4500-CO_2:1) on Scale 7; read bicarbonate alkalinity on Scale 8. (Example: For 13°C temperature, 240 mg total dissolved solids/L, pH 9.8, and 140 mg alkalinity/L, the bicarbonate content is found to be 90 mg as $CaCO_3$/L.)

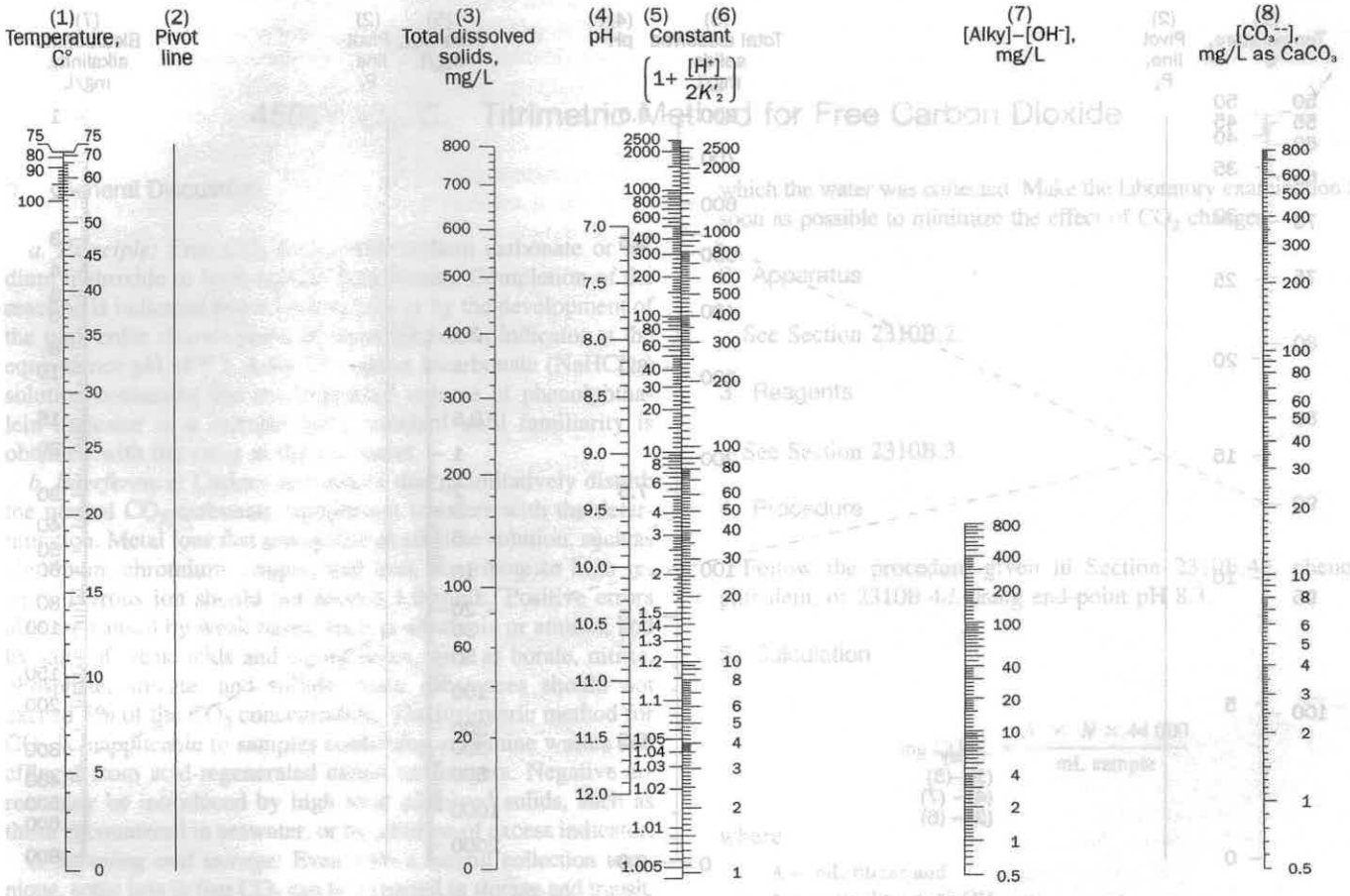

Figure 4500-CO_2:3. Nomograph for evaluation of carbonate alkalinity. To use: Align temperature (Scale 1) and total dissolved solids (Scale 3); pivot on Line 2 to proper pH (Scale 4) and read constant on Scale 5; locate constant on Scale 6 and align with nonhydroxide alkalinity (found with aid of Figure 4500-CO_2:1) on Scale 7; read carbonate alkalinity on Scale 8. (Example: For 13°C temperature, 240 mg total dissolved solids/L, pH 9.8, and 140 mg alkalinity/L, the carbonate content is found to be 50 mg as $CaCO_3$/L.)

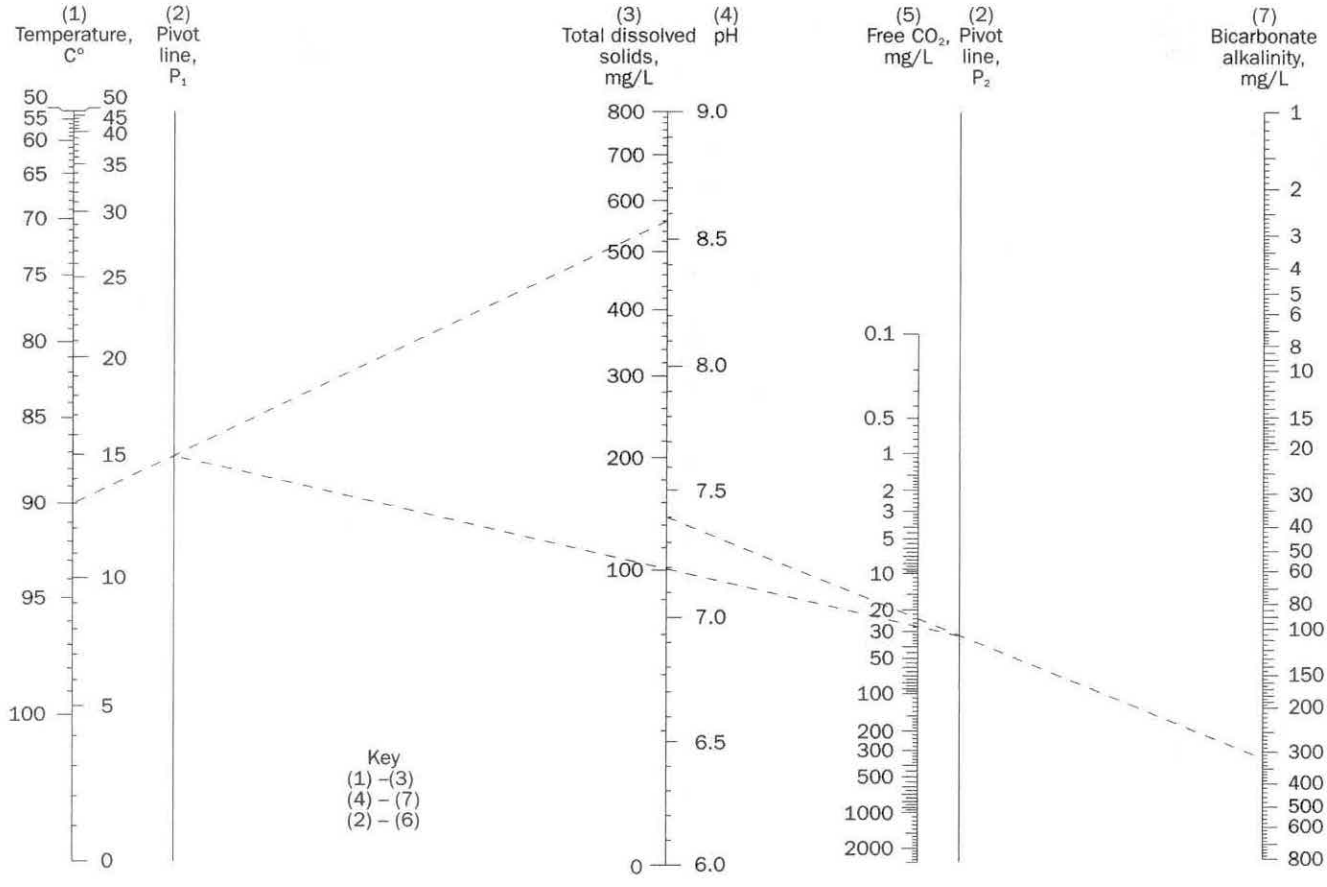

Figure 4500-CO_2:4. Nomograph for evaluation of free carbon dioxide content. To use: Align temperature (Scale 1) and total dissolved solids (Scale 3), which determines Point P_1 on Line 2; align pH (Scale 4) and bicarbonate alkalinity (Scale 7), which determines Point P_2 on Line 6; align P_1 and P_2 and read free carbon dioxide on Scale 5. (Example: For 13°C temperature, 560 mg total dissolved solids/L, pH 7.4, and 320 mg alkalinity/L, the free carbon dioxide content is found to be 28 mg/L.)

based are valid only when the salts of weak acids other than carbonic acid are absent or present in extremely small amounts.

Some treatment processes, such as superchlorination and coagulation, can affect significantly pH and total-alkalinity values of a poorly buffered water of low alkalinity and low total-dissolved-mineral content. In such instances the nomographs may not be applicable.

2. Precision and Bias

The precision possible with the nomographs depends on the size and range of the scales. With practice, the recommended nomographs can be read with a precision of 1%. However, the overall bias of the results depends on the bias of the analytical data applied to the nomographs and the validity of the theoretical equations and the numerical constants on which the nomographs are based. An approximate check of the bias of the calculations can be made by summing the three forms of alkalinity. Their sum should equal the total alkalinity.

3. Bibliography

MOORE, E.W. 1939. Graphic determination of carbon dioxide and the three forms of alkalinity. *J. Amer. Water Works Assoc.* 31:51.

4500-CO_2 C. Titrimetric Method for Free Carbon Dioxide

1. General Discussion

a. Principle: Free CO_2 reacts with sodium carbonate or sodium hydroxide to form sodium bicarbonate. Completion of the reaction is indicated potentiometrically or by the development of the pink color characteristic of phenolphthalein indicator at the equivalence pH of 8.3. A 0.01*N* sodium bicarbonate ($NaHCO_3$) solution containing the recommended volume of phenolphthalein indicator is a suitable color standard until familiarity is obtained with the color at the end point.

b. Interference: Cations and anions that quantitatively disturb the normal CO_2-carbonate equilibrium interfere with the determination. Metal ions that precipitate in alkaline solution, such as aluminum, chromium, copper, and iron, contribute to high results. Ferrous ion should not exceed 1.0 mg/L. Positive errors also are caused by weak bases, such as ammonia or amines, and by salts of weak acids and strong bases, such as borate, nitrite, phosphate, silicate, and sulfide. Such substances should not exceed 5% of the CO_2 concentration. The titrimetric method for CO_2 is inapplicable to samples containing acid mine wastes and effluent from acid-regenerated cation exchangers. Negative errors may be introduced by high total dissolved solids, such as those encountered in seawater, or by addition of excess indicator.

c. Sampling and storage: Even with a careful collection technique, some loss in free CO_2 can be expected in storage and transit. This occurs more frequently when the gas is present in large amounts. Occasionally a sample may show an increase in free CO_2 content on standing. Consequently, determine free CO_2 immediately at the point of sampling. Where a field determination is impractical, fill completely a bottle for laboratory examination. Keep the sample, until tested, at a temperature lower than that at which the water was collected. Make the laboratory examination as soon as possible to minimize the effect of CO_2 changes.

2. Apparatus

See Section 2310B.2.

3. Reagents

See Section 2310B.3.

4. Procedure

Follow the procedure given in Section 2310B.4*b*, phenolphthalein, or 2310B.4*d*, using end-point pH 8.3.

5. Calculation

$$\text{mg } CO_2/\text{L} = \frac{A \times N \times 44\,000}{\text{mL sample}}$$

where:

A = mL titrant and
N = normality of NaOH.

6. Precision and Bias

Precision and bias of the titrimetric method are on the order of ±10% of the known CO_2 concentration.

4500-CO_2 D. Carbon Dioxide and Forms of Alkalinity by Calculation

1. General Discussion

When the total alkalinity of a water (Section 2320) is due almost entirely to hydroxides, carbonates, or bicarbonates, and the total dissolved solids (Section 2540) is not greater than 500 mg/L, the alkalinity forms and free CO_2 can be calculated from the sample pH and total alkalinity. The calculation is subject to the same limitations as the nomographic procedure given above and the additional restriction of using a single temperature, 25°C. The calculations are based on the ionization constants:

$$K_1 = \frac{[H^+][HCO_3^-]}{[H_2CO_3^*]} \quad (K_1 = 10^{-6.36})$$

and

$$K_2 = \frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} \quad (K_2 = 10^{-10.33})$$

where:

$$[H_2CO_3^*] = [H_2CO_3] + [CO_2(aq)]$$

Activity coefficients are assumed equal to unity.

2. Calculation

Compute the forms of alkalinity and sample pH and total alkalinity using the following equations:

a. Bicarbonate alkalinity:

$$HCO_3^- \text{ as mg } CaCO_3/L = \frac{T - 5.0 \times 10^{(pH-10)}}{1 + 0.94 \times 10^{(pH-10)}}$$

where:

T = total alkalinity, mg $CaCO_3$/L.

b. Carbonate alkalinity:

$$CO_3^{2-} \text{ as mg } CaCO_3/L = 0.94 \times B \times 10^{(pH-10)}$$

where:

B = bicarbonate alkalinity, from *a*.

c. Hydroxide alkalinity:

$$OH^- \text{ as mg } CaCO_3/L = 5.0 \times 10^{(pH-10)}$$

d. Free carbon dioxide:

$$\text{mg } CO_2/L = 2.0 \times B \times 10^{(6-pH)}$$

where:

B = bicarbonate alkalinity, from *a*.

e. Total carbon dioxide:

$$\text{mg total } CO_2/L = A + 0.44\,(2B + C)$$

where:

A = mg free CO_2/L,
B = bicarbonate alkalinity from *a*, and
C = carbonate alkalinity from *b*.

3. Bibliography

DYE, J.F. 1958. Correlation of the two principal methods of calculating the three kinds of alkalinity. *J. Amer. Water Works Assoc.* 50:812.

4500-CN^- CYANIDE*

4500-CN^- A. Introduction

1. General Discussion

"Cyanide" in Section 4500-CN^- refers to inorganic cyanide (CN^-) in water. In aqueous media cyanide can exist as the undissociated hydrogen cyanide (HCN), the free cyanide ion (CN^-), and anionic complexes of cyanide with a variety of metal cations.

Hydrogen cyanide is a very weak acid. The pK_a of the dissociation of HCN is 9.2. Thus, for approximately neutral pH water samples, HCN is the predominant form[1] rather than the free CN^-.

Simple cyanide salts (NaCN and KCN) are completely dissociated in water; however, in the presence of other metal cations, anionic complexes with cyanide are formed. These anionic cyanide complexes exhibit a wide variety of stabilities, with ther-

* Approved by Standard Methods Committee, 1999.
Joint Task Group: (4500-CN^-. A, B, C) — Roy-Keith Smith (chair), Steve A. Carr, Michael F. Delaney, Donalea Dinsmore, Martin S. Frant; (4500-CN^-. N, O)—Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

modynamic formation constants ranging from $10^{-41.5}$ for $Hg(CN)_4^{2-}$ to $10^{-16.7}$ for $Zn(CN)_4^{2-}$. The stability of these complexes varies with pH, most exhibiting appreciable dissociation in acidic solutions. The most stable complex is that formed with iron, and in the presence of excess cyanide in water, all available iron will be bound in either the ferrocyanide, $Fe(CN)_6^{4-}$ or ferricyanide complex, $Fe(CN)_6^{3-}$.

The great toxicity to aquatic life of molecular HCN is well known;[2–5] it is formed in solutions of cyanide by hydrolytic reaction of CN^- with water. The toxicity of CN^- is less than that of HCN; it usually is unimportant because most of the free cyanide (CN group present as CN^- or as HCN) exists as HCN,[2–5] because the pH of most natural waters is substantially lower than the pK_a for molecular HCN. The toxicity to fish of most tested solutions of complex cyanides is attributable mainly to the HCN resulting from dissociation of the complexes.[2,4,5] Analytical distinction between HCN and other cyanide species in solutions of complex cyanides is possible.[2,5–9,10]

Most metallocyanide complexes achieve dissociation equilibrium slowly, and the toxicity of these complexes is inversely related to their highly variable stability.[2,4,5] The degree of dissociation also is related to the dilution, and increases with decreasing pH. Cyanide complexes of zinc and cadmium are dissociated almost totally in very dilute solutions and can be acutely toxic to fish, even at pH characteristic of natural aquatic environments. In equally dilute solutions there is much less dissociation of nickel and other more stable cyanide complexes. Although the complexed cyanide exhibits much less toxicity than HCN, dilute solutions of copper and silver cyanide complexes may still demonstrate acute toxicity to fish.[2,5]

The iron cyanide complexes are very stable and are generally regarded as not materially toxic. Acutely toxic levels of HCN, however, may be reached in aged solutions of moderate to high concentrations in the dark. Dilute solutions of iron cyanide complexes are subject to rapid and extensive photolysis when exposed to ultraviolet radiation.[2,11] The latter process is limited in deep, turbid, or shaded receiving waters.

Historically, the generally accepted physicochemical technique for industrial waste treatment of cyanide compounds is alkaline chlorination:

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

The first reaction product on chlorination is cyanogen chloride (CNCl), a highly toxic gas of limited solubility. The toxicity of CNCl may exceed that of equal concentrations of cyanide.[2,3,12] At an alkaline pH, CNCl hydrolyzes to the cyanate ion (CNO^-), which has only limited toxicity.

There is no known natural reduction reaction that may convert CNO^- to CN^-.[13] On the other hand, breakdown of toxic CNCl is pH- and time-dependent. At pH 9, with no excess chlorine present, CNCl may persist for 24 h.[14,15]

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

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

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

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

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

The alkaline chlorination of cyanide compounds is relatively fast, but depends equally on the dissociation constant, which also governs toxicity. Metal cyanide complexes, such as nickel, cobalt, silver, and gold, do not dissociate readily. The chlorination reaction therefore requires more time and a significant chlorine excess.[16] Iron cyanides, because they do not dissociate to any degree, are not oxidized by chlorination. There is correlation between the refractory properties of the noted complexes, in their resistance to chlorination and lack of toxicity.

Recent literature indicates that alkaline chlorination of wastewater with high nitrogen loads, followed by dechlorination with ascorbic acid, forms additional cyanide ion.[17] This generates negative values for the cyanide amenable to chlorination test (4500-CN⁻.G). Treatment of the sample with sulfamic acid before chlorination and judicious use of sodium thiosulfate for dechlorination can correct the problem.

It is advantageous to differentiate between *total cyanide* and *cyanides amenable to chlorination*. When total cyanide is determined, the almost nondissociable cyanides, as well as cyanide bound in complexes that are readily dissociable and complexes of intermediate stability, are measured. Cyanide compounds that are amenable to chlorination include free cyanide as well as those complex cyanides that are potentially dissociable, almost wholly or in large degree, and therefore, potentially toxic at low concentrations, even in the dark. The chlorination test procedure is carried out under rigorous conditions appropriate for measurement of the more dissociable forms of cyanide.

The free and potentially dissociable cyanides also may be estimated when using the *weak acid dissociable* procedure. These methods depend on a rigorous distillation, but the solution is only slightly acidified, and elimination of iron cyanides is insured by the earlier addition of precipitation chemicals to the distillation flask and by the avoidance of ultraviolet irradiation.

The *cyanogen chloride* procedure is common with the colorimetric test for cyanides amenable to chlorination. This test is based on the addition of chloramine-T and subsequent color complex formation with pyridine-barbituric acid solution. Without the addition of chloramine-T, only existing CNCl is measured. CNCl is a gas that hydrolyzes to CNO^-; sample preservation is not possible. Because of this, spot testing of CNCl levels may be best. This procedure can be adapted and used when the sample is collected.

There may be analytical requirements for the determination of CNO^-, even though the reported toxicity level is low. On acidification, CNO^- decomposes to ammonia (NH_3).[3] Molecular ammonia and metal-ammonia complexes are toxic to aquatic life.[18]

Thiocyanate (SCN^-) is not very toxic to aquatic life.[2,19] However, upon chlorination, toxic CNCl is formed, as discussed above.[2,3,12] At least where subsequent chlorination is anticipated, the determination of SCN^- is desirable. Thiocyanate is biodegradable; ammonium ion is released in this reaction. Although the typical detoxifying agents used in cyanide poisoning induce thiocyanate formation, biochemical cyclic reactions with cyanide are possible, resulting in detectable levels of cyanide from exposure to thiocyanate.[19] Thiocyanate may be analyzed in samples properly preserved for determination of cyanide; however,

thiocyanate also can be preserved in samples by acidification with H_2SO_4 to pH ≤ 2.

2. Cyanide in Solid Waste

a. Soluble cyanide: Determination of soluble cyanide requires sample leaching with distilled water until solubility equilibrium is established. One hour of stirring in distilled water should be satisfactory. Cyanide analysis is then performed on the leachate. Low cyanide concentration in the leachate may indicate presence of sparingly soluble metal cyanides. The cyanide content of the leachate is indicative of residual solubility of insoluble metal cyanides in the waste.

High levels of cyanide in the leachate indicate soluble cyanide in the solid waste. When 500 mL distilled water are stirred into a 500-mg solid waste sample, the cyanide concentration (mg/L) of the leachate multiplied by 1000 will give the solubility level of the cyanide in the solid waste in milligrams per kilogram. The leachate may be analyzed for total cyanide and/or cyanide amenable to chlorination.

b. Insoluble cyanide: The insoluble cyanide of the solid waste can be determined with the total cyanide method by placing a 500-mg sample with 500 mL distilled water in the distillation flask and in general following the distillation procedure (Section 4500-CN^-.C). In calculating, multiply by 1000 to give the cyanide content of the solid sample in milligrams per kilogram. Insoluble iron cyanides in the solid can be leached out earlier by stirring a weighed sample for 12 to 16 h in a 10% NaOH solution. The leached and wash waters of the solid waste will give the iron cyanide content with the distillation procedure. Prechlorination will have eliminated all cyanide amenable to chlorination. Do not expose sample to sunlight.

3. Selection of Method

a. Total cyanide after distillation: After removal of interfering substances, cyanide is converted to HCN gas, which is distilled and absorbed in sodium hydroxide (NaOH) solution.[20] Because of the catalytic decomposition of cyanide in the presence of cobalt at high temperature in a strong acid solution,[21,22] cobalticyanide is not recovered completely. Indications are that cyanide complexes of the noble metals, i.e., gold, platinum, and palladium, are not recovered fully by this procedure either. Distillation also separates cyanide from other color-producing and possibly interfering organic or inorganic contaminants. Subsequent analysis is for the simple salt, sodium cyanide (NaCN). Some organic cyanide compounds, such as cyanohydrins, are decomposed by the distillation. Aldehydes react with cyanide to form cyanohydrins.

The absorption liquid is analyzed by a titrimetric, colorimetric, or cyanide-ion-selective electrode procedure:

1) The titration method (D) is suitable for cyanide concentrations above 1 mg/L.

2) The colorimetric methods (E, N, and O) are suitable for cyanide concentrations as low as 1 to 5 μg/L under ideal conditions. Method N uses flow injection analysis of the distillate. Method O uses flow injection analysis following transfer through a semipermeable membrane for separating gaseous cyanide, and colorimetric analysis. Method E uses conventional colorimetric analysis of the distillate from Method C.

3) The ion-selective electrode method (F) using the cyanide ion electrode is applicable in the concentration range of 0.05 to 10 mg/L.

b. Cyanide amenable to chlorination:

1) Distillation of two samples is required, one that has been chlorinated to destroy all amenable cyanide present and the other unchlorinated. Analyze absorption liquids from both tests for total cyanide. The observed difference equals cyanides amenable to chlorination.

2) The colorimetric methods, by conversion of amenable cyanide and SCN^- to CNCl and developing the color complex with pyridine-barbituric acid solution, are used for the determination of the total of these cyanides (H, N, and O). Repeating the test with the cyanide masked by the addition of formaldehyde provides a measure of the SCN^- content. When subtracted from the earlier results this provides an estimate of the amenable CN^- content. This method is useful for natural and ground waters, clean metal finishing, and heat treating effluents. Sanitary wastes may exhibit interference.

3) The *weak acid dissociable cyanides* procedure approximately measures the cyanide amenable to chlorination by freeing HCN from the dissociable cyanide. After being collected in a NaOH absorption solution, CN^- may be determined by one of the finishing procedures given for the total cyanide determination. An automated procedure (O) also is presented.

It should be noted that although cyanide amenable to chlorination and weak acid dissociable cyanide appear to be identical, certain industrial effluents (e.g., pulp and paper, petroleum refining industry effluents) contain some poorly understood substances that may produce interference. Application of the procedure for cyanide amenable to chlorination yields negative values. For natural waters and metal-finishing effluents, the direct colorimetric determination appears to be the simplest and most economical.

c. Cyanogen chloride: The colorimetric method for measuring cyanide amenable to chlorination may be used, but omit the chloramine-T addition. The spot test also may be used.

d. Spot test for sample screening: This procedure allows a quick sample screening to establish whether more than 50 μg/L cyanide amenable to chlorination is present. The test also may be used to estimate the CNCl content at the time of sampling.

e. Cyanate: CNO^- is converted to ammonium carbonate, $(NH_4)_2CO_3$, by acid hydrolysis at elevated temperature. Ammonia (NH_3) is determined before the conversion of the CNO^- and again afterwards. The CNO^- is estimated from the difference in NH_3 found in the two tests.[23–25] Measure NH_3 by either:

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

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

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

4. References

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4500-CN⁻ B. Preliminary Treatment of Samples

Caution—*Use care in manipulating cyanide-containing samples because of toxicity. Process in a hood or other well-ventilated area. Avoid contact, inhalation, or ingestion.*

1. General Discussion

The nature of the preliminary treatment will vary according to the interfering substance present. Sulfides, fatty acids, oxidizing agents, nitrites, and nitrates are removed by pre-treatment procedures. Most other interfering substances are removed by distillation.

2. Preservation of Samples

Oxidizing agents, such as chlorine, decompose most cyanides. For chlorine residuals down to 2 mg/L, test by placing a drop of sample on a strip of fresh potassium iodide-starch paper moistened with acetate buffer (4500-Cl.C.3*e*). A blue-purple color indicates that oxidants are present. Add small portions of sodium thiosulfate solution (0.02 g/L) with constant re-testing until the oxidizers are neutralized. Avoid any excess thiosulfate solution. Sodium arsenite solution (0.1 g/L) may be used in lieu of thiosulfate. For chlorine residuals below 2 mg/L, determine residual by DPD-FAS titration (4500-Cl.F) or DPD colorimetric method (4500-Cl.G) and add a stoichiometric amount of sodium thiosulfate solutions (4500-Cl.B.2*d*).

Ascorbic acid is no longer being recommended for preservation of samples for cyanide analysis. Ascorbic acid functions as a carbon donor in the presence of nitrite or nitrate, and generates cyanide during the distillation.[1] Sodium thiosulfate is an adequate dechlorinating agent as long as it is not used in excess. Sodium arsenite also may be used, but it is a hazardous material. If ascorbic acid must be used, add sulfamic acid (2 g/500 mL sample) before adding ascorbic acid and sodium hydroxide.

If sulfides are suspected as present in the sample, remove them. Oxidized products of sulfide convert cyanide to thiocyanate rapidly, especially at elevated pH.[2] Test for S^{2-} by placing a drop of sample on lead acetate test paper previously moistened with acetic acid buffer solutions, pH 4 (Section 4500-Cl.C.3*e*). Darkening of the paper indicates presence of S^{2-}. Add lead acetate, or if the S^{2-} concentration is too high, add powdered lead carbonate, $PbCO_3$, to avoid significantly reducing pH. Repeat test until a drop of treated sample no longer darkens the acidified lead acetate test paper. Filter sample before raising pH for stabilization. When particulate, metal cyanide complexes are suspected, filter solution before removing S^{2-}. Reconstitute sample by returning filtered particulates to the sample bottle after S^{2-} removal. Homogenize particulates before analyses.

Aldehydes convert cyanide to cyanohydrin. Longer contact times between cyanide and the aldehyde and the higher ratios of aldehyde to cyanide both result in increasing losses of cyanide that are not reversible during analysis. If the presence of alde-

hydes is suspected, stabilize with NaOH at time of collection and add 2 mL 3.5% ethylenediamine solution per 100 mL of sample.

Because most cyanides are very reactive and unstable, analyze samples as soon as possible. If sample cannot be analyzed immediately, add NaOH pellets or a strong NaOH solution to raise sample pH to 12 to 12.5. Add dechlorinating agent before pH adjustment if sample is disinfected. Store in a closed, dark bottle in a cool place.

To analyze for CNCl collect a separate sample and omit NaOH addition because CNCl is converted rapidly to CNO^- at high pH. Make colorimetric estimation immediately after sampling.

3. Interferences

a. Oxidizing agents may destroy most of the cyanide during storage and manipulation. Add $NaAsO_2$ or $Na_2S_2O_3$ as directed above; avoid excess $Na_2S_2O_3$.

b. Sulfide will distill over with cyanide and, therefore, adversely affect colorimetric, titrimetric, and electrode procedures. Test for and remove S^{2-} as directed above. Treat 25 mL more than required for the distillation to provide sufficient filtrate volume.

c. Fatty acids that distill and form soaps under alkaline titration conditions make the end point almost impossible to detect. Remove fatty acids by extraction.[3] Acidify sample with acetic acid (1 + 9) to pH 6.0 to 7.0. (CAUTION—*Perform this operation in a hood as quickly as possible.*) Immediately extract with iso-octane, hexane, or $CHCl_3$ (preference in order named). Use a solvent volume equal to 20% of sample volume. One extraction usually is adequate to reduce fatty acid concentration below the interference level. Avoid multiple extractions or a long contact time at low pH to minimize loss of HCN. When extraction is completed, immediately raise pH to >12 with NaOH solution.

d. Carbonate in high concentration may affect the distillation procedure by causing the violent release of carbon dioxide with excessive foaming when acid is added before distillation and by reducing pH of the absorption solution. Use calcium hydroxide to preserve such samples.[4] Add calcium hydroxide slowly, with stirring, to pH 12 to 12.5. After precipitate settles, decant supernatant liquid for determining cyanide.

Insoluble complex cyanide compounds will not be determined. If such compounds are present, filter a measured amount of well-mixed treated sample through a glass fiber or membrane filter (47-mm diam or less). Rinse filter with dilute (1 to 9) acetic acid until effervescence ceases. Treat entire filter with insoluble material as insoluble cyanide (4500-CN^-.A.2*b*) or add to filtrate before distillation.

e. Other possible interferences include substances that might contribute color or turbidity. In most cases, distillation will remove these.

Note, however, that the strong acid distillation procedure requires using sulfuric acid with various reagents. With certain wastes, these conditions may result in reactions that otherwise would not occur in the aqueous sample. As a quality control measure, periodically conduct addition and recovery tests with industrial waste samples.

f. Aldehydes convert cyanide to cyanohydrin, which forms nitrile under the distillation conditions. Only direct titration without distillation can be used, which reveals only non-complex cyanides. Formaldehyde interference is noticeable in concentrations exceeding 0.5 mg/L. Use the following spot test to establish absence or presence of aldehydes (detection level 0.05 mg/L):[5–7]

1) Reagents

a) *MBTH indicator solution:* Dissolve 0.05 g 3-methyl, 2-benzothiazolone hydrazone hydrochloride in 100 mL water. Filter if turbid.

b) *Ferric chloride oxidizing solution:* Dissolve 1.6 g sulfamic acid and 1 g $FeCl_3 \cdot 6H_2O$ in 100 mL water.

c) *Ethylenediamine solution,* 3.5%: Dilute 3.5 mL pharmaceutical-grade anhydrous $NH_2CH_2CH_2NH_2$ to 100 mL with water.

2) Procedure—If the sample is alkaline, add 1+ 1 H_2SO_4 to 10 mL sample to adjust pH to less than 8. Place 1 drop of sample and 1 drop distilled water for a blank in separate cavities of a white spot plate. Add 1 drop MBTH solution and then 1 drop $FeCl_3$ oxidizing solution to each spot. Allow 10 min for color development. The color change will be from a faint green-yellow to a deeper green with blue-green to blue at higher concentrations of aldehyde. The blank should remain yellow.

To minimize aldehyde interference, add 2 mL 3.5% ethylenediamine solution/100 mL sample. This quantity overcomes the interference caused by up to 50 mg/L formaldehyde.

When using a known addition in testing, 100% recovery of the CN^- is not necessarily to be expected. Recovery depends on the aldehyde excess, time of contact, and sample temperature.

g. Glucose and other sugars, especially at the pH of preservation, lead to cyanohydrin formation by reaction of cyanide with aldose.[8] Reduce cyanohydrin to cyanide with ethylenediamine (see *f* above). MBTH is not applicable.

h. Nitrite may form HCN during distillation in Methods C, G, and L through reaction with organic compounds.[9,10] Nitrate also may reduce to nitrite, which can react further with thiocyanate. Add at least 2 g sulfamic acid at time of sample collection and before dechlorination or basification to avoid nitrite interference.

i. Some sulfur compounds may decompose during distillation, releasing S, H_2S, or SO_2. Sulfur compounds may convert cyanide to thiocyanate and also may interfere with the analytical procedures for CN^-. To avoid this potential interference, add 50 mg $PbCO_3$ to the absorption solution before distillation. Filter sample before proceeding with the colorimetric or titrimetric determination.

Absorbed SO_2 forms Na_2SO_3, which consumes chloramine-T added in the colorimetric determination. The volume of chloramine-T added is sufficient to overcome 100 to 200 mg SO_3^{2-}/L. Test for presence of chloramine-T after adding it by placing a drop of sample on KI-starch test paper; add more chloramine-T if the test paper remains blank, or use Method F.

Some wastewaters, such as those from coal gasification or chemical extraction mining, contain high concentrations of sulfites. Pretreat sample to avoid overloading the absorption solution with SO_3^{2-}. Titrate a suitable sample iodometrically (Section 4500-O) with dropwise addition of 30% H_2O_2 solution to determine volume of H_2O_2 needed for the 500 mL distillation sample. Subsequently, add H_2O_2 dropwise while stirring, but in only such volume that not more than 300 to 400 mg SO_3^{2-}/L will remain. Adding a lesser quantity than calculated is required to avoid oxidizing any CN^- that may be present.

j. Alternate procedure: The strong acid distillation procedure uses concentrated acid with magnesium chloride to dissociate metal-cyanide complexes. In some instances, particularly with industrial wastes, it may be susceptible to interferences such as those from conversion of thiocyanate to cyanide in the presence

of an oxidant, e.g., nitrate. If such interferences are present use a ligand displacement procedure with a mildly acidic medium with EDTA to dissociate metal-cyanide complexes.[11] Under such conditions thiocyanate is relatively stable and many oxidants, including nitrate, are weaker.

If any cyanide procedure is revised to meet specific requirements, obtain recovery data by the addition of known amounts of cyanide. Multiple interferences are present in some samples. When the sample is from a source being tested for the first time, fortify with known cyanide amounts (laboratory-fortified matrix procedure) to check for the presence of interferences.

4. Quality Control

Use protocols specified in Section 4020 to verify performance. These include daily use of reagent blanks, laboratory-fortified blanks, and known additions. Analyses for regulatory purposes may require additional quality control measures.

5. References

1. Carr, S.A., R.B. Baird & B.T. Lin. 1997. Wastewater derived interferences in cyanide analysis. *Water Res.* 31:7.
2. Luthy, R.G. & S.G. Bruce, Jr. 1979. Kinetics of reaction of cyanide and reduced sulfur species in aqueous solution. *Environ. Sci. Technol.* 13:1481.
3. Kruse, J.M. & M.G. Mellon. 1951. Colorimetric determination of cyanides. *Sewage Ind. Wastes* 23:1402.
4. Luthy, R.G., S.G. Bruce, R.W. Walters & D.V. Nakles. 1979. Cyanide and thiocyanate in coal gasification wastewater. *J. Water Pollut. Control Fed.* 51:2267.
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6. Hauser, T.R. & R.L. Cummins. 1964. Increasing sensitivity of 3-methyl-2-benzothiazone hydrazone test for analysis of aliphatic aldehydes in air. *Anal. Chem.* 36:679.
7. Methods of Air Sampling and Analysis, 1st ed. 1972. Inter Society Committee, Air Pollution Control Assoc., pp. 199–204.
8. Raaf, S.F., W.G. Characklis, M.A. Kessick & C.H. Ward. 1977. Fate of cyanide and related compounds in aerobic microbial systems. *Water Res.* 11:477.
9. Rapean, J.C., T. Hanson & R.A. Johnson. 1980. Biodegradation of cyanide-nitrate interference in the standard test for total cyanide. Proc. 35th Ind. Waste Conf., Purdue Univ., Lafayette, Ind., p. 430.
10. Casey, J.P. 1980. Nitrosation and cyanohydrin decomposition artifacts in distillation test for cyanide. Extended Abs. American Chemical Soc., Div. Environmental Chemistry, Aug. 24–29, 1980. Las Vegas, Nev.
11. Csikai, N.J. & A.J. Barnard, Jr. 1983. Determination of total cyanide in thiocyanate-containing waste water. *Anal. Chem.* 55: 1677.

4500-CN⁻ C. Total Cyanide after Distillation

1. General Discussion

Hydrogen cyanide (HCN) is liberated from an acidified sample by distillation and purging with air. The HCN gas is collected by passing it through an NaOH scrubbing solution. Cyanide concentration in the scrubbing solution is determined by titrimetric, colorimetric, or potentiometric procedures.

Note: The requirement to use magnesium chloride in the distillation first appeared in the 15th Edition of *Standard Methods*. Review of data[1] demonstrates that it is not essential. Use of magnesium chloride in the distillation is left to the discretion of the analyst.

2. Apparatus

The apparatus described in ¶s *a* through *d* below is shown in Figure 4500-CN⁻:1. Note: Other equivalent apparatus, including scaled-down apparatus, may be used if the scaled-down apparatus volumes preserve the same ratios of sample volume to digestion reagents volume as specified in the procedure (¶ 4). Evaluate any apparatus being considered for use by experiments to show acceptable recovery of total cyanide.

a. Boiling flask, 1 L, with inlet tube and provision for water-cooled condenser.

b. Gas absorber, with gas dispersion tube equipped with medium-porosity fritted outlet.

c. Heating element, adjustable.

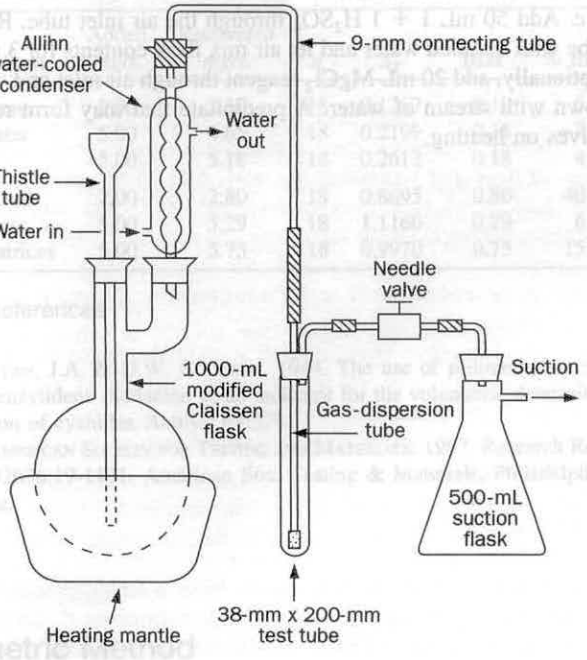

Figure 4500-CN⁻:1. Cyanide distillation apparatus.

d. Ground glass ST joints, TFE-sleeved or with an appropriate lubricant for the boiling flask and condenser. Neoprene stopper and plastic threaded joints also may be used.

3. Reagents

a. Sodium hydroxide solution: Dissolve 40 g NaOH in water and dilute to 1 L.

b. Magnesium chloride reagent (optional): Dissolve 510 g $MgCl_2 \cdot 6H_2O$ in water and dilute to 1 L.

c. Sulfuric acid, H_2SO_4, 1 + 1.

d. Lead carbonate, $PbCO_3$, powdered.

e. Sulfamic acid, NH_2SO_3H.

4. Procedure

a. Add 500 mL sample, containing not more than 10 mg CN^-/L (diluted if necessary with distilled water) to the boiling flask. If a higher CN^- content is anticipated, use the spot test (4500-CN^-.K) to approximate the required dilution. Add 10 mL NaOH solution to the gas scrubber and dilute, if necessary, with distilled water to obtain an adequate liquid depth in the absorber. Do not use more than 225 mL total volume of absorber solution. When S^{2-} generation from the distilling flask is anticipated add 50 or more mg powdered $PbCO_3$ to the absorber solution to precipitate S^{2-}. Connect the train, consisting of boiling flask air inlet, flask, condenser, gas washer, suction flask trap, and aspirator. Adjust suction so that at least 1 air bubble/s enters the boiling flask. This air rate will carry HCN gas from flask to absorber and usually will prevent a reverse flow of HCN through the air inlet. If this air rate does not prevent sample backup in the delivery tube, increase air-flow rate to 2 air bubbles/s. Observe air purge rate in the absorber where the liquid level should be raised not more than 6.5 to 10 mm. Maintain air flow throughout the reaction.

b. Add 2 g sulfamic acid through the air inlet tube and wash down with distilled water.

c. Add 50 mL 1 + 1 H_2SO_4 through the air inlet tube. Rinse tube with distilled water and let air mix flask contents for 3 min. Optionally, add 20 mL $MgCl_2$ reagent through air inlet and wash down with stream of water. A precipitate that may form redissolves on heating.

d. Heat with rapid boiling, but do not flood condenser inlet or permit vapors to rise more than halfway into condenser. Adequate refluxing is indicated by a reflux rate of 40 to 50 drops/min from the condenser lip. Reflux for at least 1 h. Discontinue heating but continue air flow for 15 min. Cool and quantitatively transfer absorption solution to a 250-mL volumetric flask. Rinse absorber and its connecting tubing sparingly with distilled water and add to flask. Dilute to volume with distilled water and mix thoroughly.

e. Determine cyanide concentration in the absorption solution by procedure of 4500-CN^-, D, E, or F.

f. Distillation gives quantitative recovery of even refractory cyanides such as iron complexes. To obtain complete recovery of cobalticyanide use ultraviolet radiation pretreatment.[2,3] If incomplete recovery is suspected, distill again by refilling the gas washer with a fresh charge of NaOH solution and refluxing 1 h more. The cyanide from the second reflux, if any, will indicate completeness of recovery.

g. As a quality control measure, periodically (daily) test apparatus, reagents, and other potential variables in the concentration range of interest by distilling a sample containing a known amount of cyanide. As an example, at least 100 ± 4% recovery from 1 mg CN^-/L standard should be obtained. Distillation of a complex cyanide such as potassium ferricyanide is preferred over the simple sodium or potassium cyanide. Additionally, use QC protocols from Section 4020 to verify performance.

5. References

1. Elly, C.T. 1968. Recovery of cyanides by modified Serfass distillation. *J. Water Pollut. Control Fed.* 40:848.
2. Casapieri, P., R. Scott & E.A. Simpson. 1970. The determination of cyanide ions in waters and effluents by an Auto Analyzer procedure. *Anal. Chim. Acta* 49:188.
3. Goulden, P.D., K.A. Badar & P. Brooksbank. 1972. Determination of nanogram quantities of simple and complex cyanides in water. *Anal. Chem.* 44:1845.

4500-CN⁻ D. Titrimetric Method

1. General Discussion

a. Principle: CN^- in the alkaline distillate from the preliminary treatment procedure is titrated with standard silver nitrate ($AgNO_3$) to form the soluble cyanide complex, $Ag(CN)_2^-$. As soon as all CN^- has been complexed and a small excess of Ag^+ has been added, the excess Ag^+ is detected by the silver-sensitive indicator, *p*-dimethylaminobenzalrhodanine, which immediately turns from a yellow to a salmon color.[1] The distillation has provided a 2:1 concentration. The indicator is sensitive to about 0.1 mg Ag/L. If titration shows that CN^- is below 1 mg/L, examine another portion colorimetrically or potentiometrically.

2. Apparatus

Koch microburet, 10-mL capacity.

3. Reagents

a. Indicator solution: Dissolve 20 mg *p*-dimethylaminobenzalrhodanine in 100 mL acetone.

b. Standard silver nitrate titrant: Dissolve 3.27 g $AgNO_3$ in 1 L distilled water. Standardize against standard NaCl solution, using the argentometric method with K_2CrO_4 indicator, as directed in Chloride, Section 4500-Cl^-.B.

Dilute 500 mL $AgNO_3$ solution according to the titer found so that 1.00 mL is equivalent to 1.00 mg CN^-.

c. Sodium hydroxide dilution solution: Dissolve 1.6 g NaOH in 1 L distilled water.

4. Procedure

a. From the absorption solution take a measured volume of sample so that the titration will require approximately 1 to 10 mL $AgNO_3$ titrant. Dilute to 100 mL using the NaOH dilution solution or to some other convenient volume to be used for all titrations. For samples with low cyanide concentration (≤5 mg/L) do not dilute. Add 0.5 mL indicator solution.

b. Titrate with standard $AgNO_3$ titrant to the first change in color from a canary yellow to a salmon hue. Titrate a blank containing the same amount of alkali and water, i.e., 100 mL NaOH dilution solution (or volume used for sample). As the analyst becomes accustomed to the end point, blank titrations decrease from the high values usually experienced in the first few trials to 1 drop or less, with a corresponding improvement in precision.

5. Calculation

$$\text{mg } CN^-/L = \frac{(A - B) \times 1000}{\text{mL original sample}} \times \frac{250}{\text{mL portion used}}$$

where:

A = mL standard $AgNO_3$ for sample and
B = mL standard $AgNO_3$ for blank.

6. Precision and Bias[2]

Based on the results of six operators in three laboratories, the overall and single-operator precision of this method within its designated range may be expressed as follows:

Reagent water: $S_T = 0.04x + 0.038$

$S_o = 0.01x + 0.018$

Selected water matrices: $S_T = 0.06x + 0.711$

$S_o = 0.04x + 0.027$

where:

S_T = overall precision, mg/L,
S_o = single-operator precision, mg/L, and
x = cyanide concentration, mg/L.

Recoveries of known amounts of cyanide from reagent water and selected water matrices are:

Medium	Added mg/L	Recovered mg/L	n	S_T	Bias	% Bias
Reagent water	2.00	2.10	18	0.1267	0.10	5
	5.00	4.65	18	0.2199	−0.35	−7
	5.00	5.18	18	0.2612	0.18	4
Selected water matrices	2.00	2.80	18	0.8695	0.80	40
	5.00	5.29	18	1.1160	0.29	6
	5.00	5.75	18	0.9970	0.75	15

7. References

1. Ryan, J.A. & G.W. Culshaw. 1944. The use of *p*-dimethylaminobenzylidene rhodanine as an indicator for the volumetric determination of cyanides. *Analyst* 69:370.
2. American Society for Testing and Materials. 1987. Research Rep. D2036:19-1131. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN⁻ E. Colorimetric Method

1. General Discussion

a. Principle: CN^- in the alkaline distillate from preliminary treatment is converted to CNCl by reaction with chloramine-T at pH <8 without hydrolyzing to CNO^-.[1] (Caution—*CNCl is a toxic gas; avoid inhalation.*) After the reaction is complete, CNCl forms a red-blue color on addition of a pyridine-barbituric acid reagent. Maximum color absorbance in aque-

ous solution is between 575 and 582 nm. To obtain colors of comparable intensity, have the same salt content in sample and standards.

b. Interference: All known interferences are eliminated or reduced to a minimum by distillation.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 578 nm, providing a light path of 10 mm or longer.

b. Filter photometer, providing a light path of at least 10 mm and equipped with a red filter having maximum transmittance at 570 to 580 nm.

3. Reagents

a. Chloramine-T solution: Dissolve 1.0 g white, water-soluble powder in 100 mL water. Prepare weekly and store in refrigerator.

b. Stock cyanide solution: Dissolve approximately 1.6 g NaOH and 2.51 g KCN in 1 L distilled water. (CAUTION—*KCN is highly toxic; avoid contact or inhalation.*) Standardize against standard silver nitrate ($AgNO_3$) titrant as described in Section 4500-CN^-D.4, using 25 mL KCN solution. Check titer weekly because the solution gradually loses strength; 1 mL = 1 mg CN^-.

c. Standard cyanide solution: Based on the concentration determined for the KCN stock solution (¶ 3*b*) calculate volume required (approximately 10 mL) to prepare 1 L of a 10 μg CN^-/mL solution. Dilute with the NaOH dilution solution. Dilute 10 mL of the 10 μg CN^-/mL solution to 100 mL with the NaOH dilution solution; 1.0 mL = 1.0 μg CN^-. Prepare fresh daily and keep in a glass-stoppered bottle. (CAUTION—*Toxic; take care to avoid ingestion.*)

d. Pyridine-barbituric acid reagent: Place 15 g barbituric acid in a 250-mL volumetric flask and add just enough water to wash sides of flask and wet barbituric acid. Add 75 mL pyridine and mix. Add 15 mL conc hydrochloric acid (HCl), mix, and cool to room temperature. Dilute to volume and mix until barbituric acid is dissolved. The solution is stable for approximately 6 months if stored in an amber bottle under refrigeration; discard if precipitate develops.

e. Acetate buffer: Dissolve 410 g sodium acetate trihydrate, $NaC_2H_3O_2 \cdot 3H_2O$, in 500 mL of water. Add glacial acetic acid to adjust to pH 4.5, approximately 500 mL.

f. Sodium hydroxide dilution solution: Dissolve 1.6 g NaOH in 1 L distilled water.

4. Procedure

a. Preparation of standard curve: Pipet a series of standards containing 1 to 10 μg CN^- into 50-mL volumetric flasks (0.02 to 0.2 μg CN^-/mL). Dilute to 40 mL with NaOH dilution solution. Use 40 mL of NaOH dilution solution as blank. Develop and measure absorbance in 10-mm cells as described in ¶ *b* for both standards and blank. For concentrations lower than 0.02 μg CN^-/mL use 100-mm cells.

Recheck calibration curve periodically and each time a new reagent is prepared.

b. Color development: Pipet a portion of absorption solution into a 50-mL volumetric flask and dilute to 40 mL with NaOH dilution solution. Add 1 mL acetate buffer and 2 mL chloramine-T solution, stopper, and mix by inversion twice. Let stand exactly 2 min.

Add 5 mL pyridine-barbituric acid reagent, dilute to volume with distilled water, mix thoroughly, and let stand exactly 8 min. Measure absorbance against distilled water at 578 nm.

Measure absorbance of blank (0.0 mg CN^-/L) using 40 mL NaOH dilution solution and procedures for color development.

5. Calculation

Use the linear regression feature available on most scientific calculators, or compute slope and intercept of standard curve as follows:

$$m = \frac{n \Sigma ca - \Sigma c \Sigma a}{n \Sigma a^2 - (\Sigma a)^2}$$

$$b = \frac{\Sigma a^2 \Sigma c - \Sigma a \Sigma ac}{n \Sigma a^2 - (\Sigma a)^2}$$

where:

a = absorbance of standard solution,
c = concentration of CN^- in standard, mg/L,
n = number of standard solutions,
m = slope of standard curve, and
b = intercept on c axis.

Include the blank concentration, 0.0 mg CN^-/L and blank absorbance in the calculations above.

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

where:

X = absorption solution, mL,
Y = original sample, mL, and
a_1 = absorbance of sample solution.

6. Precision and Bias[2]

Based on the results of nine operators in nine laboratories, the overall and single-operator precision of this method within its designated ranges may be expressed as follows:

Reagent water: $S_T = 0.06x + 0.003$

$$S_o = 0.11x + 0.010$$

Selected water matrices: $S_T = 0.04x + 0.018$

$$S_o = 0.04x + 0.008$$

where:

S_T = overall precision, mg/L,
S_o = single-operator precision, mg/L, and
x = cyanide concentration, mg/L.

Recoveries of known amounts of cyanide from reagent water and selected water matrices (coke plant and refinery wastes, sewage, and surface water) are:

Medium	Added *mg/L*	Recovered *mg/L*	*n*	S_T	Bias	% Bias
Reagent water	0.060	0.060	26	0.0101	0.000	0
	0.500	0.480	23	0.0258	−0.020	−4
	0.900	0.996	27	0.0669	0.096	11
Selected water matrices	0.060	0.060	25	0.0145	0.000	0
	0.500	0.489	26	0.0501	−0.011	−3
	0.900	0.959	24	0.0509	0.059	7

7. References

1. Amus, E. & H. Garschagen. 1953. Über die Verwendung der Barbitsäure für die photometrische Bestimmund von Cyanid und Rhodanid. *Z. Anal. Chem.* 138:414.
2. American Society for Testing and Materials. 1987. Research Rep. D2036:19-1131. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN⁻ F. Cyanide-Selective Electrode Method

1. General Discussion

CN^- in the alkaline distillate from the preliminary treatment procedures can be determined potentiometrically by using a CN^--selective electrode in combination with a double-junction reference electrode and a pH meter having an expanded millivolt scale, or a specific ion meter. This method can be used to determine CN^- concentration in place of either the colorimetric or titrimetric procedures in the concentration range of 0.05 to 10 mg CN^-/L.[1-3] If the CN^--selective electrode method is used, the previously described titration screening step can be omitted.

2. Apparatus

a. Expanded-scale pH meter or specific-ion meter.
*b. Cyanide-ion-selective electrode.**
c. Reference electrode, double-junction.
d. Magnetic mixer with TFE-coated stirring bar.
e. Koch microburet, 10-mL capacity.

3. Reagents

a. Stock standard cyanide solution: See Section 4500-CN⁻.E.3*b*.

b. Sodium hydroxide dilution solution: Dissolve 1.6 g NaOH in water and dilute to 1 L.

c. Standard cyanide solution: Dilute a calculated volume (approximately 25 mL) of stock KCN solution, based on the determined concentration, to 1000 mL with NaOH diluent. Mix thoroughly; 1 mL 25 μg CN^-.

d. Dilute standard cyanide solution: Dilute 100.0 mL standard CN^- solution to 1000 mL with NaOH diluent; 1.00 mL = 2.5 μg CN^-. Prepare daily and keep in a dark, glass-stoppered bottle.

e. Potassium nitrate solution: Dissolve 100 g KNO_3 in water and dilute to 1 L. Adjust to pH 12 with KOH. This is the outer filling solution for the double-junction reference electrode.

4. Procedure

a. Calibration: Using Koch microburet and standard CN^- solution, prepare four (or more) additional solutions containing 2.5, 0.25, 0.125, and 0.025 μg CN^-/mL in NaOH dilution solution. Transfer approximately 100 mL of each of these standard solutions into a 250-mL beaker prerinsed with a small portion of standard being tested. Immerse CN^- and double-junction reference electrodes. Mix well on a magnetic stirrer at 25°C, maintaining as closely as possible the same stirring rate for all solutions.

Always progress from the lowest to the highest concentration of standard because otherwise equilibrium is reached only slowly. The electrode membrane dissolves in solutions of high CN^- concentration; do not use with a concentration above 25 μg CN^-/mL. After making measurements remove electrode and soak in water.

After equilibrium is reached (at least 5 min and not more than 10 min), record potential (millivolt) readings. Plot CN^- concentration on logarithmic axis of semilogarithmic paper versus potential developed in solution on linear axis. A straight line with a slope of approximately 59 mV per decade indicates that the instrument and electrodes are operating properly. Record slope of line obtained (millivolts/decade of concentration). The slope may vary somewhat from the theoretical value of 59.2 mV per decade because of manufacturing variation and reference electrode (liquid-junction) potentials. The slope should be a straight line and is the basis for calculating sample concentration. Follow manufacturer's instructions for direct-reading ion meters.

b. Measurement of sample: Place 100 mL of absorption liquid obtained in Section 4500-CN⁻.C.4*d* (or an accurately measured portion diluted to 100 mL with NaOH dilution solution) into a 250-mL beaker. When measuring low CN^- concentrations, first rinse beaker and electrodes with a small volume of sample. Immerse CN^- and double-junction reference electrodes and mix on a magnetic stirrer at the same stirring rate used for calibration. After equilibrium is reached (at least 5 min and not more than 10 min), record values indicated on ion meter or found from graph prepared as above. Calculate concentration as directed below.

5. Calculations

$$\text{mg } CN^-/L = \mu g\ CN^-/mL \text{ from graph or meter} \times \frac{100}{x} \times \frac{250}{y}$$

where:

x = volume of absorption solution, mL, and
y = volume of original sample, mL.

* Orion Model 94-06A or equivalent.

6. Precision and Bias[4]

The precision of the CN^--ion-selective electrode method using the absorption solution from total cyanide distillation has been found in collaborative testing to be linear within its designated range.

Based on the results of six operators in five laboratories, the overall and single-operator precision of this method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.06x + 0.003$$

$$S_o = 0.03x + 0.008$$

$$\text{Selected water matrices: } S_T = 0.05x + 0.008$$

$$S_o = 0.03x + 0.012$$

where:

S_T = overall precision, mg/L,
S_o = single-operator precision, mg/L, and
x = cyanide concentration, mg/L.

Recoveries of known amounts of cyanide from reagent water and selected water matrices are:

Medium	Added *mg/L*	Recovered *mg/L*	*n*	S_T	Bias	% Bias
Reagent water	0.060	0.059	18	0.0086	−0.001	2
	0.500	0.459	18	0.0281	−0.041	−8
	0.900	0.911	18	0.0552	0.011	1
	5.00	5.07	18	0.297	0.07	1
Selected water matrices	0.060	0.058	14	0.0071	−0.002	−3
	0.500	0.468	21	0.0414	−0.032	−6
	0.900	0.922	19	0.0532	0.022	2
	5.00	5.13	20	0.2839	0.13	3

7. References

1. Orion Research, Inc. 1975. Cyanide Ion Electrode Instruction Manual. Cambridge, Mass.
2. Frant, M.S., J.W. Ross & J.H. Riseman. 1972. An electrode indicator technique for measuring low levels of cyanide. *Anal. Chem.* 44:2227.
3. Sekerka, J. & J.F. Lechner. 1976. Potentiometric determination of low levels of simple and total cyanides. *Water Res.* 10:479.
4. American Society for Testing and Materials. 1987. Research Rep. D2036:19-1131. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN^- G. Cyanides Amenable to Chlorination after Distillation

1. General Discussion

This method is applicable to the determination of cyanides amenable to chlorination.

After part of the sample is chlorinated to decompose the cyanides, both the chlorinated and the untreated sample are subjected to distillation as described in Section 4500-CN^-.C. The difference between the CN^- concentrations found in the two samples is expressed as cyanides amenable to chlorination.

Some unidentified organic chemicals may oxidize or form breakdown products during chlorination, giving higher results for cyanide after chlorination than before chlorination. This may lead to a negative value for cyanides amenable to chlorination after distillation for wastes from, for example, the steel industry, petroleum refining, and pulp and paper processing. Where such interferences are encountered use Method 4500-CN^-.I for determining dissociable cyanide.

Protect sample from exposure to ultraviolet radiation, and perform manipulations under incandescent light, to prevent photodecomposition of some metal-cyanide complexes by ultraviolet light.

2. Apparatus

a. Distillation apparatus: See Section 4500-CN^-.C.2.

b. Apparatus for determining cyanide by either the titrimetric method, Section 4500-CN^-.D.2, the colorimetric method, Section 4500-CN^-.E.2, or the electrode method, Section 4500-CN^-.F.2.

3. Reagents

a. All reagents listed in Section 4500-CN^-.C.3.

b. All reagents listed in Section 4500-CN^-.D.3, 4500-CN^-.E.3, or 4500-CN^-.F.3, depending on method of estimation.

c. Calcium hypochlorite solution: Dissolve 5 g $Ca(OCl)_2$ in 100 mL distilled water. Store in an amber-colored glass bottle in the dark. Prepare monthly.

d. Potassium iodide(KI)-starch test paper.

4. Procedure

a. Divide sample into two equal portions of 500 mL (or equal portions diluted to 500 mL) and chlorinate one as in ¶ *b* below. Analyze both portions for CN^-. The difference in determined concentrations is the cyanide amenable to chlorination.

b. Place one portion in a 1-L beaker covered with aluminum foil or black paper. Keep beaker covered with a wrapped watch glass during chlorination. Add $Ca(OCl)_2$ solution dropwise to sample while agitating and maintaining pH between 11 and 12 by adding NaOH solution. Test for chlorine by placing a drop of treated sample on a strip of KI-starch paper. A distinct blue color indicates sufficient chlorine (approximately 50 to 100 mg Cl_2/L). Maintain excess residual chlorine for 1 h while agitating. If necessary, add more $Ca(OCl)_2$ and/or NaOH.

c. After 1 h remove any residual chlorine by dropwise addition of $NaAsO_2$ solution (2 g/100 mL) or by addition of 8 drops H_2O_2 (3%) followed by 4 drops $Na_2S_2O_3$ solution (500 g/L). Test with KI-starch paper until there is no color change.

d. Distill both chlorinated and unchlorinated samples as in Section 4500-CN$^-$.C. Test according to Methods D, E, or F.

5. Calculation

$$\text{mg CN}^- \text{ amenable to chlorination/L} = G - H$$

where:

G = mg CN$^-$/L found in unchlorinated portion of sample and
H = mg CN$^-$/L found in chlorinated portion of sample.

For samples containing significant quantities of iron cyanides, it is possible that the second distillation will give a higher value for CN$^-$ than the test for total cyanide, leading to a negative result. When the difference is within the precision limits of the method, report, "no determinable quantities of cyanide amenable to chlorination." If the difference is greater than the precision limit, ascertain the cause such as presence of interferences, manipulation of the procedure, etc., or use Method I.

6. Precision and Bias[1]

The precision and bias information given in this section may not apply to waters of untested matrices.

a. Precision:

1) Colorimetric—Based on the results of eight operators in seven laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.18x + 0.005$$
$$S_o = 0.06x + 0.003$$

$$\text{Selected water matrices: } S_T = 0.20x + 0.009$$
$$S_o = 0.05x + 0.005$$

2) Titrimetric—Based on the results of six operators in three laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.01x + 0.439$$
$$S_o = 0.241 - 0.03x$$

$$\text{Selected water matrices: } S_T = 0.12x + 0.378$$
$$S_o = 0.209 - 0.01x$$

where:

S_T = overall precision, mg/L,
S_o = single-operator precision, mg/L, and
x = cyanide concentration, mg CN$^-$/L

b. Bias: Recoveries of known amount of cyanide amenable to chlorination from reagent water and selected water matrices are shown below:

Medium	Technique	Added *mg/L*	Recovered *mg/L*	*n*	S_T	Bias	% Bias
Reagent water	Colorimetric	0.008	0.009	21	0.0033	0.001	13
		0.019	0.023	20	0.0070	0.004	21
		0.080	0.103	20	0.0304	0.018	23
		0.191	0.228	21	0.0428	0.037	19
	Titrimetric	1.00	0.73	18	0.350	−0.27	−27
		1.00	0.81	18	0.551	−0.19	−19
		4.00	3.29	18	0.477	−0.71	−18
Selected water matrices	Colorimetric	0.008	0.013	17	0.0077	0.005	63
		0.019	0.025	18	0.0121	0.006	32
		0.080	0.100	18	0.0372	0.020	25
		0.191	0.229	18	0.0503	0.038	20
	Titrimetric	1.00	1.20	18	0.703	0.20	20
		1.00	1.10	18	0.328	0.10	10
		4.00	3.83	18	0.818	−0.17	−4

7. Reference

1. American Society for Testing and Materials. 1987. Research Rep. D2036:19-1131. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN^- H. Cyanides Amenable to Chlorination without Distillation (Short-Cut Method)

1. General Discussion

This method covers the determination of HCN and of CN complexes that are amenable to chlorination and also thiocyanates (SCN^-). The procedure does not measure cyanates (CNO^-) or iron cyanide complexes, but does determine cyanogen chloride (CNCl). It may be modified for use in presence of thiocyanates. The method requires neither lengthy distillation nor the chlorination of one sample before distillation. The recovery of CN^- from metal cyanide complexes will be comparable to that in Methods G and I.

The cyanides are converted to CNCl by chloramine-T after the sample has been heated. In the absence of nickel, copper, silver, and gold cyanide complexes or SCN^-, the CNCl may be developed at room temperature. The pyridine-barbituric acid reagent produces a red-blue color in the sample. The color can be estimated visually against standards or photometrically at 578 nm. The dissolved salt content in the standards used for the development of the calibration curve should be near the salt content of the sample, including the added NaOH and phosphate buffer.

The method's usefulness is limited by thiocyanate interference. Although the procedure allows the specific determination of CN^- amenable to chlorination (see 4500-CN^-.H.2 and 5) by masking the CN^- content and thereby establishing a correction for the thiocyanide content, the ratio of SCN^- to CN^- should not exceed 3 to be applicable. In working with unknown samples, screen the sample for SCN^- by the spot test (4500-CN^-.K).

2. Interferences

a. Remove interfering agents as described in Section 4500-CN^-.B with the exception of NO_2^- and NO_3^- (4500-CN^-.B.3*h*).

b. The SCN^- ion reacts with chloramine-T to give a positive error equivalent to its concentration. The procedure allows the separate determination of SCN^- and subtraction of this value from the results for the total. Use the spot test (4500-CN^-.K) for SCN^- when its presence is suspected. If the SCN^- content is more than three times the CN^- content, use Method G or I.

c. Reducing chemical compounds, such as SO_3^{2-}, may interfere by consuming chlorine in the chloramine-T addition. A significant excess of chlorine is provided, but the procedure prescribes a test (4500-CN^-.H.5*d*) to avoid this interference.

d. Color and turbidity may interfere with the colorimetric determination. Overcome this interference by extraction with chloroform (4500-CN^-.B.3*c*) but omit reduction of the pH. Otherwise, use Method G or I.

Compensation for color and turbidity may be made by determining absorbance of a second sample solution to which all reagents except chloramine-T have been added.

e. Color intensity and absorption are affected by wide variations in total dissolved solids content of the sample.

For samples containing high concentrations of dissolved solids (3000 to 10 000 mg/L), add 6 g NaCl/L NaOH solution (1.6 g/L) used to prepare standards. For samples containing dissolved solids concentrations greater than 10 000 mg/L, add sufficient NaCl to the NaOH solution to approximate the dissolved solids content.

3. Apparatus

a. Apparatus listed in 4500-CN^-.E.2.

b. Hot water bath.

4. Reagents

a. Reagents listed in Sections 4500-CN^-.B and E.3.

b. Sodium chloride, NaCl, crystals.

c. Sodium carbonate, Na_2CO_3, crystals.

d. Sulfuric acid solution, H_2SO_4, 1*N*.

e. EDTA solution, 0.05*M:* Dissolve 18.5 g disodium salt of ethylenediamine tetraacetic acid in water and dilute to 1 L.

f. Formaldehyde solution, 10%: Dilute 27 mL formaldehyde (37% pharmaceutical grade) to 100 mL.

g. Phosphate buffer: Dissolve 138 g sodium dihydrogen phosphate monohydrate, $NaH_2PO_4 \cdot H_2O$, in water and dilute to 1 L. Refrigerate.

5. Procedure

a. Calibrate as directed in Section 4500-CN^-.E.1*a* and 4*a*. For samples with more than 3000 mg total dissolved solids/L, prepare a calibration curve from standards and blank NaOH solutions containing 6 g NaCl/L. Samples containing total dissolved solids exceeding 10 000 mg/L require appropriate standards and a new calibration curve.

b. Adjust pH of 50 mL sample to between 11.4 and 11.8. If acid is needed, add a small amount (0.2 to 0.4 g) of sodium carbonate and stir to dissolve. Then add HCl solution (1+9) dropwise while stirring. For raising the pH, use NaOH solution (40 g/L).

c. Pipet 20.0 mL of adjusted sample into a 50-mL volumetric flask. If the cyanide concentration is greater than 0.3 mg/L, use a smaller portion and dilute to 20 mL with NaOH solution. Do not exceed the concentration limit of 0.3 mg/L.

d. To insure uniform color development, both in calibration and testing, maintain a uniform temperature. Immerse flasks in a water bath held at 27 ± 1°C for 10 min before adding reagents and keep samples in water bath until all reagents have been added.

Add 4 mL phosphate buffer and swirl to mix. Add one drop of EDTA solution, and mix.

e. Add 2 mL chloramine-T solution and swirl to mix. Place 1 drop of sample on potassium iodide-starch test paper that has been moistened previously with acetate buffer solution. Repeat the chloramine-T addition if required. After exactly 3 min, add 5 mL pyridine-barbituric acid reagent and swirl to mix.

f. Remove samples from water bath, dilute to volume, and mix. Allow 8 min from the addition of the pyridine-barbituric acid reagent for color development.

Determine absorbance at 578 nm in a 1.0-cm cell versus distilled water.

Calculate concentration of cyanide, mg/L in the original sample following instructions given in 4500-CN^-.E.

g. If the presence of thiocyanate is suspected, pipet a second 20-mL portion of pH-adjusted sample into a 50-mL volumetric flask. Add 3 drops 10% formaldehyde solution. Mix and let stand

10 min. Place in a water bath at 27 ± 1°C for an additional 10 min before the addition of the reagent chemicals and hold in the bath until all reagents have been added.

Continue with *b* above.

Calculate the concentration of cyanide, as milligrams per liter, in the original sample following instructions given in 4500-CN⁻. E.

h. In the presence of thiocyanate, cyanide amenable to chlorination is equal to the difference between the concentrations of cyanide obtained in *f* and *g*.

6. Calculation

See 4500-CN⁻.E.5.

Deduct SCN^- value from the results found when the CN^- has not been masked by formaldehyde addition (total) for cyanide content.

7. Precision and Bias[1]

This precision and bias information may not apply to waters of untested matrices.

a. Precision: Based on the results of 14 operators in nine laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

Reagent water: $S_T = 0.10x + 0.006$
$S_o = 0.07x + 0.005$

Selected water matrices: $S_T = 0.11x + 0.007$
$S_o = 0.02x + 0.005$

where:

S_T = overall precision, mg/L,
S_o = single-operator precision, mg/L, and
x = cyanide concentration, mg/L.

b. Bias: Recoveries of known amounts of cyanide from reagent water and selected water matrices including creek waters, diluted sewage (1 to 20), and industrial wastewater are shown below.

	Added mg/L		Recovered				%
Medium	CN^-	SCN^-	mg/L	*n*	S_T	Bias	Bias
Reagent water	0.005		0.007	42	0.0049	0.002	40
	0.027		0.036	41	0.0109	0.009	25
	0.090		0.100	42	0.0167	0.010	11
	0.090	0.080	0.080	39	0.0121	−0.010	11
	0.270		0.276	42	0.0320	0.006	2
Selected water matrices	0.005		0.003	40	0.0042	−0.002	40
	0.027		0.026	42	0.0093	−0.001	4
	0.090		0.087	42	0.0202	−0.003	3
	0.090	0.080	0.068	37	0.0146	−0.022	24
	0.270		0.245	41	0.0319	−0.025	9

8. Reference

1. American Society for Testing and Materials. 1987. Research Rep. D2036:19-1074. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN⁻ I. Weak Acid Dissociable Cyanide

1. General Discussion

Hydrogen cyanide (HCN) is liberated from a slightly acidified (pH 4.5 to 6.0) sample under the prescribed distillation conditions. The method does not recover CN^- from tight complexes that would not be amenable to oxidation by chlorine. The acetate buffer used contains zinc salts to precipitate iron cyanide as a further assurance of the selectivity of the method. In other respects the method is similar to 4500-CN⁻.C.

2. Interferences

See 4500-CN⁻.B.3.

Protect sample and apparatus from ultraviolet light to prevent photodecomposition of some metal-cyanide complexes and an increase in concentration of weak acid dissociable cyanide.

If procedure is used to determine low concentrations of cyanide in samples of ferri- and ferrocyanide, add more, e.g., fivefold excess, zinc acetate solution before adding acid and distilling.

3. Apparatus

See Section 4500-CN⁻.C.2 and Figure 4500-CN⁻:1, and also Section 4500-CN⁻.D.2, 4500-CN⁻.E.2, or 4500-CN⁻.F.2, depending on method of estimation.

4. Reagents

a. Reagents listed in Section 4500-CN⁻.C.3.

b. Reagents listed in Section 4500-CN⁻.D.3, 4500-CN⁻.E.3, or 4500-CN⁻.F.3, depending on method of estimation.

c. Acetic acid, 1 + 9: Mix 1 volume of glacial acetic acid with 9 volumes of water.

d. Acetate buffer: Dissolve 410 g sodium acetate trihydrate ($NaC_2H_3O_2 \cdot 3H_2O$) in 500 mL water. Add glacial acetic acid to yield a solution pH of 4.5 (approximately 500 mL).

e. Zinc acetate solution, 100 g/L: Dissolve 120 g $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ in 500 mL water. Dilute to 1 L.

f. Methyl red indicator.

5. Procedure

Follow procedure described in 4500-CN⁻.C.4, but with the following modifications:

a. Do not add sulfamic acid, because NO_2^- and NO_3^- do not interfere.

b. Instead of H_2SO_4 and $MgCl_2$ reagents, add 20 mL each of the acetate buffer and zinc acetate solutions through air inlet tube. Also add 2 to 3 drops methyl red indicator. Rinse air inlet tube with water and let air mix contents. If the solution is not pink, add acetic acid (1 + 9) dropwise through air inlet tube until a pink color persists.

c. Follow instructions beginning with 4500-CN^-.C.4*d*.

d. For determining CN^- in the absorption solution, use the preferred finish method (4500-CN^-.D, E, or F).

6. Precision and Bias[1]

The precision and bias information given in this section may not apply to waters of untested matrices.

a. Precision:

1) Colorimetric—Based on the results of nine operators in nine laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.09x + 0.010$$
$$S_o = 0.08x + 0.005$$
$$\text{Selected water matrices: } S_T = 0.08x + 0.012$$
$$S_o = 0.05x + 0.008$$

2) Electrode—Based on the results of six operators in five laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.09x + 0.004$$
$$S_o = 0.02x - 0.009$$
$$\text{Selected water matrices: } S_T = 0.08x + 0.005$$
$$S_o = 0.02x + 0.004$$

3) Titrimetric—Based on the results of six operators in three laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.532 - 0.10x$$
$$S_o = 0.151 - 0.01x$$
$$\text{Selected water matrices: } S_T = 0.604 - 0.06x$$
$$S_o = 0.092 + 0.02x$$

where:

S_T = overall precision,
S_o = single-operator precision, and
x = cyanide concentration, mg/L.

b. Bias: Recoveries of known amounts of cyanide from reagent water and selected water matrices are shown below.

Medium	Technique	Added mg/L	Recovered mg/L	n	S_T	Bias	% Bias
Reagent water	Colorimetric	0.030	0.030	25	0.0089	0.000	0
		0.100	0.117	27	0.0251	0.017	17
		0.400	0.361	27	0.0400	−0.039	−10
	Electrode	0.030	0.030	21	0.0059	0.000	0
		0.100	0.095	21	0.0163	−0.005	−5
		0.400	0.365	21	0.0316	−0.035	−9
		1.000	0.940	21	0.0903	−0.060	−6
	Titrimetric	1.00	1.35	18	0.4348	0.35	35
		1.00	1.38	18	0.3688	0.38	38
		4.00	3.67	18	0.1830	−0.33	−8
Selected water matrices	Colorimetric	0.030	0.029	15	0.0062	0.001	3
		0.100	0.118	24	0.0312	0.018	18
		0.400	0.381	23	0.0389	−0.019	−5
	Electrode	0.030	0.029	20	0.0048	−0.001	−3
		0.100	0.104	21	0.0125	0.004	4
		0.400	0.357	21	0.0372	−0.043	−11
		1.000	0.935	21	0.0739	−0.065	−7
	Titrimetric	1.00	1.55	18	0.5466	0.55	55
		1.00	1.53	18	0.4625	0.53	53
		4.00	3.90	18	0.3513	−0.10	−3

7. Reference

1. American Society for Testing and Materials. 1987. Research Rep. D2036:19-1131. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN^- J. Cyanogen Chloride

1. General Discussion

Cyanogen chloride (CNCl) is the first reaction product when cyanide compounds are chlorinated. It is a volatile gas, only slightly soluble in water, but highly toxic even in low concentrations. (Caution: *Avoid inhalation or contact.*) A mixed pyridine-barbituric acid reagent produces a red-blue color with CNCl.

Because CNCl hydrolyzes to cyanate (CNO^-) at a pH of 12 or more, collect a separate sample for CNCl analysis (See Section 4500-CN^-.B.2) in a closed container without sodium hydroxide (NaOH). A quick test with a spot plate or comparator as soon as the sample is collected may be the only procedure for avoiding hydrolysis of CNCl due to time lapse between sampling and analysis.

If starch-iodide (KI) test paper indicates presence of chlorine or other oxidizing agents, add sodium thiosulfate ($Na_2S_2O_3$) immediately as directed in Section 4500-CN^-.B.2.

2. Apparatus

See Section 4500-CN^-.E.2.

3. Reagents

a. Reagents listed in Sections 4500-CN^-.E.3 and 4500-CN^-.H.4.

b. Phosphate buffer: Dissolve 138 g sodium dihydrogen phosphate monohydrate, $NaH_2PO_4 \cdot H_2O$, in water and dilute to 1 L. Refrigerate.

4. Procedure

a. Preparation of standard curve: Pipet a series of standards containing 1 to 10 μg CN^- into 50-mL volumetric flasks (0.02 to 0.2 μg CN^-/mL). Dilute to 20 mL with NaOH dilution solution. Use 20 mL of NaOH dilution solution for the blank. Add 2 mL chloramine-T solution and 4 mL phosphate buffer; stopper and mix by inversion two or three times. Add 5 mL pyridine-barbituric acid reagent, dilute to volume with water, mix thoroughly, and let stand exactly 8 min for color development. Measure absorbance at 578 nm in a 10-mm cell using distilled water as a reference. Calculate slope and intercept of the curve.

b. If sample pH is above 8, reduce it to 8.0 to 8.5 by careful addition of phosphate buffer. Measure 20 mL sample portion into 50-mL volumetric flask. If more than 0.20 mg CNCl-CN^-/L is present use a smaller portion diluted to 20 mL with water. Add 1 mL phosphate buffer, stopper and mix by inversion *one* time. Let stand 2 min. Add 5 mL pyridine-barbituric acid reagent, stopper and mix by inversion *one* time. Let color develop 3 min, dilute to volume with water, mix thoroughly, and let stand an additional 5 min. Measure absorbance at 578 nm in 10-mm cell using distilled water as a reference.

5. Calculation

Compute slope (m) and intercept (b) of standard curve as directed in 4500-CN^-.E.5.

$$\text{Cyanogen chloride as } CN^-, \text{ mg/L} = (ma_1 + b) \times \frac{50}{\text{mL sample}}$$

where:

a_1 = absorbance of sample solution.

6. Precision[1]

Cyanogen chloride is unstable and round-robin testing is not possible. Single-operator precision is as follows:

Six operators made 70 duplicate analyses on samples of different concentrations within the applicable range of the method. The overall single-operator precision within its designated range may be expressed as follows:

$$\log S_o = (0.5308 \log c) - 1.9842$$
$$\log R = (0.5292 \log c) - 1.8436$$

where:

c = mg CNCl-CN^-/L,
S_o = single-operator precision in the range of the method (precision is dependent on concentration), and
R = range between duplicate determinations.

The collaborative test data were obtained on reagent-grade water. For other matrices, these data may not apply.

7. Reference

1. American Society for Testing and Materials. 1989. Research Rep. D4165:19-1100. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN^- K. Spot Test for Sample Screening

1. General Discussion

The spot test procedure permits quick screening to establish whether more than 50 μg/L of cyanide amenable to chlorination is present. The test also establishes the presence or absence of cyanogen chloride (CNCl). With practice and dilution, the test reveals the approximate concentration range of these compounds by the color development compared with that of similarly treated standards.

When chloramine-T is added to cyanides amenable to chlorination, CNCl is formed. CNCl forms a red-blue color with the mixed reagent pyridine-barbituric acid. When testing for CNCl omit the chloramine-T addition. (Caution: *CNCl is a toxic gas; avoid inhalation.*)

The presence of formaldehyde in excess of 0.5 mg/L interferes with the test. A spot test for the presence of aldehydes and a method for removal of this interference are given in Section 4500-CN^-.B.3.

Thiocyanate (SCN^-) reacts with chloramine-T, thereby creating a positive interference. The CN^- can be masked with formaldehyde and the sample retested. This makes the spot test specific for SCN^-. In this manner it is possible to determine whether the spot discoloration is due to the presence of CN^-, SCN^-, or both.

2. Apparatus

a. Porcelain spot plate with 6 to 12 cavities.
b. Dropping pipets.
c. Glass stirring rods.

3. Reagents

a. Chloramine-T solution: See Section 4500-CN^-.E.3*a*.
b. Stock cyanide solution: See Section 4500-CN^-.E.3*b*.
c. Pyridine-barbituric acid reagent: See Section 4500-CN^-.E.3*d*.
d. Hydrochloric acid, HCl, 1 + 9.
e. Phenolphthalein indicator aqueous solution.
f. Sodium carbonate, Na_2CO_3, anhydrous.
g. Formaldehyde, 37%, pharmaceutical grade.

4. Procedure

If the solution to be tested has a pH greater than 10, neutralize a 20- to 25-mL portion. Add about 250 mg Na_2CO_3 and swirl to

dissolve. Add 1 drop phenolphthalein indicator. Add 1 + 9 HCl dropwise with constant swirling until the solution becomes colorless. Place 3 drops sample and 3 drops distilled water (for blanks) in separate cavities of the spot plate. To each cavity, add 1 drop chloramine-T solution and mix with a clean stirring rod. Add 1 drop pyridine-barbituric acid solution to each cavity and again mix. After 1 min, the sample spot will turn pink to red if 50 μg/L or more of CN^- are present. The blank spot will be faint yellow because of the color of the reagents. Until familiarity with the spot test is gained, use, in place of the water blank, a standard solution containing 50 μg CN^-/L for color comparison. This standard can be made by diluting the stock cyanide solution (¶3*b*).

If SCN^- is suspected, test a second sample pretreated as follows: Heat a 20- to 25-mL sample in a water bath at 50°C; add 0.1 mL formaldehyde and hold for 10 min. This treatment will mask up to 5 mg CN^-/L, if present. Repeat spot testing procedure. Color development indicates presence of SCN^-. Comparing color intensity in the two spot tests is useful in judging relative concentration of CN^- and SCN^-. If deep coloration is produced, serial dilution of sample and additional testing may allow closer approximation of the concentrations.

4500-CN^- L. Cyanates

1. General Discussion

Cyanate (CNO^-) may be of interest in analysis of industrial waste samples because the alkaline chlorination process used for the oxidation of cyanide yields cyanate in the second reaction.

Cyanate is unstable at neutral or low pH; therefore, stabilize the sample as soon as collected by adding sodium hydroxide (NaOH) to pH >12. Remove residual chlorine by adding sodium thiosulfate ($Na_2S_2O_3$) (see Section 4500-CN^-.B.2).

a. Principle: Cyanate hydrolyzes to ammonia when heated at low pH.

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

The ammonia concentration must be determined on one sample portion before acidification. The ammonia content before and after hydrolysis of cyanate may be measured by phenate (4500-NH_3.F), or ammonia-selective electrode (4500-NH_3.D) method.[1] The test is applicable to cyanate compounds in natural waters and industrial waste.

b. Interferences:

1) Organic nitrogenous compounds may hydrolyze to ammonia (NH_3) upon acidification. To minimize this interference, control acidification and heating closely.

2) Reduce oxidants that oxidize cyanate to carbon dioxide and nitrogen with $Na_2S_2O_3$ (see Section 4500-CN^-.G).

3) Industrial waste containing organic material may contain unknown interferences.

c. Detection limit: 1 to 2 mg CNO^-/L.

2. Apparatus

a. Expanded-scale pH meter or selective-ion meter.

*b. Ammonia-selective electrode.**

c. Magnetic mixer, with TFE-coated stirring bar.

d. Heat barrier: Use a 3-mm-thick insulator under beaker to insulate against heat produced by stirrer motor.

* Orion Model 95-12, EIL Model 8002-2, Beckman Model 39565, or equivalent.

3. Reagents

a. Stock ammonium chloride solution: Dissolve 3.819 g anhydrous NH_4Cl, dried at 100°C, in water, and dilute to 1 L; 1.00 mL = 1.00 mg N = 1.22 mg NH_3.

b. Standard ammonium chloride solution: From the stock NH_4Cl solution prepare standard solutions containing 1.0, 10.0, and 100.0 mg NH_3-N/L by diluting with ammonia-free water.

c. Sodium hydroxide, 10*N*: Dissolve 400 g NaOH in water and dilute to 1 L.

d. Sulfuric acid solution, H_2SO_4, 1 + 1.

e. Ammonium chloride solution: Dissolve 5.4 g NH_4Cl in distilled water and dilute to 1 L. (Use only for soaking electrodes.)

4. Procedure

a. Calibration: Daily, calibrate the ammonia electrode as in 4500-NH_3.F.4*b* and *c* using standard NH_4Cl solutions.

b. Treatment of sample: Dilute sample, if necessary, so that the CNO^- concentration is 1 to 200 mg/L or NH_3-N is 0.5 to 100 mg/L. Take or prepare at least 200 mL. From this 200 mL, take a 100-mL portion and, following the calibration procedure, establish the potential (millivolts) developed from the sample. Check electrode reading with prepared standards and adjust instrument calibration setting daily. Record NH_3-N content of untreated sample (*B*).

Acidify 100 mL of prepared sample by adding 0.5 mL 1 + 1 H_2SO_4 to a pH of 2.0 to 2.5. Heat sample to 90 to 95°C and maintain temperature for 30 min. Cool to room temperature and restore to original volume by adding ammonia-free water. Pour into a 150-mL beaker, immerse electrode, start magnetic stirrer, then add 1 mL 10*N* NaOH solution. With pH paper check that pH is greater than 11. If necessary, add more NaOH until pH 11 is reached.

After equilibrium has been reached (30 s) record the potential reading. Estimate NH_3-N content from calibration curve.

5. Calculations

$$\text{mg } NH_3\text{-N derived from } CNO^-/L = A - B$$

where:

A = mg NH_3-N/L found in the acidified and heated sample portion and

B = mg NH_3-N/L found in untreated portion.

$$\text{mg } CNO^-/L = 3.0 \times (A - B)$$

4500-CN⁻ M. Thiocyanate

1. General Discussion

When wastewater containing thiocyanate (SCN^-) is chlorinated, highly toxic cyanogen chloride (CNCl) is formed. At an acidic pH, ferric ion (Fe^{3+}) and SCN^- form an intense red color suitable for colorimetric determination.

a. Interference:

1) Hexavalent chromium (Cr^{6+}) interferes and is removed by adding ferrous sulfate ($FeSO_4$) after adjusting to pH 1 to 2 with nitric acid (HNO_3). Raising the pH to 9 with 1*N* sodium hydroxide (NaOH) precipitates Fe^{3+} and Cr^{3+}, which are then filtered out.

2) Reducing agents that reduce Fe^{3+} to Fe^{2+}, thus preventing formation of ferric thiocyanate complex, are destroyed by adding a few drops of hydrogen peroxide (H_2O_2). Avoid excess H_2O_2 to prevent reaction with SCN^-.

3) Industrial wastes may be highly colored or contain various interfering organic compounds. To eliminate these interferences,[1] use the pretreatment procedure given in ¶4*c* below. It is the analyst's responsibility to validate the method's applicability without pretreatment (¶ 4*b*). If in doubt, pretreat sample before proceeding with analysis (¶ 4*c*).

4) If sample contains cyanide amenable to chlorination and would be preserved for the cyanide determination at a high pH, sulfide could interfere by converting cyanide to SCN^-. To preserve SCN^- and CN^-, precipitate the sulfide by adding lead salts according to 4500-CN^-.B.2 before adding alkali; filter to remove precipitate.

5) Thiocyanate is biodegradable. Preserve samples at pH <2 by adding mineral acid and refrigerate.

6) If interferences from industrial wastes are not removed as directed in ¶ 4*c* below, consider adopting a solvent extraction technique with colorimetric or atomic absorption analysis of the extract.[2,3]

b. Application: 0.1 to 2.0 mg SCN^-/L in natural or wastewaters. For higher concentrations, use a portion of diluted sample.

2. Apparatus

a. Spectrophotometer or filter photometer, for use at 460 nm, providing a light path of 5 cm.

b. Glass adsorption column: Use a 50-mL buret with a glass-wool plug, and pack with macroreticular resin (¶3*f*) approximately 40 cm high. For convenience, apply a powder funnel of the same diameter as the buret to the top with a short piece of plastic tubing.

3. Reagents

a. Ferric nitrate solution: Dissolve 404 g $Fe(NO_3)_3 \cdot 9H_2O$ in about 800 mL distilled water. Add 80 mL conc HNO_3 and dilute to 1 L.

b. Nitric acid solution, 0.1*N*: Mix 6.4 mL conc HNO_3 in about 800 mL distilled water and dilute to 1 L.

c. Stock thiocyanate solution: Dissolve 1.673 g potassium thiocyanate (KSCN) in distilled water and dilute to 1000 mL; 1.00 mL = 1.00 mg SCN^-.

d. Standard thiocyanate solution: Dilute 10 mL stock solution to 1 L with distilled water; 1.00 mL = 0.01 mg SCN^-.

e. Sodium hydroxide solution, 4 g/L: Dissolve 4 g NaOH in about 800 mL distilled water and dilute to 1 L.

f. Macroreticular resin, 18 to 50 mesh:* The available resin may not be purified. Some samples have shown contamination with waxes and oil, giving poor permeability and adsorption. Purify as follows:

Place sufficient resin to fill the column or columns in a beaker and add 5 times the resin volume of acetone. Stir gently for 1 h. Pour off fines and acetone from settled resin and add 5 times the resin volume of hexane. Stir for 1 h. Pour off fines and hexane and add 5 times the resin volume of methanol. Stir for 15 min. Pour off methanol and add 3 times the resin volume of 0.1*N* NaOH. Stir for 15 min. Pour off NaOH solution and add 3 times the resin volume of 0.1*N* HNO_3. Stir for 15 min. Pour off HNO_3 solution and add 3 times the resin volume of distilled water. Stir for 15 min. Drain excess water and use purified resin to fill the column. Store excess purified resin after covering it with distilled water. Keep in a closed jar.

g. Methyl alcohol.

4. Procedure

a. Preparation of calibration curve: Prepare a series of standards containing from 0.02 mg to 0.40 mg SCN^- by pipetting

*Amberlite® XAD-7, or equivalent.

6. Precision

No data on precision of this method are available. See 4500-NH_3.A.4 for precision of ammonia-selective electrode method.

7. Reference

1. Thomas, R.F. & R.L. Booth. 1973. Selective electrode determination of ammonia in water and wastes. *Environ. Sci. Technol.* 7:523.

measured volumes of standard KSCN solution into 200-mL volumetric flasks and diluting with water. Mix well. Develop color according to ¶ *b* below. Plot absorbance against SCN^- concentration expressed as mg/50 mL sample. The absorbance plot should be linear.

b. Color development: Use a filtered sample or portion from a diluted solution so that the concentration of SCN^- is between 0.1 and 2 mg/L. Adjust pH to 2 with conc HNO_3 added dropwise. Pipet 50-mL portion to a beaker, add 2.5 mL ferric nitrate, and mix.

Fill a 5-cm absorption cell and measure absorbance against a reagent blank at 460 nm or close to the maximum absorbance found with the instrument being used. Measure absorbance of the developed color against a reagent blank within 5 min from adding the reagent. (The color develops within 30 s and fades on standing in light.)

c. Sample pretreatment:

1) Color and various organic compounds interfere with absorbance measurement. At pH 2, macroreticular resin removes these interfering materials by adsorption without affecting thiocyanate.

2) To prepare the adsorption column, fill it with resin, rinse with 100 mL methanol, and follow by rinses with 100 mL 0.1*N* NaOH, 100 mL 0.1*N* HNO_3, and finally with 100 mL distilled water. If previously purified resin is used, omit these preparatory steps.

3) When washing, regenerating, or passing a sample through the column, as solution level approaches resin bed, add and drain five separate 5-mL volumes of solution or water (depending on which is used in next step) to approximate bed height. After last 5-mL volume, fill column with remaining liquid. This procedure prevents undue mixing of solutions and helps void the column of the previous solution.

4) Acidify 150 mL sample (or a dilution) to pH 2 by adding conc HNO_3 dropwise while stirring. Pass it through the column at a flow rate not to exceed 20 mL/min. If the resin becomes packed and the flow rate falls to 4 to 5 mL/min, use gentle pressure through a manually operated hand pump or squeeze bulb on the column. In this case, use a separator funnel for the liquid reservoir instead of the powder funnel. Alternatively use a vacuum bottle as a receiver and apply gentle vacuum. Do not let liquid level drop below the adsorbent in the column.

5) When passing a sample through the column, measure 90 mL of sample in a graduated cylinder, and from this use the five 5-mL additions as directed in ¶ 3), then pour the remainder of the 90 mL into the column. Add rest of sample and collect 60 mL eluate to be tested after the first 60 mL has passed through the column.

6) Prepare a new calibration curve using standards prepared according to ¶ 4*a*, but acidify standards according to ¶ 4*b*, and pass them through the adsorption column. Develop color and measure absorbance according to ¶ 4*b* against a reagent blank prepared by passing acidified, distilled water through the adsorption column.

7) Pipet 50 mL from the collected eluate to a beaker, add 2.5 mL ferric nitrate solution, and mix. Measure absorbance according to ¶ 4*b* against a reagent blank [see ¶ 6) above].

8) From the measured absorbance value, determine thiocyanate content of the sample or dilution using the absorbance plot.

9) Each day the column is in use, test a mid-range standard to check absorption curve.

10) Regenerate column between samples by rinsing with 100 mL 0.1*N* NaOH; 50 mL 0.1*N* HNO_3; and 100 mL water. Insure that the water has rinsed empty glass section of the buret. Occasionally rinse with 100 mL methanol for complete regeneration. Adsorbed weak organic acids and thiocyanate residuals from earlier tests are eluted by the NaOH rinse. Leave the column covered with the last rinse water for storage.

5. Calculation

Compute slope (m) and intercept (b) of standard curve as directed in 4500-CN^-.E.5.

Calculate thiocyanate concentration as follows:

$$\text{mg } SCN^-/L = (ma_1 + b) \times \text{dilution factor}$$

where:

a_1 = absorbance of sample solution.

6. Precision and Bias[4]

a. Precision: Based on the results of twelve operators in nine laboratories, at four levels of concentration, the precision of the test method within its designated range is linear with concentration and may be expressed as follows:

$$\text{Reagent water: } S_T = 0.093x + 0.0426$$
$$S_o = 0.045x + 0.010$$

$$\text{Water matrix: } S_T = 0.055x + 0.0679$$
$$S_o = 0.024x + 0.182$$

where:

S_T = overall precision, mg/L,
S_o = pooled single-operator precision, mg/L, and
x = thiocyanate concentration, mg/L.

b. Bias: Recoveries of known amounts of thiocyanate from reagent water and selected water matrices including natural waters, laboratory effluent, steel mill effluent, and dechlorinated and treated sanitary effluents were as follows:

Medium	Added *mg/L*	Recovered *mg/L*	*n*	S_T	Bias	% Bias
Reagent water	1.42	1.411	30	0.181	−0.009	−0.6
	0.71	0.683	27	0.091	−0.027	−4
	0.35	0.329	30	0.084	−0.021	−6
	0.07	0.068	30	0.052	−0.002	−3
Selected water matrices	1.42	1.408	26	0.151	−0.012	−0.8
	0.71	0.668	29	0.096	−0.042	−6
	0.35	0.320	29	0.085	−0.030	−9
	0.07	0.050	29	0.079	−0.020	−29

For other matrices these data may not apply.

7. References

1. Spencer, R.R., J. Leenheer & V.C. Marti. 1980. Automated colorimetric determination of thiocyanate, thiosulfate and tetrathionate in

water. 94th Annu. Meeting. Assoc. Official Agricultural Chemists, Washington, D.C. 1981.
2. Danchick, R.S. & D.F. Boltz. 1968. Indirect spectrophotometric and atomic absorption spectrometric methods in determination of thiocyanate. *Anal. Chem.* 43:2215.
3. Luthy, R.G. 1978. Manual of Methods: Preservation and Analysis of Coal Gasification Wastewaters. FE-2496-16, U.S. Dep. Energy, National Technical Information Serv., Springfield, Va.
4. American Society for Testing and Materials. 1989. Research Rep. D4193:19-1099. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN⁻ N. Total Cyanide after Distillation, by Flow Injection Analysis

1. General Discussion

a. Principle: Total cyanides are digested and steam-distilled from the sample as in 4500-CN⁻.C, cyanides amenable to chlorination are digested and steam-distilled from the sample as in 4500-CN⁻.G, or weak acid dissociable cyanides are digested and steam-distilled from the sample as in 4500-CN⁻.I, by using the apparatus described in 4500-CN⁻.C or an equivalent distillation apparatus. In any case, the distillate should consist of cyanide in 0.25*M* NaOH. The cyanide in this distillate is converted to cyanogen chloride, CNCl, by reaction with chloramine-T at pH less than 8. The CNCl then forms a red-blue dye by reacting with pyridine-barbituric acid reagent. The absorbance of this red dye is measured at 570 nm and is proportional to the total or weak acid dissociable cyanide in the sample.

Also see Sections 4500-CN⁻.A and E, and Section 4130, Flow Injection Analysis (FIA).

b . Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Nonvolatile interferences are eliminated or minimized by the distillation procedure. Some of the known interferences are aldehydes, nitrate-nitrite, and oxidizing agents such as chlorine, thiocyanate, thiosulfate, and sulfide. Multiple interferences may require the analysis of a series of laboratory fortified sample matrices (LFM) to verify the suitability of the chosen treatment. See Section 4500-CN⁻.B for a discussion of preliminary treatment of samples to be distilled.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold (Figure 4500-CN⁻:2) with tubing heater and flow cell. Relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*

d. Absorbance detector, 570 nm, 10-nm bandpass.

e. Injection valve control and data acquisition system.

*Teflon or equivalent.

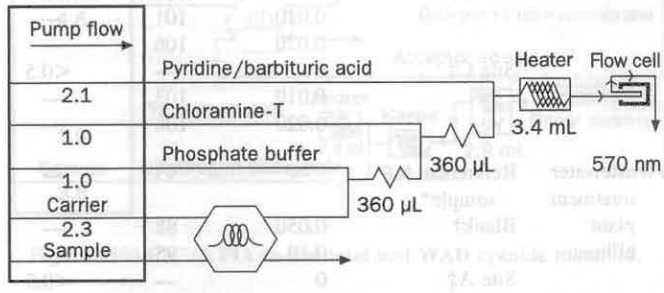

Figure 4500-CN⁻:2. FIA cyanide manifold.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and all reagents with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. Carrier solution, 0.25*M*: In a 1-L plastic container dissolve 10.0 g NaOH in 1.00 L water.

b. Phosphate buffer, 0.71*M*: To a 1-L tared container add 97.0 g potassium phosphate, monobasic, anhydrous, KH_2PO_4, and 975 g water. Stir or shake until dissolved. Prepare fresh monthly.

c. Chloramine-T: Dissolve 2.0 g chloramine-T hydrate (mol wt 227.65) in 500 mL water. Prepare fresh daily.

d. Pyridine/barbituric acid: In fume hood, place 15.0 g barbituric acid in a tared 1-L container and add 100 g water, rinsing down sides of beaker to wet the barbituric acid. Add 73 g pyridine (C_5H_5N) with stirring and mix until barbituric acid dissolves. Add 18 g conc HCl, then an additional 825 g water, and mix. Prepare fresh weekly.

e. Stock cyanide standard, 100 mg CN⁻/L: In a 1-L container, dissolve 2.0 g potassium hydroxide (KOH) in approximately 800 mL water. Add 0.250 g potassium cyanide (KCN). Caution: *KCN is highly toxic. Avoid inhalation of dust or contact with the solid or solutions.* Make to final weight of 1000 g with water and mix. Prepare fresh weekly or standardize weekly using procedure in Section 4500-CN⁻.D.4.

f. Standard cyanide solution: Prepare cyanide standards in the desired concentration range, using the stock cyanide standard (¶ 3*e*) and diluting with the 0.25*M* NaOH carrier (¶ 3*a*).

TABLE 4500-CN^-:I. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition *mg CN^-/L*	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	94	—
	Blank†	0.050	96	—
		0.10	96	—
	Site A‡	0	—	<0.5
		0.010	104	—
		0.020	104	—
	Site B‡	0	—	<0.5
		0.010	101	—
		0.020	106	—
	Site C‡	0	—	<0.5
		0.010	103	—
		0.020	108	—
Wastewater treatment plant effluent	Reference sample*	—	95	—
	Blank†	0.050	88	—
		0.10	95	—
	Site A‡	0	—	<0.5
		0.010	112	—
		0.020	106	—
	Site B‡	0	—	<0.5
		0.010	110	—
		0.020	105	—
	Site C‡	0	—	<0.5
		0.010	101	—
		0.020	106	—
Landfill leachate	Reference sample*	—	98	—
	Blank†	0.050	96	—
		0.10	98	—
	Site A‡	0	—	<0.5
		0.050	114	—
		0.10	106	—
	Site B‡	0	—	<0.5
		0.050	104	—
		0.10	104	—
	Site C‡	0	—	<0.5
		0.050	103	—
		0.10	107	—

* U.S. EPA QC sample, 0.498 mg CN^-/L, diluted five-fold.
† Determined in duplicate.
‡ Samples diluted five-fold. Samples without known additions determined four times; sample with known additions determined in duplicate; typical relative differences between duplicates <0.5%.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-CN^-:2 and follow method supplied by manufacturer or laboratory standard operating procedure for this method. Follow quality control guidelines outlined in Section 4020.

5. Calculation

Prepare standard curves by plotting absorbance of standards processed through manifold versus cyanide concentration. The calibration curve is linear.

6. Precision and Bias

a. Recovery and relative standard deviation: The results of single-laboratory studies with various matrices are given in Table 4500-CN^-:I.

b. MDL without distillation: Using a published MDL method,[1] analysts ran 21 replicates of an undistilled 0.010-mg CN^-/L standard with a 780-μL sample loop. These gave a mean of 0.010 mg CN^-/L, a standard deviation of 0.00012 mg CN^-/L, and an MDL of 0.0003 mg CN^-/L. A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagent flow rate.

c. MDL with distillation: Using a published MDL method,[1] analysts ran 21 replicates of a 0.0050-mg CN^-/L standard distilled using the distillation device† equivalent to the apparatus specified in 4500-CN^-.C. When the 0.25*M* NaOH distillates were determined with a 780-μL sample loop, they gave a mean of 0.0045 mg CN^-/L, a standard deviation of 0.0002 mg CN^-/L, and an MDL of 0.0006 mg CN^-/L.

d. Precision study: Ten injections of an undistilled 0.050-mg CN^-/L standard gave a relative standard deviation of 0.21%.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 12.11 amended June 30, 1986. 49 CFR 43430.

†MICRO DIST, Lachat Instruments, Milwaukee, WI.

4500-CN⁻ O. Total Cyanide and Weak Acid Dissociable Cyanide by Flow Injection Analysis

1. General Discussion

a. Principle: Total cyanide consists of various metal-cyanide complexes. To break down or digest these complexes to yield HCN, the sample is mixed with heated phosphoric acid and then irradiated with ultraviolet radiation. The resulting "donor stream" contains the product HCN (aq). This donor stream is passed over a silicone rubber gas permeation membrane. The HCN from the donor stream is extracted by the membrane as HCN (g) and is trapped in a parallel "acceptor stream" that consists of dilute sodium hydroxide, the equivalent of the distillate resulting from the digesting distillations in the sample preparation methods 4500-CN⁻.C, G, and I.

As in 4500-CN⁻.N, the cyanide in this acceptor stream or distillate is converted to cyanogen chloride, CNCl, by reaction with chloramine-T at pH less than 8. The CNCl then forms a red-blue dye by reacting with pyridine-barbituric acid reagent. The absorbance of this red dye is measured at 570 nm and is proportional to the total or weak acid dissociable cyanide in the sample.

The weak acid dissociable (WAD) cyanide method is similar except that ultraviolet radiation and phosphoric acid are not used in the donor stream. Instead, a solution of dihydrogen phosphate is used as the donor stream.

Also see Sections 4500-CN⁻.A, E, and N and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering the sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Nonvolatile interferences are eliminated or minimized by the gas-permeable membrane.

Multiple interferences may require the analysis of a series of sample matrices with known additions to verify the suitability of the chosen treatment. See Section 4500-CN⁻.B for a discussion of preliminary treatment of samples that will be distilled.

1) Total cyanide interferences—Sulfide up to a concentration of 10 mg/L and thiocyanate up to a concentration of 20 mg/L do not interfere in the determination of 100 μg CN^-/L. When a sample containing nitrate at 100 mg NO_3^--N/L and 20 mg/L thiocyanate was treated with sulfamic acid, the determined value was 138.2 μg CN^-/L for a known concentration of 100 μg CN^-/L. When pretreated with ethylenediamine, a sample containing 50 mg formaldehyde/L did not interfere in the determination of cyanide.

2) WAD interferences—Sulfide up to 10 mg/L and thiocyanate up to 50 mg/L do not interfere in the determination of 0.1 mg/L cyanide.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold (Figure 4500-CN⁻:3) with tubing heater, in-line ultraviolet digestion fluidics, a gas-permeable silicone rubber membrane and its holder, and flow cell. In Figure 4500-CN⁻:3, relative flow rates only are shown. The tubing volumes

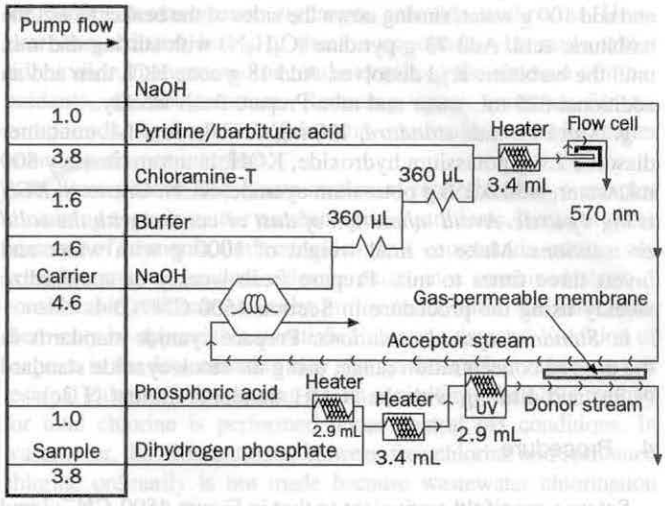

Figure 4500-CN⁻:3. FIA in-line total and WAD cyanide manifold.

are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE. The ultraviolet unit should consist of TFE tubing irradiated by a mercury discharge ultraviolet lamp emitting radiation at 254 nm.

d. Absorbance detector, 570 nm, 10-nm bandpass.

e. Injection valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and all reagents with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L of solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. Phosphoric acid donor stream (total cyanide): To a 1-L volumetric flask, add approximately 700 mL water, then add 30 mL conc phosphoric acid, H_3PO_4. Mix and let solution cool. Dilute to mark. Prepare fresh monthly.

b. Dihydrogen phosphate donor stream (WAD cyanide): To a tared 1-L container add 97 g anhydrous potassium dihydrogen phosphate, KH_2PO_4, and 975 g water. Stir for 2 h or until the potassium phosphate has gone into solution. Degas with helium. Prepare fresh monthly.

c. NaOH acceptor stream, carrier, and diluent (total and WAD cyanide), 0.025*M* NaOH: To a 1-L container add 1.0 g sodium hydroxide (NaOH) and 999 g water. Mix with a magnetic stirrer for about 5 min. Cover with a laboratory film. Degas with helium. Prepare fresh daily.

d. Buffer (total and WAD cyanide), 0.71*M* phosphate: To a 1-L tared container add 97.0 g potassium phosphate, monobasic, anhydrous, KH_2PO_4, and 975 g water. Stir or shake until dissolved. Prepare fresh monthly.

e. Chloramine-T solution (total and WAD cyanide): Dissolve 3 g chloramine-T hydrate in 500 mL water. Degas with helium.

Prepare fresh daily. NOTE: Chloramine-T is an air-sensitive solid. Preferably discard this chemical 6 months after opening.

f. Pyridine/barbituric acid solution (total and WAD cyanide): In the fume hood, place 15.0 g barbituric acid in a tared 1-L container and add 100 g water, rinsing down the sides of the beaker to wet the barbituric acid. Add 73 g pyridine (C_5H_5N) with stirring and mix until the barbituric acid dissolves. Add 18 g conc HCl, then add an additional 825 mL water and mix. Prepare fresh weekly.

g. Stock cyanide standard, 100 mg CN^-/L: In a 1-L container dissolve 2.0 g potassium hydroxide, KOH, in approximately 800 mL water. Add 0.250 g potassium cyanide, KCN. CAUTION: *KCN is highly toxic. Avoid inhalation of dust or contact with the solid or solutions.* Make to final weight of 1000 g with water and invert three times to mix. Prepare fresh weekly or standardize weekly using the procedure in Section 4500-CN^--D.4.

h. Standard cyanide solutions: Prepare cyanide standards in the desired concentration range, using the stock cyanide standard (¶ 3*g*) and diluting with the NaOH standards diluent (¶ 3*c*).

4. Procedure

Set up a manifold equivalent to that in Figure 4500-CN^-:3 and follow the method supplied by the manufacturer or laboratory standard operating procedure for this method. Follow quality control guidelines outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold vs. cyanide concentration. The calibration curve is linear.

6. Precision and Bias

a. MDL, total cyanide: A 420-µL sample loop was used in the total cyanide method. Using a published MDL method[1], analysts ran 21 replicates of a 10.0-µg CN^-/L standard. These gave a mean of 9.69 µg CN^-/L, a standard deviation of 0.86 µg CN^-/L, and an MDL of 2.7 µg CN^-/L.

b. MDL, WAD cyanide: A 420-µL sample loop was used in the WAD cyanide method. Using a published MDL method[1], analysts ran 21 replicates of a 10.0-µg CN^-/L standard. These gave a mean of 11.5 µg CN^-/L, a standard deviation of 0.73 µg CN^-/L, and an MDL of 2.3 µg CN^-/L.

c. Precision study, total cyanide: Seven injections of a 100.0-µg CN^-/L standard gave a relative standard deviation (RSD) of 1.0%.

d. Precision study, WAD cyanide: Ten injections of a 200.0-µg CN^-/L standard gave an RSD of 1.3%.

e. Recovery of total cyanide: Two injections each were made of solutions of potassium ferricyanide and potassium ferrocyanide, both at a concentration equivalent to 100 µg CN^-/L. Both gave an average recovery of 98%.

7. Reference

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4500-Cl CHLORINE (RESIDUAL)*

4500-Cl A. Introduction

1. Effects of Chlorination

The chlorination of water supplies and polluted waters serves primarily to destroy or deactivate disease-producing microorganisms. A secondary benefit, particularly in treating drinking water, is the overall improvement in water quality resulting from the reaction of chlorine with ammonia, iron, manganese, sulfide, and some organic substances.

Chlorination may produce adverse effects. Taste and odor characteristics of phenols and other organic compounds present in a water supply may be intensified. Potentially carcinogenic chloroorganic compounds such as chloroform may be formed. Combined chlorine formed on chlorination of ammonia- or amine-bearing waters adversely affects some aquatic life. To fulfill the primary purpose of chlorination and to minimize any adverse effects, it is essential that proper testing procedures be used with a foreknowledge of the limitations of the analytical determination.

2. Chlorine Forms and Reactions

Chlorine applied to water in its molecular or hypochlorite form initially undergoes hydrolysis to form free chlorine consisting of aqueous molecular chlorine, hypochlorous acid, and hypochlorite ion. The relative proportion of these free chlorine forms is pH- and temperature-dependent. At the pH of most waters, hypochlorous acid and hypochlorite ion will predominate.

Free chlorine reacts readily with ammonia and certain nitrogenous compounds to form combined chlorine. With ammonia, chlorine reacts to form the chloramines: monochloramine, dichloramine, and nitrogen trichloride. The presence and concentrations of these combined forms depend chiefly on pH, temperature, initial chlorine-to-nitrogen ratio, absolute chlorine de-

* Approved by Standard Methods Committee, 2000.

mand, and reaction time. Both free and combined chlorine may be present simultaneously. Combined chlorine in water supplies may be formed in the treatment of raw waters containing ammonia or by the addition of ammonia or ammonium salts. Chlorinated wastewater effluents, as well as certain chlorinated industrial effluents, normally contain only combined chlorine. Historically, the principal analytical problem has been to distinguish between free and combined forms of chlorine.

3. Selection of Method

In two separate but related studies, samples were prepared and distributed to participating laboratories to evaluate chlorine methods. Because of poor accuracy and precision and a high overall (average) total error in these studies, all orthotolidine procedures except one were dropped in the 14th edition of this work. The useful stabilized neutral orthotolidine method was deleted from the 15th edition because of the toxic nature of orthotolidine. The leuco crystal violet (LCV) procedure was dropped from the 17th edition because of its relative difficulty and the lack of comparative advantages.

a. Natural and treated waters: The iodometric methods (B and C) are suitable for measuring total chlorine concentrations greater than 1 mg/L, but the amperometric end point of Methods C and D gives greater sensitivity. All acidic iodometric methods suffer from interferences, generally in proportion to the quantity of potassium iodide (KI) and H^+ added.

The amperometric titration method (D) is a standard of comparison for the determination of free or combined chlorine. It is affected little by common oxidizing agents, temperature variations, turbidity, and color. The method is not as simple as the colorimetric methods and requires greater operator skill to obtain the best reliability. Loss of chlorine can occur because of rapid stirring in some commercial equipment. Clean and conditioned electrodes are necessary for sharp end points.

A low-level amperometric titration procedure (E) has been added to determine total chlorine at levels below 0.2 mg/L. This method is recommended only when quantification of such low residuals is necessary. The interferences are similar to those found with the standard amperometric procedure (D). The DPD methods (Methods F and G) are operationally simpler for determining free chlorine than the amperometric titration. Procedures are given for estimating the separate mono- and dichloramine and combined fractions. High concentrations of monochloramine interfere with the free chlorine determination unless the reaction is stopped with arsenite or thioacetamide. In addition, the DPD methods are subject to interference by oxidized forms of manganese unless compensated for by a blank.

The amperometric and DPD methods are unaffected by dichloramine concentrations in the range of 0 to 9 mg Cl as Cl_2/L in the determination of free chlorine. Nitrogen trichloride, if present, may react partially as free chlorine in the amperometric, DPD, and FACTS methods. The extent of this interference in the DPD methods does not appear to be significant.

The free chlorine test, syringaldazine (FACTS, Method H) was developed specifically for free chlorine. It is unaffected by significant concentrations of monochloramine, dichloramine, nitrate, nitrite, and oxidized forms of manganese.[1]

Sample color and turbidity may interfere in all colorimetric procedures.

Organic contaminants may produce a false free chlorine reading in most colorimetric methods (see ¶ 3*b* below). Many strong oxidizing agents interfere in the measurement of free chlorine in all methods. Such interferences include bromine, chlorine dioxide, iodine, permanganate, hydrogen peroxide, and ozone. However, the reduced forms of these compounds—bromide, chloride, iodide, manganous ion, and oxygen, in the absence of other oxidants, do not interfere. Reducing agents such as ferrous compounds, hydrogen sulfide, and oxidizable organic matter generally do not interfere.

b. Wastewaters: The determination of total chlorine in samples containing organic matter presents special problems. Because of the presence of ammonia, amines, and organic compounds, particularly organic nitrogen, residual chlorine exists in a combined state. A considerable residual may exist in this form, but at the same time there may be appreciable unsatisfied chlorine demand. Addition of reagents in the determination may change these relationships so that residual chlorine is lost during the analysis. Only the DPD method for total chlorine is performed under neutral pH conditions. In wastewater, the differentiation between free chlorine and combined chlorine ordinarily is not made because wastewater chlorination seldom is carried far enough to produce free chlorine.

The determination of residual chlorine in industrial wastes is similar to that in domestic wastewater when the waste contains organic matter, but may be similar to the determination in water when the waste is low in organic matter.

None of these methods is applicable to estuarine or marine waters because the bromide is converted to bromine and bromamines, which are detected as free or total chlorine. A procedure for estimating this interference is available for the DPD method.

Although the methods given below are useful for the determination of residual chlorine in wastewaters and treated effluents, select the method in accordance with sample composition. Some industrial wastes, or mixtures of wastes with domestic wastewater, may require special precautions and modifications to obtain satisfactory results.

Determine free chlorine in wastewater by any of the methods provided that known interfering substances are absent or appropriate correction techniques are used. The amperometric method is the method of choice because it is not subject to interference from color, turbidity, iron, manganese, or nitrite nitrogen. The DPD method is subject to interference from high concentrations of monochloramine, which is avoided by adding thioacetamide immediately after reagent addition. Oxidized forms of manganese at all levels encountered in water will interfere in all methods except in the free chlorine measurement of amperometric titrations and FACTS, but a blank correction for manganese can be made in Methods F and G.

The FACTS method is unaffected by concentrations of monochloramine, dichloramine, nitrite, iron, manganese, and other interfering compounds normally found in domestic wastewaters.

For total chlorine in samples containing significant amounts of organic matter, use either the DPD methods (F and G), amperometric, or iodometric back titration method (C) to prevent contact between the full concentration of liberated iodine and the sample. With Method C, do not use the starch-iodide end point if the concentration is less than 1 mg/L. In the absence of interference, the amperometric and starch-iodide end points give concordant results. The amperometric end point is inherently more sensitive and is free of interference from color and turbid-

ity, which can cause difficulty with the starch-iodide end point. On the other hand, certain metals, surface-active agents, and complex anions in some industrial wastes interfere in the amperometric titration and indicate the need for another method for such wastewaters. Silver in the form of soluble silver cyanide complex, in concentrations of 1.0 mg Ag/L, poisons the cell at pH 4.0 but not at 7.0. The silver ion, in the absence of the cyanide complex, gives extensive response in the current at pH 4.0 and gradually poisons the cell at all pH levels. Cuprous copper in the soluble copper cyanide ion, in concentrations of 5 mg Cu/L or less, poisons the cell at pH 4.0 and 7.0. Although iron and nitrite may interfere with this method, minimize the interference by buffering to pH 4.0 before adding KI. Oxidized forms of manganese interfere in all methods for total chlorine including amperometric titration. An unusually high content of organic matter may cause uncertainty in the end point.

Regardless of end-point detection, either phenylarsine oxide or thiosulfate may be used as the standard reducing reagent at pH 4. The former is more stable and is preferred.

The DPD titrimetric and colorimetric methods (F and G, respectively) are applicable to determining total chlorine in polluted waters. In addition, both DPD procedures and the amperometric titration method allow for estimating monochloramine and dichloramine fractions. Because all methods for total chlorine depend on the stoichiometric production of iodine, waters containing iodine-reducing substances may not be analyzed accurately by these methods, especially where iodine remains in the solution for a significant time. This problem occurs in Methods B and D. The back titration procedure (C) and Methods F and G cause immediate reaction of the iodine generated so that it has little chance to react with other iodine-reducing substances.

In all colorimetric procedures, compensate for color and turbidity by using color and turbidity blanks.

A method (I) for total residual chlorine using a potentiometric iodide electrode is proposed. This method is suitable for analysis of chlorine residuals in natural and treated waters and wastewater effluents. No differentiation of free and combined chlorine is possible. This procedure is an adaptation of other iodometric techniques and is subject to the same inferences.

4. Sampling and Storage

Chlorine in aqueous solution is not stable, and the chlorine content of samples or solutions, particularly weak solutions, will decrease rapidly. Exposure to sunlight or other strong light or agitation will accelerate the reduction of chlorine. Therefore, start chlorine determinations immediately after sampling, avoiding excessive light and agitation. Do not store samples to be analyzed for chlorine.

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4500-Cl B. Iodometric Method I

1. General Discussion

a. Principle: Chlorine will liberate free iodine from potassium iodide (KI) solutions at pH 8 or less. The liberated iodine is titrated with a standard solution of sodium thiosulfate ($Na_2S_2O_3$) with starch as the indicator. Titrate at pH 3 to 4 because the reaction is not stoichiometric at neutral pH due to partial oxidation of thiosulfate to sulfate.

b. Interference: Oxidized forms of manganese and other oxidizing agents interfere. Reducing agents such as organic sulfides also interfere. Although the neutral titration minimizes the interfering effect of ferric and nitrite ions, the acid titration is preferred because some forms of combined chlorine do not react at pH 7. Use only acetic acid for the acid titration; sulfuric acid (H_2SO_4) will increase interferences; *never use hydrochloric acid (HCl).* See Section A.3 for discussion of other interferences.

c. Minimum detectable concentration: The minimum detectable concentration approximates 40 μg Cl as Cl_2/L if 0.01*N* $Na_2S_2O_3$ is used with a 1000-mL sample. Concentrations below 1 mg/L cannot be determined accurately by the starch-iodide end point used in this method. Lower concentrations can be measured with the amperometric end point in Methods C and D.

2. Reagents

a. Acetic acid, conc (glacial).

b. Potassium iodide, KI, crystals.

c. Standard sodium thiosulfate, 0.1*N:* Dissolve 25 g $Na_2S_2O_3 \cdot 5H_2O$ in 1 L freshly boiled distilled water and standardize against potassium bi-iodate or potassium dichromate after at least 2 weeks storage. This initial storage is necessary to allow oxidation of any bisulfite ion present. Use boiled distilled

water and add a few milliliters chloroform ($CHCl_3$) to minimize bacterial decomposition.

Standardize 0.1*N* $Na_2S_2O_3$ by one of the following:

1) Iodate method—Dissolve 3.249 g anhydrous potassium bi-iodate, $KH(IO_3)_2$, primary standard quality, or 3.567 g KIO_3 dried at 103 ± 2°C for 1 h, in distilled water and dilute to 1000 mL to yield a 0.1000*N* solution. Store in a glass-stoppered bottle.

To 80 mL distilled water, add, with constant stirring, 1 mL conc H_2SO_4, 10.00 mL 0.1000*N* $KH(IO_3)_2$, and 1 g KI. Titrate immediately with 0.1*N* $Na_2S_2O_3$ titrant until the yellow color of the liberated iodine almost is discharged. Add 1 mL starch indicator solution and continue titrating until the blue color disappears.

2) Dichromate method—Dissolve 4.904 g anhydrous potassium dichromate, $K_2Cr_2O_7$, of primary standard quality, in distilled water and dilute to 1000 mL to yield a 0.1000*N* solution. Store in a glass-stoppered bottle.

Proceed as in the iodate method, with the following exceptions: Substitute 10.00 mL 0.1000*N* $K_2Cr_2O_7$ for iodate and let reaction mixture stand 6 min in the dark before titrating with 0.1*N* $Na_2S_2O_3$ titrant.

$$\text{Normality } Na_2S_2O_3 = \frac{1}{\text{mL } Na_2S_2O_3 \text{ consumed}}$$

d. Standard sodium thiosulfate titrant, 0.01*N* or 0.025*N:* Improve the stability of 0.01*N* or 0.025*N* $Na_2S_2O_3$ by diluting an aged 0.1*N* solution, made as directed above, with freshly boiled distilled water. Add 4 g sodium borate and 10 mg mercuric iodide/L solution. For accurate work, standardize this solution daily in accordance with the directions given above, using 0.01*N* or 0.025*N* iodate or $K_2Cr_2O_7$. Use sufficient volumes of these standard solutions so that their final dilution is not greater than 1 + 4. To speed up operations where many samples must be titrated use an automatic buret of a type in which rubber does not come in contact with the solution. Standard titrants, 0.0100*N* and 0.0250*N*, are equivalent, respectively, to 354.5 µg and 886.3 µg Cl as Cl_2/1.00 mL.

e. Starch indicator solution: To 5 g starch (potato, arrowroot, or soluble), add a little cold water and grind in a mortar to a thin paste. Pour into 1 L of boiling distilled water, stir, and let settle overnight. Use clear supernate. Preserve with 1.25 g salicylic acid, 4 g zinc chloride, or a combination of 4 g sodium propionate and 2 g sodium azide/L starch solution. Some commercial starch substitutes are satisfactory.

f. Standard iodine, 0.1*N:* See C.3*g*.

g. Dilute standard iodine, 0.0282*N:* See C.3*h*.

3. Procedure

a. Volume of sample: Select a sample volume that will require no more than 20 mL 0.01*N* $Na_2S_2O_3$ and no less than 0.2 mL for the starch-iodide end point. For a chlorine range of 1 to 10 mg/L, use a 500-mL sample; above 10 mg/L, use proportionately less sample. Use smaller samples and volumes of titrant with the amperometric end point.

b. Preparation for titration: Place 5 mL acetic acid, or enough to reduce the pH to between 3.0 and 4.0, in a flask or white porcelain casserole. Add about 1 g KI estimated on a spatula. Pour sample in and mix with a stirring rod.

c. Titration: Titrate away from direct sunlight. Add 0.025*N* or 0.01*N* $Na_2S_2O_3$ from a buret until the yellow color of the liberated iodine almost is discharged. Add 1 mL starch solution and titrate until blue color is discharged.

If the titration is made with 0.025*N* $Na_2S_2O_3$ instead of 0.01*N*, then, with a 1-L sample, 1 drop is equivalent to about 50 µg/L. It is not possible to discern the end point with greater accuracy.

d. Blank titration: Correct result of sample titration by determining blank contributed by oxidizing or reducing reagent impurities. The blank also compensates for the concentration of iodine bound to starch at the end point.

Take a volume of distilled water corresponding to the sample used for titration in ¶s 3*a–c*, add 5 mL acetic acid, 1 g KI, and 1 mL starch solution. Perform blank titration as in 1) or 2) below, whichever applies.

1) If a blue color develops, titrate with 0.01*N* or 0.025*N* $Na_2S_2O_3$ to disappearance of blue color and record result. *B* (see ¶ 4, below) is negative.

2) If no blue color occurs, titrate with 0.0282*N* iodine solution until a blue color appears. Back-titrate with 0.01*N* or 0.025*N* $Na_2S_2O_3$ and record the difference. *B* is positive.

Before calculating the chlorine concentration, subtract the blank titration of ¶ 1) from the sample titration; or, if necessary, add the net equivalent value of the blank titration of ¶ 2).

4. Calculation

For standardizing chlorine solution for temporary standards:

$$\text{mg Cl as } Cl_2/\text{mL} = \frac{(A \pm B) \times N \times 35.45}{\text{mL sample}}$$

For determining total available residual chlorine in a water sample:

$$\text{mg Cl as } Cl_2/\text{L} = \frac{(A \pm B) \times N \times 35\,450}{\text{mL sample}}$$

where:

A = mL titration for sample,
B = mL titration for blank (positive or negative), and
N = normality of $Na_2S_2O_3$.

5. Precision and Bias

Published studies[1,2] give the results of nine methods used to analyze synthetic water samples without interferences; variations of some of the methods appear in this edition. More current data are not now available.

6. References

1. Water Chlorine (Residual) No. 1. 1969. Analytical Reference Service Rep. No. 35, U.S. Environmental Protection Agency, Cincinnati, Ohio.
2. Water Chlorine (Residual) No. 2. 1971. Analytical Reference Service Rep. No. 40, U.S. Environmental Protection Agency, Cincinnati, Ohio.

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4500-Cl C. Iodometric Method II

1. General Discussion

a. Principle: In this method, used for wastewater analysis, the end-point signal is reversed because the unreacted standard reducing agent (phenylarsine oxide or thiosulfate) remaining in the sample is titrated with standard iodine or standard iodate, rather than the iodine released being titrated directly. This indirect procedure is necessary regardless of the method of end-point detection, to avoid contact between the full concentration of liberated iodine and the wastewater.

When iodate is used as a back titrant, use only phosphoric acid. Do not use acetate buffer.

b. Interference: Oxidized forms of manganese and other oxidizing agents give positive interferences. Reducing agents such as organic sulfides do not interfere as much as in Method B. Minimize iron and nitrite interference by buffering to pH 4.0 before adding potassium iodide (KI). An unusually high content of organic matter may cause some uncertainty in the end point. Whenever manganese, iron, and other interferences definitely are absent, reduce this uncertainty and improve precision by acidifying to pH 1.0. Control interference from more than 0.2 mg nitrite/L with phosphoric acid-sulfamic acid reagent. A larger fraction of organic chloramines will react at lower pH along with interfering substances. See Section A.3 for a discussion of other interferences.

2. Apparatus

For a description of the amperometric end-point detection apparatus and a discussion of its use, see D.2*a*.

3. Reagents

a. Standard phenylarsine oxide solution, 0.005 64*N*: Dissolve approximately 0.8 g phenylarsine oxide powder in 150 mL 0.3*N* NaOH solution. After settling, decant 110 mL into 800 mL distilled water and mix thoroughly. Bring to pH 6 to 7 with 6*N* HCl and dilute to 950 mL with distilled water. CAUTION: *Severe poison, cancer suspect agent.*

Standardization—Accurately measure 5 to 10 mL freshly standardized 0.0282*N* iodine solution into a flask and add 1 mL KI solution. Titrate with phenylarsine oxide solution, using the amperometric end point (Method D) or starch solution (see B.2*e*) as an indicator. Adjust to 0.005 64*N* and recheck against the standard iodine solution; 1.00 mL = 200 μg available chlorine. (CAUTION: *Toxic—take care to avoid ingestion.*)

b. Standard sodium thiosulfate solution, 0.1*N*: See B.2*c*.

c. Standard sodium thiosulfate solution, 0.005 64*N*: Prepare by diluting 0.1*N* $Na_2S_2O_3$. For maximum stability of the dilute solution, prepare by diluting an aged 0.1*N* solution with freshly boiled distilled water (to minimize bacterial action) and add 4 g $Na_4B_4O_7$/L. To inhibit mold formation optionally add either 10 mg HgI_2 or 2 drops toluene per liter of solution. Standardize daily as directed in B.2*c* using 0.005 64*N* $K_2Cr_2O_7$ or iodate solution. Use sufficient volume of sample so that the final dilution does not exceed 1 + 2. Use an automatic buret of a type in which rubber does not come in contact with the solution. 1.00 mL = 200 μg available chlorine.

d. Potassium iodide, KI, crystals.

e. Acetate buffer solution, pH 4.0: Dissolve 146 g anhydrous $NaC_2H_3O_2$, or 243 g $NaC_2H_3O_2 \cdot 3H_2O$, in 400 mL distilled water, add 480 g conc acetic acid, and dilute to 1 L with chlorine-demand-free water.

f. Standard arsenite solution, 0.1*N*: Accurately weigh a stoppered weighing bottle containing approximately 4.95 g arsenic trioxide, As_2O_3. Transfer without loss to a 1-L volumetric flask and again weigh bottle. Do not attempt to brush out adhering oxide. Moisten As_2O_3 with water and add 15 g NaOH and 100 mL distilled water. Swirl flask contents gently to dissolve. Dilute to 250 mL with distilled water and saturate with CO_2, thus converting all NaOH to $NaHCO_3$. Dilute to mark, stopper, and mix thoroughly. This solution will preserve its titer almost indefinitely. (CAUTION: *Severe poison. Cancer suspect agent.*)

$$\text{Normality} = \frac{\text{g } As_2O_3}{49.455}$$

g. Standard iodine solution, 0.1*N*: Dissolve 40 g KI in 25 mL chlorine-demand-free water, add 13 g resublimed iodine, and stir until dissolved. Transfer to a 1-L volumetric flask and dilute to mark.

Standardization—Accurately measure 40 to 50 mL 0.1*N* arsenite solution into a flask and titrate with 0.1*N* iodine solution, using starch solution as indicator. To obtain accurate results, insure that the solution is saturated with CO_2 at end of titration by passing current of CO_2 through solution for a few minutes just before end point is reached, or add a few drops of HCl to liberate sufficient CO_2 to saturate solution. Alternatively standardize against $Na_2S_2O_3$; see B.2*c*1).

Optionally, prepare 0.1000*N* iodine solution directly as a standard solution by weighing 12.69 g primary standard resublimed iodine. Because I_2 may be volatilized and lose from both solid and solution, transfer the solid immediately to KI as spec-

ified above. Never let solution stand in open containers for extended periods.

h. Standard iodine titrant, 0.0282*N*: Dissolve 25 g KI in a little distilled water in a 1-L volumetric flask, add correct amount of 0.1*N* iodine solution exactly standardized to yield a 0.0282*N* solution, and dilute to 1 L with chlorine-demand-free water. For accurate work, standardize daily according to directions in ¶ 3*g* above, using 5 to 10 mL of arsenite or $Na_2S_2O_3$ solution. Store in amber bottles or in the dark; protect solution from direct sunlight at all times and keep from all contact with rubber.

i. Starch indicator: See B.2*e*.

j. Standard iodate titrant, 0.005 64*N:* Dissolve 201.2 mg primary standard grade KIO_3, dried for 1 h at 103°C, or 183.3 mg primary standard anhydrous potassium bi-iodate in distilled water and dilute to 1 L.

k. Phosphoric acid solution, H_3PO_4, 1 + 9.

l. Phosphoric acid-sulfamic acid solution: Dissolve 20 g NH_2SO_3H in 1 L 1 + 9 phosphoric acid.

m. Chlorine-demand-free water: Prepare chlorine-demand-free water from good-quality distilled or deionized water by adding sufficient chlorine to give 5 mg/L free chlorine. After standing 2 d this solution should contain at least 2 mg/L free chlorine; if not, discard and obtain better-quality water. Remove remaining free chlorine by placing container in sunlight or irradiating with an ultraviolet lamp. After several hours take sample, add KI, and measure total chlorine with a colorimetric method using a nessler tube to increase sensitivity. Do not use before last trace of free and combined chlorine has been removed.

Distilled water commonly contains ammonia and also may contain reducing agents. Collect good-quality distilled or deionized water in a sealed container from which water can be drawn by gravity. To the air inlet of the container add an H_2SO_4 trap consisting of a large test tube half filled with 1 + 1 H_2SO_4 connected in series with a similar but empty test tube. Fit both test tubes with stoppers and inlet tubes terminating near the bottom of the tubes and outlet tubes terminating near the top of the tubes. Connect outlet tube of trap containing H_2SO_4 to the distilled water container, connect inlet tube to outlet of empty test tube. The empty test tube will prevent discharge to the atmosphere of H_2SO_4 due to temperature-induced pressure changes. Stored in such a container, chlorine-demand-free water is stable for several weeks unless bacterial growth occurs.

4. Procedure

a. Preparation for titration:

1) Volume of sample—For chlorine concentration of 10 mg/L or less, titrate 200 mL. For greater chlorine concentrations, use proportionately less sample and dilute to 200 mL with chlorine-demand-free water. Use a sample of such size that not more than 10 mL phenylarsine oxide solution is required.

2) Preparation for titration—Measure 5 mL 0.005 64*N* phenylarsine oxide or thiosulfate for chlorine concentrations from 2 to 5 mg/L, and 10 mL for concentrations of 5 to 10 mg/L, into a flask or casserole for titration with standard iodine or iodate. Start stirring. For titration by amperometry or standard iodine, also add excess KI (approximately 1 g) and 4 mL acetate buffer solution or enough to reduce the pH to between 3.5 and 4.2.

b. Titration: Use one of the following:

1) Amperometric titration—Add 0.0282*N* iodine titrant in small increments from a 1-mL buret or pipet. Observe meter needle response as iodine is added: the pointer remains practically stationary until the end point is approached, whereupon each iodine increment causes a temporary deflection of the microammeter, with the pointer dropping back to its original position. Stop titration at end point when a small increment of iodine titrant gives a definite pointer deflection upscale and the pointer does not return promptly to its original position. Record volume of iodine titrant used to reach end point.

2) Colorimetric (iodine) titration—Add 1 mL starch solution and titrate with 0.0282*N* iodine to the first appearance of blue color that persists after complete mixing.

3) Colorimetric (iodate) titration—To suitable flask or casserole add 200 mL chlorine-demand-free water and add, with agitation, the required volume of reductant, an excess of KI (approximately 0.5 g), 2 mL 10% H_3PO_4 solution, and 1 mL starch solution in the order given, and titrate immediately* with 0.005 64*N* iodate solution to the first appearance of a blue color that persists after complete mixing. Designate volume of iodate solution used as *A*. Repeat procedure, substituting 200 mL sample for the 200 mL chlorine-demand-free water. If sample is colored or turbid, titrate to the first change in color, using for comparison another portion of sample with H_3PO_4 added. Designate this volume of iodate solution as *B*.

5. Calculation

a. Titration with standard iodine:

$$\text{mg Cl as } Cl_2/\text{L} = \frac{(A - 5B) \times 200}{C}$$

where:

A = mL 0.005 64*N* reductant,
B = mL 0.0282 *N* I_2, and
C = mL sample.

b. Titration with standard iodate:

$$\text{mg Cl as } Cl_2/\text{L} = \frac{(A - B) \times 200}{C}$$

where:

A = mL $Na_2S_2O_3$,
B = mL iodate required to titrate $Na_2S_2O_3$, and
C = mL sample.

6. Bibliography

See B.7.

* Titration may be delayed up to 10 min without appreciable error if H_3PO_4 is not added until immediately before titration.

4500-Cl D. Amperometric Titration Method

1. General Discussion

Amperometric titration requires a higher degree of skill and care than the colorimetric methods. Chlorine residuals over 2 mg/L are measured best by means of smaller samples or by dilution with water that has neither residual chlorine nor a chlorine demand. The method can be used to determine total chlorine and can differentiate between free and combined chlorine. A further differentiation into monochloramine and dichloramine fractions is possible by control of KI concentration and pH.

a. Principle: The amperometric method is a special adaptation of the polarographic principle. Free chlorine is titrated at a pH between 6.5 and 7.5, a range in which the combined chlorine reacts slowly. The combined chlorine, in turn, is titrated in the presence of the proper amount of KI in the pH range 3.5 to 4.5. When free chlorine is determined, the pH must not be greater than 7.5 because the reaction becomes sluggish at higher pH values, nor less than 6.5 because at lower pH values some combined chlorine may react even in the absence of iodide. When combined chlorine is determined, the pH must not be less than 3.5 because of increased interferences at lower pH values, nor greater than 4.5 because the iodide reaction is not quantitative at higher pH values. The tendency of monochloramine to react more readily with iodide than does dichloramine provides a means for further differentiation. The addition of a small amount of KI in the neutral pH range enables estimation of monochloramine content. Lowering the pH into the acid range and increasing the KI concentration allows the separation determination of dichloramine.

Organic chloramines can be measured as free chlorine, monochloramine, or dichloramine, depending on the activity of chlorine in the organic compound.

Phenylarsine oxide is stable even in dilute solution and each mole reacts with two equivalents of halogen. A special amperometric cell is used to detect the end point of the residual chlorine-phenylarsine oxide titration. The cell consists of a nonpolarizable reference electrode that is immersed in a salt solution and a readily polarizable noble-metal electrode that is in contact with both the salt solution and the sample being titrated. In some applications, end-point selectivity is improved by adding +200 mV to the platinum electrode versus silver, silver chloride. Another approach to end-point detection uses dual platinum electrodes, a mercury cell with voltage divider to impress a potential across the electrodes, and a microammeter. If there is no chlorine residual in the sample, the microammeter reading will be comparatively low because of cell polarization. The greater the residual, the greater the microammeter reading. The meter acts merely as a null-point indicator—that is, the actual meter reading is not important, but rather the relative readings as the titration proceeds. The gradual addition of phenylarsine oxide causes the cell to become more and more polarized because of the decrease in chlorine. The end point is recognized when no further decrease in meter reading can be obtained by adding more phenylarsine oxide.

b. Interference: Accurate determinations of free chlorine cannot be made in the presence of nitrogen trichloride, NCl_3, or chlorine dioxide, which titrate partly as free chlorine. When present, NCl_3 can titrate partly as free chlorine and partly as dichloramine, contributing a positive error in both fractions at a rate of approximately 0.1%/min. Some organic chloramines also can be titrated in each step. Monochloramine can intrude into the free chlorine fraction and dichloramine can interfere in the monochloramine fraction, especially at high temperatures and prolonged titration times. Free halogens other than chlorine also will titrate as free chlorine. Combined chlorine reacts with iodide ions to produce iodine. When titration for free chlorine follows a combined chlorine titration, which requires addition of KI, erroneous results may occur unless the measuring cell is rinsed thoroughly with distilled water between titrations.

Interference from copper has been noted in samples taken from copper pipe or after heavy copper sulfate treatment of reservoirs, with metallic copper plating out on the electrode. Silver ions also poison the electrode. Interference occurs in some highly colored waters and in waters containing surface-active agents. Very low temperatures slow response of measuring cell and longer time is required for the titration, but precision is not affected. A reduction in reaction rate is caused by pH values above 7.5; overcome this by buffering all samples to pH 7.0 or less. On the other hand, some substances, such as manganese, nitrite, and iron, do not interfere. The violent stirring of some commercial titrators can lower chlorine values by volatilization. When dilution is used for samples containing high chlorine content, take care that the dilution water is free of chlorine and ammonia and possesses no chlorine demand.

See A.3 for a discussion of other interferences.

2. Apparatus

a. End-point detection apparatus, consisting of a cell unit connected to a microammeter, with necessary electrical accessories. The cell unit includes a noble-metal electrode of sufficient surface area, a salt bridge to provide an electrical connection without diffusion of electrolyte, and a reference electrode of silver-silver chloride in a saturated sodium chloride solution connected into the circuit by means of the salt bridge. Numerous commercial systems are available.

Keep platinum electrode free of deposits and foreign matter. Vigorous chemical cleaning generally is unnecessary. Occasional mechanical cleaning with a suitable abrasive usually is sufficient. Keep salt bridge in good operating condition; do not allow it to become plugged nor permit appreciable flow of electrolyte through it. Keep solution surrounding reference electrode free of contamination and maintain it at constant composition by insuring an adequate supply of undissolved salt at all times. A cell with two metal electrodes polarized by a small DC potential also may be used. (See Bibliography.)

b. Agitator, designed to give adequate agitation at the noble-metal electrode surface to insure proper sensitivity. Thoroughly clean agitator and exposed electrode system to remove all chlorine- consuming contaminants by immersing them in water containing 1 to 2 mg/L free chlorine for a few minutes. Add KI to the same water and let agitator and electrodes remain immersed for 5 min. After thorough rinsing with chlorine-demand-free

water or the sample to be tested, sensitized electrodes and agitator are ready for use. Remove iodide reagent completely from cell.

c. Buret: Commercial titrators usually are equipped with suitable burets (1 mL). Manual burets are available.*

d. Glassware, exposed to water containing at least 10 mg/L chlorine for 3 h or more before use and rinsed with chlorine-demand-free water.

3. Reagents

a. Standard phenylarsine oxide titrant: See C.3*a*.

b. Phosphate buffer solution, pH 7: Dissolve 25.4 g anhydrous KH_2PO_4 and 34.1 g anhydrous Na_2HPO_4 in 800 mL distilled water. Add 2 mL sodium hypochlorite solution containing 1% chlorine and mix thoroughly. Protect from sunlight for 2 d. Determine that free chlorine still remains in the solution. Then expose to sunlight until no chlorine remains. If necessary, carry out the final dechlorination with an ultraviolet lamp. Determine that no total chlorine remains by adding KI and measuring with one of the colorimetric tests. Dilute to 1 L with distilled water and filter if any precipitate is present.

c. Potassium iodide solution: Dissolve 50 g KI and dilute to 1 L with freshly boiled and cooled distilled water. Store in the dark in a brown glass-stoppered bottle, preferably in the refrigerator. Discard when solution becomes yellow.

d. Acetate buffer solution, pH 4: See C.3*e*.

4. Procedure

a. Sample volume: Select a sample volume requiring no more than 2 mL phenylarsine oxide titrant. Thus, for chlorine concentrations of 2 mg/L or less, take a 200-mL sample; for chlorine levels in excess of 2 mg/L, use 100 mL or proportionately less.

b. Free chlorine: Unless sample pH is known to be between 6.5 and 7.5, add 1 mL pH 7 phosphate buffer solution to produce a pH of 6.5 to 7.5. Titrate with standard phenylarsine oxide titrant, observing current changes on microammeter. Add titrant in progressively smaller increments until all needle movement ceases. Make successive buret readings when needle action becomes sluggish, signaling approach of end point. Subtract last very small increment that causes no needle response because of overtitration. Alternatively, use a system involving continuous current measurements and determine end point mathematically.

Continue titrating for combined chlorine as described in ¶ 4*c* below or for the separate monochloramine and dichloramine fractions as detailed in ¶s 4*e* and 4*f*.

c. Combined chlorine: To sample remaining from free-chlorine titration add 1.00 mL KI solution and 1 mL acetate buffer solution, in that order. Titrate with phenylarsine oxide titrant to the end point, as above. Do not refill buret but simply continue titration after recording figure for free chlorine. Again subtract last increment to give amount of titrant actually used in reaction with chlorine. (If titration was continued without refilling buret, this figure represents total chlorine. Subtracting free chlorine from total gives combined chlorine.) Wash apparatus and sample cell thoroughly to remove iodide ion to avoid inaccuracies when the titrator is used subsequently for a free chlorine determination.

d. Separate samples: If desired, determine total chlorine and free chlorine on separate samples. If sample pH is between 3.5 and 9.5 and total chlorine alone is required, treat sample immediately with 1 mL KI solution followed by 1 mL acetate buffer solution, and titrate with phenylarsine oxide titrant as described in ¶ 4*c* preceding.

e. Monochloramine: After titrating for free chlorine, add 0.2 mL KI solution to same sample and, without refilling buret, continue titration with phenylarsine oxide titrant to end point. Subtract last increment to obtain net volume of titrant consumed by monochloramine.

f. Dichloramine: Add 1 mL acetate buffer solution and 1 mL KI solution to same sample and titrate final dichloramine fraction as described above.

5. Calculation

Convert individual titrations for free chlorine, combined chlorine, total chlorine, monochloramine, and dichloramine by the following equation:

$$\text{mg Cl as } Cl_2/\text{L} = \frac{A \times 200}{\text{mL sample}}$$

where:

A = mL phenylarsine oxide titration.

6. Precision and Bias

See B.5.

7. Bibliography

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* Kimax 17110-F, 5 mL, Kimble Products, Box 1035, Toledo, OH, or equivalent.

4500-Cl E. Low-Level Amperometric Titration Method

1. General Discussion

Detection and quantification of chlorine residuals below 0.2 mg/L require special modifications to the amperometric titration procedure. With these modifications chlorine concentrations at the 10-μg/L level can be measured. It is not possible to differentiate between free and combined chlorine forms. Oxidizing agents that interfere with the amperometric titration method (D) will interfere.

a. Principle: This method modifies D by using a more dilute titrant and a graphical procedure to determine the end point.

b. Interference: See D.1*b*.

2. Apparatus

See D.2.

3. Reagents

a. Potassium bi-iodate, 0.002 256*N*: Dissolve 0.7332 g anhydrous potassium bi-iodate, $KH(IO_3)_2$, in 500 mL chlorine-free distilled water and dilute to 1000 mL. Dilute 10.00 mL to 100.0 mL with chlorine-free distilled water. Use only freshly prepared solution for the standardization of phenylarsine oxide.

b. Potassium iodide, KI crystals.

c. Low-strength phenylarsine oxide titrant, 0.000 564*N*: Dilute 10.00 mL of 0.005 64*N* phenylarsine oxide (see C.3*a*) to 100.0 mL with chlorine-demand-free water (see C.3*m*).

Standardization—Dilute 5.00 mL 0.002 256*N* potassium bi-iodate to 200 mL with chlorine-free water. Add approximately 1.5 g KI and stir to dissolve. Add 1 mL acetate buffer and let stand in the dark for 6 min. Titrate using the amperometric titrator and determine the equivalence point as indicated below.

$$\text{Normality} = 0.002256 \times 5/A$$

where:

A = mL phenylarsine oxide titrant required to reach the equivalence point of standard bi-iodate.

d. Acetate buffer solution, pH 4: See C.3*e*.

4. Procedure

Select a sample volume requiring no more than 2 mL phenylarsine oxide titrant. A 200-mL sample will be adequate for samples containing less than 0.2 mg total chlorine/L.

Before beginning titration, rinse buret with titrant several times. Rinse sample container with distilled water and then with sample. Add 200 mL sample to sample container and approximately 1.5 g KI. Dissolve, using a stirrer or mixer. Add 1 mL acetate buffer and place container in end-point detection apparatus. When the current signal stabilizes, record the reading. Initially adjust meter to a near full-scale deflection. Titrate by adding small, known, volumes of titrant. After each addition, record cumulative volume added and current reading when the signal stabilizes. If meter reading falls to near or below 10% of full-scale deflection, record low reading, readjust meter to near full-scale deflection, and record difference between low amount and readjusted high deflection. Add this value to all deflection readings for subsequent titrant additions. Continue adding titrant until no further meter deflection occurs. If fewer than three titrant additions were made before meter deflection ceased, discard sample and repeat analysis using smaller titrant increments.

Determine equivalence point by plotting total meter deflection against titrant volume added. Draw straight line through the first several points in the plot and a second, horizontal straight line corresponding to the final total deflection in the meter. Read equivalence point as the volume of titrant added at the intersection of these two lines.

5. Calculation

$$\text{mg Cl as } Cl_2/\text{L} = \frac{A \times 200 \times N}{B \times 0.00564}$$

where:

A = mL titrant at equivalence point,
B = sample volume, mL, and
N = phenylarsine oxide normality.

6. Bibliography

BROOKS, A. S. & G. L. SEEGERT. 1979. Low-level chlorine analysis by amperometric titration. *J. Water Pollut. Control Fed.* 51:2636.

4500-Cl F. DPD Ferrous Titrimetric Method

1. General Discussion

a. Principle: N,N-diethyl-*p*-phenylenediamine (DPD) is used as an indicator in the titrimetric procedure with ferrous ammonium sulfate (FAS). Where complete differentiation of chlorine species is not required, the procedure may be simplified to give only free and combined chlorine or total chlorine.

In the absence of iodide ion, free chlorine reacts instantly with DPD indicator to produce a red color. Subsequent addition of a small amount of iodide ion acts catalytically to cause monochloramine to produce color. Addition of iodide ion to excess evokes a rapid response from dichloramine. In the presence of iodide ion, part of the nitrogen trichloride (NCl_3) is included with dichloramine and part with free chlorine. A supplementary pro-

cedure based on adding iodide ion before DPD permits estimating proportion of NCl_3 appearing with free chlorine.

Chlorine dioxide (ClO_2) appears, to the extent of one-fifth of its total chlorine content, with free chlorine. A full response from ClO_2, corresponding to its total chlorine content, may be obtained if the sample first is acidified in the presence of iodide ion and subsequently is brought back to an approximately neutral pH by adding bicarbonate ion. Bromine, bromamine, and iodine react with DPD indicator and appear with free chlorine.

Addition of glycine before determination of free chlorine converts free chlorine to unreactive forms, with only bromine and iodine residuals remaining. Subtractions of these residuals from the residual measured without glycine permits differentiation of free chlorine from bromine and iodine.

b. pH control: For accurate results careful pH control is essential. At the proper pH of 6.2 to 6.5, the red colors produced may be titrated to sharp colorless end points. *Titrate as soon as the red color is formed in each step.* Too low a pH in the first step tends to make the monochloramine show in the free-chlorine step and the dichloramine in the monochloramine step. Too high a pH causes dissolved oxygen to give a color.

c. Temperature control: In all methods for differentiating free chlorine from chloramines, higher temperatures increase the tendency for chloramines to react and lead to increased apparent free-chlorine results. Higher temperatures also increase color fading. Complete measurements rapidly, especially at higher temperature.

d. Interference: The most significant interfering substance likely to be encountered in water is oxidized manganese. To correct for this, place 5 mL buffer solution and 0.5 mL sodium arsenite solution in the titration flask. Add 100 mL sample and mix. Add 5 mL DPD indicator solution, mix, and titrate with standard FAS titrant until red color is discharged. Subtract reading from Reading *A* obtained by the normal procedure as described in ¶ 3*a*1) of this method or from the total chlorine reading obtained in the simplified procedure given in ¶ 3*a*4). If the combined reagent in powder form (see below) is used, first add KI and arsenite to the sample and mix, then add combined buffer-indicator reagent.

As an alternative to sodium arsenite use a 0.25% solution of thioacetamide, adding 0.5 mL to 100 mL sample.

Interference by copper up to approximately 10 mg Cu/L is overcome by the EDTA incorporated in the reagents. EDTA enhances stability of DPD indicator solution by retarding deterioration due to oxidation, and in the test itself, provides suppression of dissolved oxygen errors by preventing trace metal catalysis.

Chromate in excess of 2 mg/L interferes with end-point determination. Add barium chloride to mask this interference by precipitation.

High concentrations of combined chlorine can break through into the free chlorine fraction. *If free chlorine is to be measured in the presence of more than 0.5 mg/L combined chlorine, use the thioacetamide modification.* If this modification is not used, a color-development time in excess of 1 min leads to progressively greater interference from monochloramine. Adding thioacetamide (0.5 mL 0.25% solution to 100 mL) immediately after mixing DPD reagent with sample completely stops further reaction with combined chlorine in the free chlorine measurement. Continue immediately with FAS titration to obtain free chlorine. Obtain total chlorine from the normal procedure, i.e., without thioacetamide.

Because high concentrations of iodide are used to measure combined chlorine and only traces of iodide greatly increase chloramine interference in free chlorine measurements, take care to avoid iodide contamination by rinsing between samples or using separate glassware.

See A.3 for a discussion of other interferences.

e. Minimum detectable concentration: Approximately 18 μg Cl as Cl_2/L. This detection limit is achievable under ideal conditions; normal working detection limits typically are higher.

2. Reagents

a. Phosphate buffer solution: Dissolve 24 g anhydrous Na_2HPO_4 and 46 g anhydrous KH_2PO_4 in distilled water. Combine with 100 mL distilled water in which 800 mg disodium ethylenediamine tetraacetate dihydrate (EDTA) have been dissolved. Dilute to 1 L with distilled water and optionally add either 20 mg $HgCl_2$ or 2 drops toluene to prevent mold growth. Interference from trace amounts of iodide in the reagents can be negated by optional addition of 20 mg $HgCl_2$ to the solution. (CAUTION: *$HgCl_2$ is toxic—take care to avoid ingestion.*)

b. N,N-Diethyl-p-phenylenediamine (DPD) indicator solution: Dissolve 1 g DPD oxalate,* or 1.5 g DPD sulfate pentahydrate,† or 1.1 g anhydrous DPD sulfate in chlorine-free distilled water containing 8 mL 1 + 3 H_2SO_4 and 200 mg disodium EDTA. Make up to 1 L, store in a brown glass-stoppered bottle in the dark, and discard when discolored. Periodically check solution blank for absorbance and discard when absorbance at 515 nm exceeds 0.002/cm. (The buffer and indicator sulfate are available commercially as a combined reagent in stable powder form.) CAUTION: *The oxalate is toxic—take care to avoid ingestion.*

c. Standard ferrous ammonium sulfate (FAS) titrant: Dissolve 1.106 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water containing 1 mL 1 + 3 H_2SO_4 and make up to 1 L with freshly boiled and cooled distilled water. This standard may be used for 1 month, and the titer checked by potassium dichromate. For this purpose add 10 mL 1 + 5 H_2SO_4, 5 mL conc H_3PO_4, and 2 mL 0.1% barium diphenylamine sulfonate indicator to a 100-mL sample of FAS and titrate with potassium dichromate to a violet end point that persists for 30 s. FAS titrant equivalent to 100 μg Cl as Cl_2/1.00 mL requires 20.00 mL dichromate for titration.

d. Potassium iodide, KI, crystals.

e. Potassium iodide solution: Dissolve 500 mg KI and dilute to 100 mL, using freshly boiled and cooled distilled water. Store in a brown glass-stoppered bottle, preferably in a refrigerator. Discard when solution becomes yellow.

f. Potassium dichromate solution, 0.691 g to 1000 mL.

g. Barium diphenylaminesulfonate, 0.1%: Dissolve 0.1 g $(C_6H_5NHC_6H_4\text{-}4\text{-}SO_3)_2Ba$ in 100 mL distilled water.

h. Sodium arsenite solution: Dissolve 5.0 g $NaAsO_2$ in distilled water and dilute to 1 L. (CAUTION: *Toxic—take care to avoid ingestion.*)

* Eastman chemical No. 7102 or equivalent.

† Available from Gallard-Schlesinger Chemical Mfg. Corp., 584 Mineloa Avenue, Carle Place, NY 11514, or equivalent.

i. Thioacetamide solution: Dissolve 250 mg CH_3CSNH_2 in 100 mL distilled water. (CAUTION: *Cancer suspect agent. Take care to avoid skin contact or ingestion.*)

j. Chlorine-demand-free water: See C.3*m*.

k. Glycine solution: Dissolve 20 g glycine (aminoacetic acid) in sufficient chlorine-demand-free water to bring to 100 mL total volume. Store under refrigerated conditions and discard if cloudiness develops.

l. Barium chloride crystals, $BaCl_2 \cdot 2H_2O$.

3. Procedure

The quantities given below are suitable for concentrations of total chlorine up to 5 mg/L. If total chlorine exceeds 5 mg/L, use a smaller sample and dilute to a total volume of 100 mL. Mix usual volumes of buffer reagent and DPD indicator solution, or usual amount of DPD powder, with distilled water *before* adding sufficient sample to bring total volume to 100 mL. (If sample is added before buffer, test does not work.)

If chromate is present (>2 mg/L) add and mix 0.2 g $BaCl_2 \cdot 2H_2O$/100 mL sample before adding other reagents. If, in addition, sulfate is >500 mg/L, use 0.4 g $BaCl_2 \cdot 2H_2O$/100 mL sample.

a. Free chlorine or chloramine: Place 5 mL each of buffer reagent and DPD indicator solution in titration flask and mix (or use about 500 mg DPD powder). Add 100 mL sample, or diluted sample, and mix.

1) Free chlorine—Titrate rapidly with standard FAS titrant until red color is discharged (Reading *A*).

2) Monochloramine—Add one very small crystal of KI (about 0.5 mg) or 0.1 mL (2 drops) KI solution and mix. Continue titrating until red color is discharged again (Reading *B*).

3) Dichloramine—Add several crystals KI (about 1 g) and mix to dissolve. Let stand for 2 min and continue titrating until red color is discharged (Reading *C*). For dichloramine concentrations greater than 1 mg/L, let stand 2 min more if color driftback indicates slightly incomplete reaction. When dichloramine concentrations are not expected to be high, use half the specified amount of KI.

4) Simplified procedure for free and combined chlorine or total chlorine—Omit 2) above to obtain monochloramine and dichloramine together as combined chlorine. To obtain total chlorine in one reading, add full amount of KI at the start, with the specified amounts of buffer reagent and DPD indicator, and titrate after 2 min standing.

b. Nitrogen trichloride: Place one very small crystal of KI (about 0.5 mg) or 0.1 mL KI solution in a titration flask. Add 100 mL sample and mix. Add contents to a second flask containing 5 mL each of buffer reagent and DPD indicator solution (or add about 500 mg DPD powder direct to the first flask). Titrate rapidly with standard FAS titrant until red color is discharged (Reading *N*).

c. Free chlorine in presence of bromine or iodine: Determine free chlorine as in ¶ 3*a*1). To a second 100-mL sample, add 1 mL glycine solution before adding DPD and buffer. Titrate according to ¶ 3*a*1). Subtract the second reading from the first to obtain Reading *A*.

4. Calculation

For a 100-mL sample, 1.00 mL standard FAS titrant = 1.00 mg Cl as Cl_2/L.

Reading	NCl_3 Absent	NCl_3 Present
A	Free Cl	Free Cl
$B - A$	NH_2Cl	NH_2Cl
$C - B$	$NHCl_2$	$NHCl_2 + \frac{1}{2}NCl_3$
N	—	Free Cl + $\frac{1}{2}NCl_3$
$2(N - A)$	—	NCl_3
$C - N$	—	$NHCl_2$

In the event that monochloramine is present with NCl_3, it will be included in *N*, in which case obtain NCl_3 from $2(N-B)$.

Chlorine dioxide, if present, is included in *A* to the extent of one-fifth of its total chlorine content.

In the simplified procedure for free and combined chlorine, only *A* (free Cl) and *C* (total Cl) are required. Obtain combined chlorine from $C-A$.

The result obtained in the simplified total chlorine procedure corresponds to *C*.

5. Precision and Bias

See B.5.

6. Bibliography

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4500-Cl G. DPD Colorimetric Method

1. General Discussion

a. Principle: This is a colorimetric version of the DPD method and is based on the same principles. Instead of titration with standard ferrous ammonium sulfate (FAS) solution as in the titrimetric method, a colorimetric procedure is used.

b. Interference: See A.3 and F.1*d*. Compensate for color and turbidity by using sample to zero photometer. Minimize chromate interference by using the thioacetamide blank correction.

c. Minimum detectable concentration: Approximately 10 μg Cl as Cl_2/L. This detection limit is achievable under ideal conditions; normal working detection limits typically are higher.

2. Apparatus

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

1) *Spectrophotometer*, for use at a wavelength of 515 nm and providing a light path of 1 cm or longer.

2) *Filter photometer*, equipped with a filter having maximum transmission in the wavelength range of 490 to 530 nm and providing a light path of 1 cm or longer.

b. Glassware: Use separate glassware, including separate spectrophotometer cells, for free and combined (dichloramine) measurements, to avoid iodide contamination in free chlorine measurement.

3. Reagents

See F.2*a*, *b*, *c*, *d*, *e*, *h*, *i*, and *j*.

4. Procedure

a. Calibration of photometric equipment: Calibrate instrument with chlorine or potassium permanganate solutions.

1) Chlorine solutions—Prepare chlorine standards in the range of 0.05 to 4 mg/L from about 100 mg/L chlorine water standardized as follows: Place 2 mL acetic acid and 10 to 25 mL chlorine-demand-free water in a flask. Add about 1 g KI. Measure into the flask a suitable volume of chlorine solution. In choosing a convenient volume, note that 1 mL 0.025*N* $Na_2S_2O_3$ titrant (see B.2*d*) is equivalent to about 0.9 mg chlorine. Titrate with standardized 0.025*N* $Na_2S_2O_3$ titrant until the yellow iodine color almost disappears. Add 1 to 2 mL starch indicator solution and continue titrating to disappearance of blue color.

Determine the blank by adding identical quantities of acid, KI, and starch indicator to a volume of chlorine-demand-free water corresponding to the sample used for titration. Perform blank titration A or B, whichever applies, according to B.3*d*.

$$\text{mg Cl as } Cl_2/\text{mL} = \frac{(A + B) \times N \times 35.45}{\text{mL sample}}$$

where:

N = normality of $Na_2S_2O_3$,
A = mL titrant for sample,
B = mL titrant for blank (to be added or subtracted according to required blank titration. See B.3*d*).

Use chlorine-demand-free water and glassware to prepare these standards. Develop color by first placing 5 mL phosphate buffer solution and 5 mL DPD indicator reagent in flask and then adding 100 mL chlorine standard with thorough mixing as described in *b* and *c* below. Fill photometer or colorimeter cell from flask and read color at 515 nm. Return cell contents to flask and titrate with standard FAS titrant as a check on chlorine concentration.

2) Potassium permanganate solutions—Prepare a stock solution containing 891 mg $KMnO_4$/1000 mL. Dilute 10.00 mL stock solution to 100 mL with distilled water in a volumetric flask. When 1 mL of this solution is diluted to 100 mL with distilled water, a chlorine equivalent of 1.00 mg/L will be produced in the DPD reaction. Prepare a series of $KMnO_4$ standards covering the chlorine equivalent range of 0.05 to 4 mg/L. Develop color by first placing 5 mL phosphate buffer and 5 mL DPD indicator reagent in flask and adding 100 mL standard with thorough mixing as described in *b* and *c* below. Fill photometer or colorimeter cell from flask and read color at 515 nm. Return cell contents to flask and titrate with FAS titrant as a check on any absorption of permanganate by distilled water.

Obtain all readings by comparison to color standards or the standard curve before use in calculation.

b. Volume of sample: Use a sample volume appropriate to the photometer or colorimeter. The following procedure is based on using 10-mL volumes; adjust reagent quantities proportionately for other sample volumes. Dilute sample with chlorine-demand-free water when total chlorine exceeds 4 mg/L.

c. Free chlorine: Place 0.5 mL each of buffer reagent and DPD indicator reagent in a test tube or photometer cell. Add 10 mL sample and mix. Read color immediately (Reading *A*).

d. Monochloramine: Continue by adding one very small crystal of KI (about 0.1 mg) and mix. If dichloramine concentration is expected to be high, instead of small crystal add 0.1 mL (2 drops) freshly prepared KI solution (0.1 g/100 mL). Read color immediately (Reading *B*).

e. Dichloramine: Continue by adding several crystals of KI (about 0.1 g) and mix to dissolve. Let stand about 2 min and read color (Reading *C*).

f. Nitrogen trichloride: Place a very small crystal of KI (about 0.1 mg) in a clean test tube or photometer cell. Add 10 mL sample and mix. To a second tube or cell add 0.5 mL each of buffer and indicator reagents; mix. Add contents to first tube or cell and mix. Read color immediately (Reading *N*).

g. Chromate correction using thioacetamide: Add 0.5 mL thioacetamide solution (F.2*i*) to 100 mL sample. After mixing, add buffer and DPD reagent. Read color immediately. Add several crystals of KI (about 0.1 g) and mix to dissolve. Let stand about 2 min and read color. Subtract the first reading from Reading *A* and the second reading from Reading *C* and use in calculations.

h. Simplified procedure for total chlorine: Omit Step *d* above to obtain monochloramine and dichloramine together as combined chlorine. To obtain total chlorine in one reading, add the full amount of KI at the start, with the specified amounts of buffer reagent and DPD indicator. Read color after 2 min.

5. Calculation

Reading	NCl_3 Absent	NCl_3 Present
A	Free Cl	Free Cl
$B - A$	NH_2Cl	NH_2Cl
$C - B$	$NHCl_2$	$NHCl_2 + \frac{1}{2}NCl_3$
N	—	Free Cl + $\frac{1}{2}NCl_3$
$2(N - A)$	—	NCl_3
$C - N$	—	$NHCl_2$

In the event that monochloramine is present with NCl_3, it will be included in Reading N, in which case obtain NCl_3 from $2(N-B)$.

6. Bibliography

See F.6.

4500-Cl H. Syringaldazine (FACTS) Method

1. General Discussion

a. Principle: The free (available) chlorine test, syringaldazine (FACTS) measures free chlorine over the range of 0.1 to 10 mg/L. A saturated solution of syringaldazine (3,5-dimethoxy-4-hydroxybenzaldazine) in 2-propanol is used. Syringaldazine is oxidized by free chlorine on a 1:1 molar basis to produce a colored product with an absorption maximum of 530 nm. The color product is only slightly soluble in water; therefore, at chlorine concentrations greater than 1 mg/L, the final reaction mixture must contain 2-propanol to prevent product precipitation and color fading.

The optimum color and solubility (minimum fading) are obtained in a solution having a pH between 6.5 and 6.8. At a pH less than 6, color development is slow and reproducibility is poor. At a pH greater than 7, the color develops rapidly but fades quickly. A buffer is required to maintain the reaction mixture pH at approximately 6.7. Take care with waters of high acidity or alkalinity to assure that the added buffer maintains the proper pH.

Temperature has a minimal effect on the color reaction. The maximum error observed at temperature extremes of 5 and 35°C is ± 10%.

b. Interferences: Interferences common to other methods for determining free chlorine do not affect the FACTS procedure. Monochloramine concentrations up to 18 mg/L, dichloramine concentrations up to 10 mg/L, and manganese concentrations (oxidized forms) up to 1 mg/L do not interfere. Trichloramine at levels above 0.6 mg/L produces an apparent free chlorine reaction. Very high concentrations of monochloramine (≥35 mg/L) and oxidized manganese (≥2.6 mg/L) produce a color with syringaldazine slowly. Ferric iron can react with syringaldazine; however, concentrations up to 10 mg/L do not interfere. Nitrite (≤250 mg/L), nitrate (≤100 mg/L), sulfate (≤1000 mg/L), and chloride (≤1000 mg/L) do not interfere. Waters with high hardness (≥500 mg/L) will produce a cloudy solution that can be compensated for by using a blank. Oxygen does not interfere.

Other strong oxidizing agents, such as iodine, bromine, and ozone, will produce a color.

c. Minimum detectable concentration: The FACTS procedure is sensitive to free chlorine concentrations of 0.1 mg/L or less.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Filter photometer, providing a light path of 1 cm for chlorine concentrations ≤1 mg/L or a light path from 1 to 10 mm for chlorine concentrations above 1 mg/L; also equipped with a filter having a band pass of 500 to 560 nm.

b. Spectrophotometer, for use at 530 nm, providing the light paths noted above.

3. Reagents

a. Chlorine-demand-free water: See C.3*m*. Use to prepare reagent solutions and sample dilutions.

b. Syringaldazine indicator: Dissolve 115 mg 3,5-dimethoxy-4-hydroxybenzaldazine* in 1 L 2-propanol.

c. 2-Propanol: To aid in dissolution use ultrasonic agitation or gentle heating and stirring. Redistill reagent-grade 2-propanol to remove chlorine demand. Use a 30.5-cm Vigreux column and take the middle 75% fraction. Alternatively, chlorinate good-quality 2-propanol to maintain a free residual overnight; then expose to UV light or sunlight to dechlorinate. CAUTION: *2-Propanol is extremely flammable.*

d. Buffer: Dissolve 17.01 g KH_2PO_4 in 250 mL water; pH should be 4.4. Dissolve 17.75 g Na_2HPO_4 in 250 mL water; the pH should be 9.9. Mix equal volumes of these solutions to obtain FACTS buffer, pH 6.6. Verify pH with pH meter. For waters containing considerable hardness or high alkalinity other pH 6.6 buffers can be used, for example, 23.21 g maleic acid and 16.5 mL 50% NaOH per liter of water.

e. Hypochlorite solution: Dilute household hypochlorite solution, which contains about 30 000 to 50 000 mg Cl equivalent/L, to a strength between 100 and 1000 mg/L. Standardize as directed in G.4*a*1).

4. Procedure

a. Calibration of photometer: Prepare a calibration curve by making dilutions of a standardized hypochlorite solution (¶ 3*e*).

* Aldrich No. 17, 753-9, Aldrich Chemical Company, Inc., 1001 West St. Paul Ave., Milwaukee, WI 53233, or equivalent.

Develop and measure colors as described in ¶ 4*b*, below. Check calibration regularly, especially as reagent ages.

b. Free chlorine analysis: Add 3 mL sample and 0.1 mL buffer to a 5-mL-capacity test tube. Add 1 mL syringaldazine indicator, cap tube, and invert twice to mix. Transfer to a photometer tube or spectrophotometer cell and measure absorbance. Compare absorbance value obtained with calibration curve and report corresponding value as milligrams free chlorine per liter.

5. Bibliography

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4500-Cl I. Iodometric Electrode Technique

1. General Discussion

a. Principle: This method involves the direct potentiometric measurement of iodine released on the addition of potassium iodide to an acidified sample. A platinum-iodide electrode pair is used in combination with an expanded-scale pH meter.

b. Interference: All oxidizing agents that interfere with other iodometric procedures interfere. These include oxidized manganese and iodate, bromine, and cupric ions. Silver and mercuric ions above 10 and 20 mg/L interfere.

2. Apparatus

a. Electrodes: Use either a combination electrode consisting of a platinum electrode and an iodide ion-selective electrode or two individual electrodes. Both systems are available commercially.

b. pH/millivolt meter: Use an expanded-scale pH/millivolt meter with 0.1 mV readability or a direct-reading selective ion meter.

3. Reagents

a. pH 4 buffer solution: See C.3*e*.

b. Chlorine-demand-free water: See C.3*m*.

c. Potassium iodide solution: Dissolve 42 g KI and 0.2 g Na_2CO_3 in 500 mL chlorine-demand-free, distilled water. Store in a dark bottle.

d. Standard potassium iodate 0.002 81*N:* Dissolve 0.1002 g KIO_3 in chlorine-demand-free, distilled water and dilute to 1000 mL. Each 1.0 mL, when diluted to 100 mL, produces a solution equivalent to 1 mg/L as Cl_2.

4. Procedure

a. Standardization: Pipet into three 100-mL stoppered volumetric flasks 0.20, 1.00, and 5.00 mL standard iodate solution. Add to each flask, and a fourth flask to be used as a reagent blank, 1 mL each of acetate buffer solution and KI solution. Stopper, swirl to mix, and let stand 2 min before dilution. Dilute each standard to 100 mL with chlorine-demand-free distilled water. Stopper, invert flask several times to mix, and pour into separate 150-mL beakers. Stir gently without turbulence, using a magnetic stirrer, and immerse electrode(s) in the 0.2-mg/L (0.2-mL) standard. Wait for the potential to stabilize and record potential in mV. Rinse electrodes with chlorine-demand-free water and repeat for each standard and for the reagent blank. Prepare a calibration curve by plotting, on semilogarithmic paper, potential (linear axis) against concentration. Determine apparent chlorine concentration in the reagent blank from this graph (Reading B).

b. Analysis: Select a volume of sample containing no more than 0.5 mg chlorine. Pipet 1 mL acetate buffer solution and 1 mL KI into a 100-mL glass-stoppered volumetric flask. Stopper, swirl and let stand for at least 2 min. Adjust sample pH to 4 to 5, if necessary (mid-range pH paper is adequate for pH measurement), by adding acetic acid. Add pH-adjusted sample to volumetric flask and dilute to mark. Stopper and mix by inversion several times. Let stand for 2 min. Pour into a 150-mL beaker, immerse the electrode(s), wait for the potential to stabilize, and record. If the mV reading is greater than that recorded for the 5-mg/L standard, repeat analysis with a smaller volume of sample.

5. Calculation

Determine chlorine concentration (mg/L) corresponding to the recorded mV reading from the standard curve. This is Reading *A*. Determine total residual chlorine from the following:

$$\text{Total residual chlorine} = A \times 100/V$$

where V = sample volume, mL. If total residual chlorine is below 0.2 mg/L, subtract apparent chlorine in reagent blank (Reading *B*) to obtain the true total residual chlorine value.

6. Bibliography

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4500-Cl^- CHLORIDE*

4500-Cl^- A. Introduction

1. Occurrence

Chloride, in the form of chloride (Cl^-) ion, is one of the major inorganic anions in water and wastewater. The salty taste produced by chloride concentrations is variable and dependent on the chemical composition of water. Some waters containing 250 mg Cl^-/L may have a detectable salty taste if the cation is sodium. On the other hand, the typical salty taste may be absent in waters containing as much as 1000 mg/L when the predominant cations are calcium and magnesium.

The chloride concentration is higher in wastewater than in raw water because sodium chloride (NaCl) is a common article of diet and passes unchanged through the digestive system. Along the sea coast, chloride may be present in high concentrations because of leakage of salt water into the sewerage system. It also may be increased by industrial processes.

A high chloride content may harm metallic pipes and structures, as well as growing plants.

2. Selection of Method

Six methods are presented for the determination of chloride. Because the first two are similar in most respects, selection is largely a matter of personal preference. The argentometric method (B) is suitable for use in relatively clear waters when 0.15 to 10 mg Cl^- are present in the portion titrated. The end point of the mercuric nitrate method (C) is easier to detect. The potentiometric method (D) is suitable for colored or turbid samples in which color-indicated end points might be difficult to observe. The potentiometric method can be used without a pretreatment step for samples containing ferric ions (if not present in an amount greater than the chloride concentration), chromic, phosphate, and ferrous and other heavy-metal ions. The ferricyanide method (E) is an automated technique. Flow injection analysis (G), an automated colorimetric technique, is useful for analyzing large numbers of samples. Preferably determine chloride by ion chromatography (Section 4110). Chloride also can be determined by the capillary ion electrophoresis method (Section 4140). Methods (C and G) in which mercury, a highly toxic reagent, is used require special disposal practices to avoid improper sewage discharges. Follow appropriate regulatory procedures (see Section 1090).

3. Sampling and Storage

Collect representative samples in clean, chemically resistant glass or plastic bottles. The maximum sample portion required is 100 mL. No special preservative is necessary if the sample is to be stored.

* Approved by Standard Methods Committee, 1997.
Joint Task Group: 20th Edition (4500-Cl^-.G)—Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

4500-Cl^- B. Argentometric Method

1. General Discussion

a. Principle: In a neutral or slightly alkaline solution, potassium chromate can indicate the end point of the silver nitrate titration of chloride. Silver chloride is precipitated quantitatively before red silver chromate is formed.

b. Interference: Substances in amounts normally found in potable waters will not interfere. Bromide, iodide, and cyanide register as equivalent chloride concentrations. Sulfide, thiosulfate, and sulfite ions interfere but can be removed by treatment with hydrogen peroxide. Orthophosphate in excess of 25 mg/L interferes by precipitating as silver phosphate. Iron in excess of 10 mg/L interferes by masking the end point.

2. Apparatus

a. Erlenmeyer flask, 250-mL.

b. Buret, 50-mL.

3. Reagents

a. Potassium chromate indicator solution: Dissolve 50 g K_2CrO_4 in a little distilled water. Add $AgNO_3$ solution until a definite red precipitate is formed. Let stand 12 h, filter, and dilute to 1 L with distilled water.

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

c. Standard sodium chloride, 0.0141*M* (0.0141*N*): Dissolve 824.0 mg NaCl (dried at 140°C) in distilled water and dilute to 1000 mL; 1.00 mL = 500 μg Cl^-.

d. Special reagents for removal of interference:

1) *Aluminum hydroxide suspension:* Dissolve 125 g aluminum potassium sulfate or aluminum ammonium sulfate, $AlK(SO_4)_2 \cdot 12H_2O$ or $AlNH_4(SO_4)_2 \cdot 12H_2O$, in 1 L distilled water. Warm to 60°C and add 55 mL conc ammonium hydroxide (NH_4OH) slowly with stirring. Let stand about 1 h, transfer to a

large bottle, and wash precipitate by successive additions, with thorough mixing and decanting with distilled water, until free from chloride. When freshly prepared, the suspension occupies a volume of approximately 1 L.

2) *Phenolphthalein indicator solution.*

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

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

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

4. Procedure

a. Sample preparation: Use a 100-mL sample or a suitable portion diluted to 100 mL. If the sample is highly colored, add 3 mL $Al(OH)_3$ suspension, mix, let settle, and filter.

If sulfide, sulfite, or thiosulfate is present, add 1 mL H_2O_2 and stir for 1 min.

b. Titration: Directly titrate samples in the pH range 7 to 10. Adjust sample pH to 7 to 10 with H_2SO_4 or NaOH if it is not in this range. For adjustment, preferably use a pH meter with a non-chloride-type reference electrode. (If only a chloride-type electrode is available, determine amount of acid or alkali needed for adjustment and discard this sample portion. Treat a separate portion with required acid or alkali and continue analysis.) Add 1.0 mL K_2CrO_4 indicator solution. Titrate with standard $AgNO_3$ titrant to a pinkish yellow end point. Be consistent in end-point recognition.

Standardize $AgNO_3$ titrant and establish reagent blank value by the titration method outlined above. A blank of 0.2 to 0.3 mL is usual.

5. Calculation

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

where:

A = mL titration for sample,
B = mL titration for blank, and
N = normality of $AgNO_3$.

$$\text{mg NaCl/L} = (\text{mg } Cl^-/L) \times 1.65$$

6. Precision and Bias

A synthetic sample containing 241 mg Cl^-/L, 108 mg Ca/L, 82 mg Mg/L; 3.1 mg K/L, 19.9 mg Na/L, 1.1 mg NO_3^--N/L, 0.25 mg NO_2^-- N/L, 259 mg SO_4^{2-}/L, and 42.5 mg total alkalinity/L (contributed by $NaHCO_3$) in distilled water was analyzed in 41 laboratories by the argentometric method, with a relative standard deviation of 4.2% and a relative error of 1.7%.

7. Bibliography

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PAUSTIAN, P. 1987. A novel method to calculate the Mohr chloride titration. *In* Advances in Water Analysis and Treatment, Proc. 14th Annu. AWWA Water Quality Technology Conf., November 16-20, 1986, Portland, Ore., p. 673. American Water Works Assoc., Denver, Colo.

4500-Cl⁻ C. Mercuric Nitrate Method

1. General Discussion

a. Principle: Chloride can be titrated with mercuric nitrate, $Hg(NO_3)_2$, because of the formation of soluble, slightly dissociated mercuric chloride. In the pH range 2.3 to 2.8, diphenylcarbazone indicates the titration end point by formation of a purple complex with the excess mercuric ions. Xylene cyanol FF serves as a pH indicator and end-point enhancer. Increasing the strength of the titrant and modifying the indicator mixtures extend the range of measurable chloride concentrations.

b. Interference: Bromide and iodide are titrated with $Hg(NO_3)_2$ in the same manner as chloride. Chromate, ferric, and sulfite ions interfere when present in excess of 10 mg/L.

2. Apparatus

a. Erlenmeyer flask, 250-mL.

b. Microburet, 5-mL with 0.01-mL graduation intervals.

3. Reagents

a. Standard sodium chloride, 0.0141*M* (0.0141*N*): See Method B, ¶ 3*c* above.

b. Nitric acid, HNO_3, 0.1*N*.

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

d. Reagents for chloride concentrations below 100 mg/L:

1) *Indicator-acidifier reagent:* The HNO_3 concentration of this reagent is an important factor in the success of the determination and can be varied as indicated in a) or b) to suit the alkalinity range of the sample. Reagent a) contains sufficient HNO_3 to neutralize a total alkalinity of 150 mg as $CaCO_3$/L to the proper pH in a 100-mL sample. Adjust amount of HNO_3 to accommodate samples of alkalinity different from 150 mg/L.

a) Dissolve, in the order named, 250 mg s-diphenylcarbazone, 4.0 mL conc HNO_3, and 30 mg xylene cyanol FF in 100 mL 95% ethyl alcohol or isopropyl alcohol. Store in a dark bottle in a

refrigerator. This reagent is not stable indefinitely. Deterioration causes a slow end point and high results.

b) Because pH control is critical, adjust pH of highly alkaline or acid samples to 2.5 ± 0.1 with 0.1*N* HNO_3 or NaOH, not with sodium carbonate (Na_2CO_3). Use a pH meter with a nonchloride type of reference electrode for pH adjustment. If only the usual chloride-type reference electrode is available for pH adjustment, determine amount of acid or alkali required to obtain a pH of 2.5 ± 0.1 and discard this sample portion. Treat a separate sample portion with the determined amount of acid or alkali and continue analysis. Under these circumstances, omit HNO_3 from indicator reagent.

2) *Standard mercuric nitrate titrant,* 0.007 05*M* (0.0141*N*): Dissolve 2.3 g $Hg(NO_3)_2$ or 2.5 g $Hg(NO_3)_2 \cdot H_2O$ in 100 mL distilled water containing 0.25 mL conc HNO_3. Dilute to just under 1 L. Make a preliminary standardization by following the procedure described in ¶ 4*a*. Use replicates containing 5.00 mL standard NaCl solution and 10 mg sodium bicarbonate ($NaHCO_3$) diluted to 100 mL with distilled water. Adjust titrant to 0.0141*N* and make a final standardization; 1.00 mL = 500 μg Cl^-. Store away from light in a dark bottle.

e. Reagent for chloride concentrations greater than 100 mg/L:

1) *Mixed indicator reagent:* Dissolve 0.50 g diphenylcarbazone powder and 0.05 g bromphenol blue powder in 75 mL 95% ethyl or isopropyl alcohol and dilute to 100 mL with the same alcohol.

2) *Strong standard mercuric nitrate titrant,* 0.0705*M* (0.141*N*): Dissolve 25 g $Hg(NO_3)_2 \cdot H_2O$ in 900 mL distilled water containing 5.0 mL conc HNO_3. Dilute to just under 1 L and standardize by following the procedure described in ¶ 4*b*. Use replicates containing 25.00 mL standard NaCl solution and 25 mL distilled water. Adjust titrant to 0.141*N* and make a final standardization; 1.00 mL = 5.00 mg Cl^-.

4. Procedure

a. Titration of chloride concentrations less than 100 mg/L: Use a 100-mL sample or smaller portion so that the chloride content is less than 10 mg.

Add 1.0 mL indicator-acidifier reagent. (The color of the solution should be green-blue at this point. A light green indicates pH less than 2.0; a pure blue indicates pH more than 3.8.) For most potable waters, the pH after this addition will be 2.5 ± 0.1. For highly alkaline or acid waters, adjust pH to about 8 before adding indicator-acidifier reagent.

Titrate with 0.0141*N* $Hg(NO_3)_2$ titrant to a definite purple end point. The solution turns from green-blue to blue a few drops before the end point.

Determine blank by titrating 100 mL distilled water containing 10 mg $NaHCO_3$.

b. Titration of chloride concentrations greater than 100 mg/L: Use a sample portion (5 to 50 mL) requiring less than 5 mL titrant to reach the end point. Measure into a 150-mL beaker. Add approximately 0.5 mL mixed indicator reagent and mix well. The color should be purple. Add 0.1*N* HNO_3 dropwise until the color just turns yellow. Titrate with strong $Hg(NO_3)_2$ titrant to first permanent dark purple. Titrate a distilled water blank using the same procedure.

5. Calculation

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

where:

A = mL titration for sample,
B = mL titration for blank, and
N = normality of $Hg(NO_3)_2$.

$$\text{mg NaCl/L} = (\text{mg } Cl^-/L) \times 1.65$$

6. Precision and Bias

A synthetic sample containing 241 mg Cl^-/L, 108 mg Ca/L, 82 mg Mg/L, 3.1 mg K/L, 19.9 mg Na/L, 1.1 mg NO_3^--N/L, 0.25 mg NO_2^--N/L, 259 mg SO_4^{2-}/L, and 42.5 mg total alkalinity/L (contributed by $NaHCO_3$) in distilled water was analyzed in 10 laboratories by the mercurimetric method, with a relative standard deviation of 3.3% and a relative error of 2.9%.

7. Bibliography

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DOMASK, W.C. & K.A. KOBE. 1952. Mercurimetric determination of chlorides and water-soluble chlorohydrins. *Anal. Chem.* 24:989.

GOLDMAN, E. 1959. New indicator for the mercurimetric chloride determination in potable water. *Anal. Chem.* 31:1127.

4500-Cl⁻ D. Potentiometric Method

1. General Discussion

a. Principle: Chloride is determined by potentiometric titration with silver nitrate solution with a glass and silver-silver chloride electrode system. During titration an electronic voltmeter is used to detect the change in potential between the two electrodes. The end point of the titration is that instrument reading at which the greatest change in voltage has occurred for a small and constant increment of silver nitrate added.

b. Interference: Iodide and bromide also are titrated as chloride. Ferricyanide causes high results and must be removed. Chromate and dichromate interfere and should be reduced to the chromic state or removed. Ferric iron interferes if present in an amount substantially higher than the amount of chloride. Chromic ion, ferrous ion, and phosphate do not interfere.

Grossly contaminated samples usually require pretreatment. Where contamination is minor, some contaminants can be destroyed simply by adding nitric acid.

2. Apparatus

a. Glass and silver-silver chloride electrodes: Prepare in the laboratory or purchase a silver electrode coated with AgCl for use with specified instruments. Instructions on use and care of electrodes are supplied by the manufacturer.

b. Electronic voltmeter, to measure potential difference between electrodes: A pH meter may be converted to this use by substituting the appropriate electrode.

c. Mechanical stirrer, with plastic-coated or glass impeller.

3. Reagents

a. Standard sodium chloride solution, 0.0141*M* (0.0141*N*): See ¶ 4500-Cl⁻.B.3*c*.

b. Nitric acid, HNO_3, conc.

c. Standard silver nitrate titrant, 0.0141*M* (0.0141*N*): See ¶ 4500-Cl⁻.B.3*b*.

d. Pretreatment reagents:

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

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

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

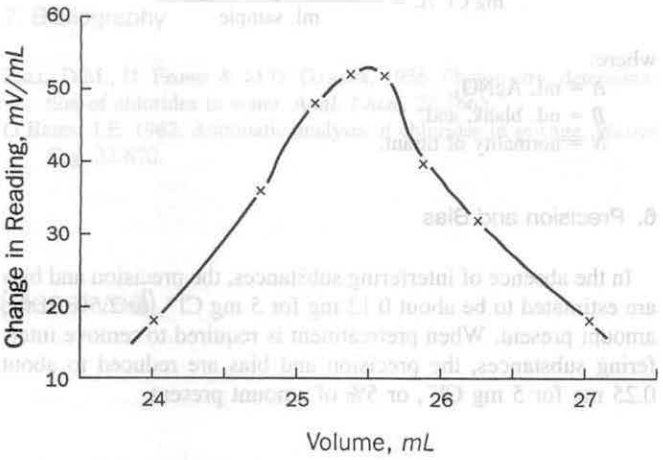
4. Procedure

a. Standardization: The various instruments that can be used in this determination differ in operating details; follow the manufacturer's instructions. Make necessary mechanical adjustments. Then, after allowing sufficient time for warmup (10 min), balance internal electrical components to give an instrument setting of 0 mV or, if a pH meter is used, a pH reading of 7.0.

1) Place 10.0 mL standard NaCl solution in a 250-mL beaker, dilute to about 100 mL, and add 2.0 mL conc HNO_3. Immerse stirrer and electrodes.

2) Set instrument to desired range of millivolts or pH units. Start stirrer.

3) Add standard $AgNO_3$ titrant, recording scale reading after each addition. At the start, large increments of $AgNO_3$ may be added; then, as the end point is approached, add smaller and equal increments (0.1 or 0.2 mL) at longer intervals, so that the exact end point can be determined. Determine volume of $AgNO_3$ used at the point at which there is the greatest change in instrument reading per unit addition of $AgNO_3$.



Experimental Data Plotted Above

Volume, mL	23.50	24.50	25.00	25.25	25.50	25.75	26.00	26.50	27.50
Change, mV/mL	18	36	48	52	52	40	32	18	

Figure 4500-Cl⁻:1. Example of differential titration curve (end point is 25.5 mL).

4) Plot a differential titration curve if the exact end point cannot be determined by inspecting the data. Plot change in instrument reading for equal increments of $AgNO_3$ against volume of $AgNO_3$ added, using average of buret readings before and after each addition. The procedure is illustrated in Figure 4500-Cl⁻:1.

b. Sample analysis:

1) Pipet 100.0 mL sample, or a portion containing not more than 10 mg Cl⁻, into a 250-mL beaker. In the absence of interfering substances, proceed with ¶ 3) below.

2) In the presence of organic compounds, sulfite, or other interferences (such as large amounts of ferric iron, cyanide, or sulfide) acidify sample with H_2SO_4, using litmus paper. Boil for 5 min to remove volatile compounds. Add more H_2SO_4, if necessary, to keep solution acidic. Add 3 mL H_2O_2 and boil for 15 min, adding chloride-free distilled water to keep the volume above 50 mL. Dilute to 100 mL, add NaOH solution dropwise until alkaline to litmus, then 10 drops in excess. Boil for 5 min, filter into a 250-mL beaker, and wash precipitate and paper several times with hot distilled water.

3) Add conc HNO_3 dropwise until acidic to litmus paper, then 2.0 mL in excess. Cool and dilute to 100 mL if necessary. Immerse stirrer and electrodes and start stirrer. Make any necessary adjustments according to the manufacturer's instructions and set selector switch to appropriate setting for measuring the difference of potential between electrodes.

4) Complete determination by titrating according to ¶ 4*a*4). If an end-point reading has been established from previous determinations for similar samples and conditions, use this predetermined end point. For the most accurate work, make a blank titration by carrying chloride-free distilled water through the procedure.

5. Calculation

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

where:

A = mL $AgNO_3$,
B = mL blank, and
N = normality of titrant.

6. Precision and Bias

In the absence of interfering substances, the precision and bias are estimated to be about 0.12 mg for 5 mg Cl^-, or 2.5% of the amount present. When pretreatment is required to remove interfering substances, the precision and bias are reduced to about 0.25 mg for 5 mg Cl^-, or 5% of amount present.

7. Bibliography

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4500-Cl^- E. Automated Ferricyanide Method

1. General Discussion

a. Principle: Thiocyanate ion is liberated from mercuric thiocyanate by the formation of soluble mercuric chloride. In the presence of ferric ion, free thiocyanate ion forms a highly colored ferric thiocyanate, of which the intensity is proportional to the chloride concentration.

b. Interferences: Remove particulate matter by filtration or centrifugation before analysis. Guard against contamination from reagents, water, glassware, and sample preservation process. No chemical interferences are significant.

c. Application: The method is applicable to potable, surface, and saline waters, and domestic and industrial wastewaters. The concentration range is 1 to 200 mg Cl^-/L; it can be extended by dilution.

2. Apparatus

a. Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-Cl^-:2.

b. Filters, 480-nm.

3. Reagents

a. Stock mercuric thiocyanate solution: Dissolve 4.17 g $Hg(SCN)_2$ in about 500 mL methanol, dilute to 1000 mL with methanol, mix, and filter through filter paper.

b. Stock ferric nitrate solution: Dissolve 202 g $Fe(NO_3)_3 \cdot 9H_2O$ in about 500 mL distilled water, then carefully add 21 mL conc HNO_3. Dilute to 1000 mL with distilled water and mix. Filter through paper and store in an amber bottle.

c. Color reagent: Add 150 mL stock $Hg(SCN)_2$ solution to 150 mL stock $Fe(NO_3)_3$ solution. Mix and dilute to 1000 mL with distilled water. Add 0.5 mL polyoxyethylene 23 lauryl ether.*

* Brij 35, available from ICI Americas, Wilmington, DE, or equivalent.

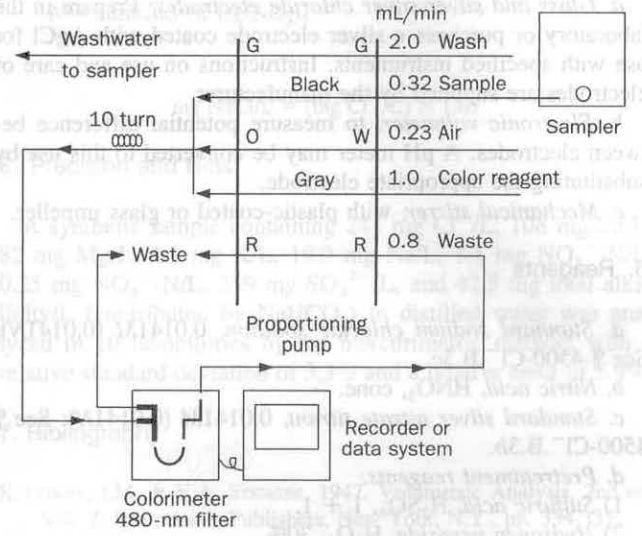

Figure 4500-Cl^-:2. Flow scheme for automated chloride analysis.

d. Stock chloride solution: Dissolve 1.6482 g NaCl, dried at 140°C, in distilled water and dilute to 1000 mL; 1.00 mL = 1.00 mg Cl^-.

e. Standard chloride solutions: Prepare chloride standards in the desired concentration range, such as 1 to 200 mg/L, using stock chloride solution.

4. Procedure

Set up manifold as shown in Figure 4500-Cl^-:2 and follow general procedure described by the manufacturer.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against chloride concentrations

in standards. Compute sample chloride concentration by comparing sample response with standard curve.

6. Precision and Bias

With an automated system in a single laboratory six samples were analyzed in septuplicate. At a concentration ranging from about 1 to 50 mg Cl^-/L the average standard deviation was 0.39 mg/L. The coefficient of variation was 2.2%. In two samples with added chloride, recoveries were 104% and 97%.

7. Bibliography

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4500-Cl⁻ F. (Reserved)

4500-Cl⁻ G. Mercuric Thiocyanate Flow Injection Analysis

1. General Discussion

a. Principle: A water sample containing chloride is injected into a carrier stream to which mercuric thiocyanate and ferric nitrate are added. The chloride complexes with the Hg(II), displacing the thiocyanate anion, which forms the highly colored ferric thiocyanate complex anion. The resulting peak's absorbance is measured at 480 nm. The peak area is proportional to the concentration of chloride in the original sample.

Also see Section 4500-Cl⁻.A and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Substances such as sulfite and thiosulfate, which reduce iron(III) to iron(II) and mercury(II) to mercury(I), can interfere. Halides, which also form strong complexes with mercuric ion (e.g., Br^-, I^-), give a positive interference.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop.

b. Multichannel proportioning pump.

c. FIA manifold with flow cell (Figure 4500-Cl⁻:3). Relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*

d. Absorbance detector, 480 nm, 10-nm bandpass.

e. Valve control and data acquisition system.

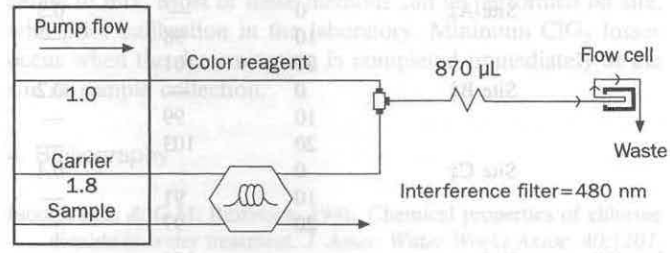

Figure 4500-Cl⁻:3. FIA chloride manifold.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions.

a. Stock mercuric thiocyanate solution: In a 1-L volumetric flask, dissolve 4.17 g mercuric thiocyanate, $Hg(SCN)_2$, in about 500 mL methanol. Dilute to mark with methanol and mix. CAUTION: *Mercuric thiocyanate is toxic. Wear gloves!*

b. Stock ferric nitrate reagent, 0.5*M*: In a 1-L volumetric flask, dissolve 202 g ferric nitrate, $Fe(NO_3)_3 \cdot 9H_2O$, in approximately 800 mL water. Add 25 mL conc HNO_3 and dilute to mark. Invert to mix.

c. Color reagent: In a 500-mL volumetric flask, mix 75 mL stock mercuric thiocyanate solution with 75 mL stock ferric nitrate reagent and dilute to mark with water. Invert to mix. Vacuum filter through a 0.45-µm membrane filter. The color reagent also is available as a commercially prepared solution that is stable for several months.

d. Stock chloride standard, 1000 mg Cl^-/L: In a 105°C oven, dry 3 g primary standard grade sodium chloride, NaCl, overnight. In a 1-L volumetric flask, dissolve 1.648 g primary standard grade sodium chloride in about 500 mL water. Dilute to mark and invert to mix.

* Teflon or equivalent.

TABLE 4500-Cl⁻:I. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg Cl⁻/L	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	101	—
	Blank†	10	104	—
		20	102	—
	Site A‡	0	—	0.4
		10	92	—
		20	101	—
	Site B‡	0	—	0.2
		10	97	—
		20	106	—
	Site C‡	0	—	0.4
		10	102	—
		20	102	—
Wastewater treatment plant effluent	Reference sample*	—	101	—
	Blank†	10	104	—
		20	102	—
	Site A‡	0	—	0.3
		10	98	—
		20	101	—
	Site B‡	0	—	0.2
		10	99	—
		20	103	—
	Site C‡	0	—	0.4
		10	91	—
		20	97	—
Landfill leachate	Reference sample*	—	100	—
	Blank†	10	101	—
		20	100	—
	Site A§	0	—	0.3
		10	97	—
		20	103	—
	Site B§	0	—	0.2
		10	89	—
		20	103	—
	Site C§	0	—	0.5
		10	89	—
		20	103	—

* U.S. EPA nutrient QC sample, 51.7 mg Cl⁻/L.
† Determined in duplicate.
‡ Samples diluted 5-fold. Samples without known additions determined four times; samples with known additions determined in duplicate. Typical relative difference between duplicates 0.2%.
§ Sample from Site A diluted 50-fold, those from B and C 100-fold. Samples without known additions determined four times; samples with known additions determined in duplicate; typical relative difference between duplicates 0.5%.

e. Standard chloride solutions: Prepare chloride standards for the calibration curve in the desired concentration range, using the stock standard (¶ 3*d*), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-Cl⁻:3 and follow method supplied by manufacturer, or laboratory standard operating procedure for this method. Follow quality control procedures described in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus chloride concentration. The calibration curve gives a good fit to a second-order polynomial.

6. Precision and Bias

a. Recovery and relative standard deviation: The results of single-laboratory studies with various matrices are given in Table 4500-Cl⁻:I.

b. MDL: A 100-μL sample loop was used in the method described above. Using a published MDL method[1] analysts ran 21 replicates of a 1.0-mg Cl⁻/L standard. These gave a mean of 1.19 mg Cl⁻/L, a standard deviation of 0.027 mg Cl⁻/L, and an MDL of 0.07 mg Cl⁻/L. This is only an estimate because the ratio of standard to the MDL is above guidelines (see Section 1030). A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagents flow rate. A higher MDL may be obtained by decreasing the sample loop volume and decreasing this ratio.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and Procedure for the Determination of Method Detection Limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-ClO_2 CHLORINE DIOXIDE*

4500-ClO_2 A. Introduction

Because the physical and chemical properties of chlorine dioxide resemble those of chlorine in many respects, read the entire discussion of Residual Chlorine (Section 4500-Cl) before attempting a chlorine dioxide determination.

1. Occurrence and Significance

Chlorine dioxide, ClO_2, has been used widely as a bleaching agent in the paper and pulp industry. It has been applied to water supplies to combat tastes and odors due to phenolic-type wastes, actinomycetes, and algae, as well as to oxidize soluble iron and manganese to a more easily removable form. It is a disinfectant, and some results suggest that it may be stronger than free chlorine or hypochlorite.

Chlorine dioxide is a deep yellow, volatile, unpleasant-smelling gas that is toxic and under certain conditions may react explosively. It should be handled with care in a vented area. The use of odor to warn of exposure to concentrations of health significance may not be adequate.

There are several methods of generating ClO_2; for laboratory purposes the acidification of a solution of sodium chlorite followed by suitable scrubbing and capture of the released gaseous ClO_2 is the most practical. CAUTION: *Sodium chlorite is a powerful oxidizer; keep out of direct contact with oxidizable material to avoid possibility of explosion.*

2. Selection of Method

The iodometric method (B) gives a very precise measure of total available strength of a solution in terms of its ability to liberate iodine from iodide. However, ClO_2, chlorine, chlorite, and hypochlorite are not distinguished easily by this technique. It is designed primarily, and best used, for standardizing ClO_2 solutions needed for preparation of temporary standards. It often is inapplicable to industrial wastes.

The amperometric methods (C and E) are useful when a knowledge of the various chlorine fractions in a water sample is desired. They distinguish various chlorine compounds of interest with good accuracy and precision, but require specialized equipment and considerable analytical skill.

3. Sampling and Storage

Determine ClO_2 promptly after collecting the sample. Do not expose sample to sunlight or strong artificial light and do not aerate to mix. Most of these methods can be performed on site, with prior calibration in the laboratory. Minimum ClO_2 losses occur when the determination is completed immediately at the site of sample collection.

4. Bibliography

INGOLS, R.S. & G.M. RIDENOUR. 1948. Chemical properties of chlorine dioxide in water treatment. *J. Amer. Water Works Assoc.* 40:1207.

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HODGDEN, H.W. & R.S. INGOLS. 1954. Direct colorimetric method for determination of chlorine dioxide in water. *Anal. Chem.* 26:1224.

FEUSS, J.V. 1964. Problems in determination of chlorine dioxide residuals. *J. Amer. Water Works Assoc.* 56:607.

MASSCHELEIN, W. 1966. Spectrophotometric determination of chlorine dioxide with acid chrome violet K. *Anal. Chem.* 38:1839.

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* Approved by Standard Methods Committee, 2000.
Joint Task Group: 20th Edition—Robert P. Fisher (chair), James M. Gindelberger, Gilbert Gordon, Robert C. Hoehn, Wayne B. Huebner, Frances Y. Saunders.

4500-ClO_2 B. Iodometric Method

1. General Discussion

a. Principle: A pure solution of ClO_2 is prepared from gaseous ClO_2 by slowly adding dilute H_2SO_4 to a sodium chlorite ($NaClO_2$) solution. Contaminants such as chlorine are removed from the gas stream by a $NaClO_2$ scrubber; the gas is passed into distilled water in a steady stream of air. *See* CAUTION, ¶ A.1.

ClO_2 releases free iodine from a KI solution acidified with acetic acid or H_2SO_4. The liberated iodine is titrated with a standard solution of sodium thiosulfate ($Na_2S_2O_3$), with starch as the indicator.

b. Interference: There is little interference in this method, but temperature and strong light affect solution stability. Minimize ClO_2 losses by storing stock ClO_2 solution in a dark refrigerator and by preparing and titrating dilute ClO_2 solutions for standardization purposes at the lowest practicable temperature and in subdued light.

c. Minimum detectable concentration: One drop (0.05 mL) of 0.01*N* (0.01*M*) $Na_2S_2O_3$ is equivalent to 20 μg ClO_2/L (or 40 μg/L in terms of available chlorine) when a 500-mL sample is titrated.

2. Reagents

All reagents listed for the determination of residual chlorine in Section 4500-Cl.B.2*a-g* are required. Also needed are the following:

a. Stock chlorine dioxide solution: Prepare a gas generating and absorbing system as illustrated in Figure 4500-ClO_2:1. Con-

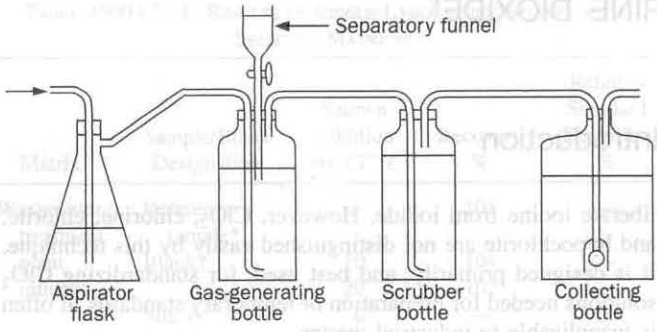

Figure 4500-ClO_2:1. Chlorine dioxide generation and absorption system.

nect aspirator flask, 500-mL capacity, with rubber tubing to a source of purified compressed air. Let air bubble through a layer of 300 mL distilled water in flask and then pass through a glass tube ending within 5 mm of the bottom of the 1-L gas-generating bottle. Conduct evolved gas via glass tubing through a scrubber bottle containing saturated $NaClO_2$ solution or a tower packed with flaked $NaClO_2$, and finally, via glass tubing, into a 2-L borosilicate glass collecting bottle where the gas is absorbed in 1500 mL distilled water. Provide an air outlet tube on collecting bottle for escape of air. Select for gas generation a bottle constructed of strong borosilicate glass and having a mouth wide enough to permit insertion of three separate glass tubes: the first leading almost to the bottom for admitting air, the second reaching below the liquid surface for gradual introduction of H_2SO_4, and the third near the top for exit of evolved gas and air. Fit to second tube a graduated cylindrical separatory funnel to contain H_2SO_4. Locate this system in a fume hood with an adequate shield.

Dissolve 10 g $NaClO_2$ in 750 mL distilled water and place in generating bottle. Carefully add 2 mL conc H_2SO_4 to 18 mL distilled water and mix. Transfer to funnel. Connect flask to generating bottle, generating bottle to scrubber, and the latter to collecting bottle. Pass a smooth current of air through the system, as evidenced by the bubbling rate in all bottles.

Introduce 5-mL increments of H_2SO_4 from funnel into generating bottle at 5-min intervals. Continue air flow for 30 min after last portion of acid has been added.

Store yellow stock solution in glass-stoppered dark-colored bottle in a dark refrigerator. The concentration of ClO_2 thus prepared varies between 250 and 600 mg/L, corresponding to approximately 500 to 1200 mg free chlorine/L.

b. Standard chlorine dioxide solution: Use this solution for preparing temporary ClO_2 standards. Dilute required volume of stock ClO_2 solution to desired strength with chlorine-demand-free water (see Section 4500-Cl.C.3*m*). Standardize solution by titrating with standard 0.01*N* (0.01*M*) or 0.025*N* (0.025*M*) $Na_2S_2O_3$ titrant in the presence of KI, acid, and starch indicator by following the procedure given in ¶ 3 below. A full or nearly full bottle of chlorine or ClO_2 solution retains its titer longer than a partially full one. When repeated withdrawals reduce volume to a critical level, standardize diluted solution at the beginning, midway in the series of withdrawals, and at the end of the series. Shake contents thoroughly before drawing off needed solution from middle of the glass-stoppered dark-colored bottle. Prepare this solution frequently.

3. Procedure

Select volume of sample, prepare for titration, and titrate sample and blank as described in Section 4500-Cl.B.3. The only exception is the following: *Let ClO_2 react in the dark with acid and KI for 5 min before starting titration.*

4. Calculations

Express ClO_2 concentrations in terms of ClO_2 or as free chlorine content. Free chlorine is defined as the total oxidizing power of ClO_2 measured by titrating iodine released by ClO_2 from an acidic solution of KI. Calculate result in terms of chlorine itself.

For standardizing ClO_2 solution:

$$\text{mg } ClO_2/\text{mL} = \frac{(A \pm B) \times N \times 13.49}{\text{mL sample titrated}}$$

For determining ClO_2 temporary standards:

$$\text{mg } ClO_2 \text{ as } Cl_2/\text{mL} = \frac{(A \pm B) \times N \times 35.45}{\text{mL sample titrated}}$$

where:

A = mL titration for sample,
B = mL titration for blank (positive or negative, see 4500-Cl.B.3*d*), and
N = normality of $Na_2S_2O_3$ = molarity of $Na_2S_2O_3$.

5. Bibliography

POST, M.A. & W.A. MOORE. 1959. Determination of chlorine dioxide in treated surface waters. *Anal. Chem.* 31:1872.

4500-ClO_2 C. Amperometric Method I

1. General Discussion

a. Principle: The amperometric titration of ClO_2 is an extension of the amperometric method for chlorine. By performing four titrations with phenylarsine oxide, free chlorine (including hypochlorite and hypochlorous acid), chloramines, chlorite, and ClO_2 may be determined separately. The first titration step consists of conversion of ClO_2 to chlorite and chlorate through addition of sufficient NaOH to produce a pH of 12, followed by neutralization to a pH of 7 and titration of free chlorine. In the second titration KI is added to a sample that has been treated similarly with alkali and had the pH readjusted to 7; titration

yields free chlorine and monochloramine. The third titration involves addition of KI and pH adjustment to 7, followed by titration of free chlorine, monochloramine, and one-fifth of the available ClO_2. In the fourth titration, addition of sufficient H_2SO_4 to lower the pH to 2 enables all available ClO_2 and chlorite, as well as the total free chlorine, to liberate an equivalent amount of iodine from the added KI and thus be titrated.

b. Interference: The interferences described in Section 4500-Cl.D.1*b* apply also to determination of ClO_2.

2. Apparatus

The apparatus required is given in Sections 4500-Cl.D.2*a* through *d*.

3. Reagents

All reagents listed for the determination of chlorine in Section 4500-Cl.D.3 are required. Also needed are the following:

a. Sodium hydroxide, NaOH, 6*N* (6*M*).

b. Sulfuric acid, H_2SO_4, 6*N* (3*M*), 1 + 5.

4. Procedure

Minimize effects of pH, time, and temperature of reaction by standardizing all conditions.

a. Titration of free available chlorine (hypochlorite and hypochlorous acid): Add sufficient 6*N* (6*M*) NaOH to raise sample pH to 12. After 10 min, add 6*N* (3*M*) H_2SO_4 to lower pH to 7. Titrate with standard phenylarsine oxide titrant to the amperometric end point as given in Section 4500-Cl.D. Record result as *A*.

b. Titration of free available chlorine and chloramine: Add 6*N* (6*M*) NaOH to raise sample pH to 12. After 10 min, add 6*N* (3*M*) H_2SO_4 to reduce pH to 7. Add 1 mL KI solution. Titrate with standard phenylarsine oxide titrant to the amperometric end point. Record result as *B*.

c. Titration of free available chlorine, chloramine, and one-fifth of available ClO_2: Adjust sample pH to 7 with pH 7 phosphate buffer solution. Add 1 mL KI solution. Titrate with standard phenylarsine oxide titrant to the amperometric end point. Record result as *C*.

d. Titration of free available chlorine, chloramines, ClO_2, and chlorite: Add 1 mL KI solution to sample. Add sufficient 6*N* (3*M*) H_2SO_4 to lower pH to 2. After 10 min, add sufficient 6*N* (6*M*) NaOH to raise pH to 7. Titrate with standard phenylarsine oxide titrant to the amperometric end point. Record result as *D*.

5. Calculation

Convert individual titrations (*A*, *B*, *C*, and *D*) into chlorine concentration by the following equation:

$$\text{mg Cl as } Cl_2/\text{L} = \frac{E \times 200}{\text{mL sample}}$$

where:

E = mL phenylarsine oxide titration for each individual sample *A*, *B*, *C*, or *D*.

Calculate ClO_2 and individual chlorine fractions as follows:

$$\begin{aligned}
\text{mg } ClO_2 \text{ as } ClO_2/\text{L} &= 1.9\,(C - B)\\
\text{mg } ClO_2 \text{ as } Cl_2/\text{L} &= 5\,(C - B)\\
\text{mg free available chlorine/L} &= A\\
\text{mg chloramine/L as chlorine} &= B - A\\
\text{mg chlorite/L as chlorine} &= 4B - 5C + D
\end{aligned}$$

6. Bibliography

HALLER, J.F. & S.S. LISTEK. 1948. Determination of chlorine dioxide and other active chlorine compounds in water. *Anal. Chem.* 20:639.

4500-ClO_2 D. (Reserved)

4500-ClO_2 E. Amperometric Method II

1. General Discussion

a. Principle: Like Amperometric Method I (Section 4500-ClO_2.C), this procedure entails successive titrations of combinations of chlorine species. Subsequent calculations determine the concentration of each species. The equilibrium for reduction of the chlorine species of interest by iodide is pH-dependent.

The analysis of a sample for chlorine, chlorine dioxide, chlorite, and chlorate requires the following steps: determination of all of the chlorine (free plus combined) and one-fifth of the chlorine dioxide at pH 7; lowering sample pH to 2 and determination of the remaining four-fifths of the ClO_2 and all of the chlorite (the chlorite measured in this step comes from the chlorite originally present in the sample and that formed in the first titration); preparation of a second sample by purging with nitrogen to remove ClO_2 and by reacting with iodide at pH 7 to remove any chlorine remaining; lowering latter sample pH to 2 and determination of all chlorite present (this chlorite only comes from the chlorite originally present in the sample); and, in a third sample, determination of all of the relevant, oxidized chlorine species— chlorine, chlorine dioxide, chlorite, and chlorate—after reduction in hydrochloric acid.[1]

This procedure can be applied to concentrated solutions (10 to 100 mg/L) or dilute solutions (0.1 to 10 mg/L) by appropriate selection of titrant concentration and sample size.

b. Interferences: At pH values above 4, significant iodate formation is possible if iodine is formed in the absence of iodide;[2] this results in a negative bias in titrating the first and second samples. Acidification of these samples causes reduction of iodate to iodine and a positive bias. To prevent formation of iodate add 1 g KI granules to stirred sample.

A positive bias results from oxidation of iodide to iodine by dissolved oxygen in strongly acidic solutions.[1] To minimize this bias, use bromide as the reducing agent in titrating the third sample (bromide is not oxidized by oxygen under these conditions). After reaction is completed, add iodide, which will be oxidized to iodine by the bromine formed from the reduction of the original chlorine species. Add iodide carefully so that bromine gas is not lost. Rapid dilution of the sample with sodium phosphate decreases sample acidity and minimizes oxidation of iodide by oxygen. The pH of the solution to be titrated should be between 1.0 and 2.0. Carry a blank through the procedure as a check on iodide oxidation.

The potential for interferences from manganese, copper, and nitrate is minimized by buffering the sample to pH ≥4.[3,4] For the method presented here, the low pH required for the chlorite and chlorate analyses provides conditions favorable to manganese, copper, and nitrite interferences.

2. Apparatus

a. Titrators: See Section 4500-Cl.D.2*a* through *d*. Amperometric titrators with a platinum-platinum electrode system are more stable and require less maintenance. (NOTE: Chlorine dioxide may attack adhesives used to connect the platinum plate to the electrode, resulting in poor readings.)

If a potentiometric titrator is used, provide a platinum sensing electrode and a silver chloride reference electrode for end-point detection.

b. Glassware: Store glassware used in this method separately from other laboratory glassware and do not use for other purposes because ClO_2 reacts with glass to form a hydrophobic surface coating. To satisfy any ClO_2 demand, before first use immerse all glassware in a strong ClO_2 solution (200 to 500 mg/L) for 24 h and rinse only with water between uses.

c. Sampling: ClO_2 is volatile and will vaporize easily from aqueous solution. When sampling a liquid stream, minimize contact with air by placing a flexible sample line to reach the bottom of the sample container, letting several container volumes overflow, slowly removing sample line, and capping container with minimum headspace. Protect from sunlight. Remove sample portions with a volumetric pipet with pipet tip placed at bottom of container. Drain pipet by placing its tip below the surface of reagent or dilution water.

3. Reagents

a. Standard sodium thiosulfate, 0.100*N* (0.100*M*): See Section 4500-Cl.B.2*c*.

b. Standard phenylarsine oxide, 0.005 64*N* (0.005 64*M*): See Section 4500-Cl.C.3*a*. (Weigh out 1.25 g phenylarsine oxide and standardize to 0.005 64*M*.)

c. Phosphate buffer solution, pH 7: See Section 4500-Cl.D.3*b*.

d. Potassium iodide, KI, granules.

e. Saturated sodium phosphate solution: Prepare a saturated solution of $Na_2HPO_4 \cdot 12H_2O$ with cold deionized-distilled water.

f. Potassium bromide solution, 5%: Dissolve 5 g KBr and dilute to 100 mL. Store in a brown glass-stoppered bottle. Make fresh weekly.

g. Hydrochloric acid, HCl, conc.

h. Hydrochloric acid, HCl, 2.5*N* (2.5*M*): Cautiously add 200 mL conc HCl, with mixing, to distilled water, diluting to 1000 mL.

i. Purge gas: Use nitrogen gas for purging ClO_2 from samples. Assure that gas is free of contaminants and pass it through a 5% KI scrub solution. Discard solution at first sign of color.

4. Procedure

Use either sodium thiosulfate or phenylarsine oxide as titrant. Select concentration on basis of concentration range expected. The total mass of oxidant species should be no greater than about 15 mg. Make appropriate sample dilutions if necessary. A convenient volume for titration is 200 to 300 mL. Preferably analyze all samples and blanks in triplicate.

Minimize effects of pH, time, and temperature of reaction by standardizing all conditions.

a. Titration of residual chlorine and one-fifth of available ClO_2: Place 1 mL pH 7 phosphate buffer in beaker and add distilled-deionized dilution water if needed. Introduce sample with minimum aeration and add 1 g KI granules while stirring. Titrate to end point (see Section 4500-Cl.D). Record reading A = mL titrant/mL sample.

b. Titration of four-fifths of available ClO_2 and chlorite: Continuing with same sample, add 2 mL 2.5*N* (2.5*M*) HCl. Let stand in the dark for 5 min. Titrate to end point. Record reading B = mL titrant/mL sample.

c. Titration of nonvolatilized chlorine: Place 1 mL pH 7 phosphate buffer in purge vessel and add distilled-deionized dilution water if needed. Add sample and purge with nitrogen gas for 15 min. Use a gas-dispersion tube to give good gas-liquid contact. Add 1 g KI granules while stirring and titrate to end point. Record reading C = mL titrant/mL sample.

d. Titration of chlorite: Continuing with same sample, add 2 mL 2.5*N* (2.5*M*) HCl. Let stand in the dark for 5 min. Titrate to end point, and record reading D = mL titrant/mL sample.

e. Titration of chlorine, ClO_2, chlorate, and chlorite: Add 1 mL KBr and 10 mL conc HCl to 50-mL reaction flask and mix. Carefully add 15 mL sample, with minimum aeration. Mix and stopper immediately. Let stand in the dark for 20 min. Rapidly add 1 g KI granules and shake vigorously for 5 s. Rapidly transfer to titration flask containing 25 mL saturated Na_2HPO_4 solution. Rinse reaction flask thoroughly and add rinse water to titration flask. Final titration volume should be about 200 to 300 mL. Titrate to end point.

Repeat procedure of preceding paragraph using distilled-deionized water in place of sample to determine blank value.

Record reading E = (mL titrant sample − mL titrant blank)/mL sample.

TABLE 4500-ClO_2:I. EQUIVALENT WEIGHTS FOR CALCULATING CONCENTRATIONS ON THE BASIS OF MASS

pH	Species	Molecular Weight *mg/mol*	Electrons Transferred	Equivalent Weight *mg/eq*
7	Chlorine dioxide	67 452	1	67 452
2, 0.1	Chlorine dioxide	67 452	5	13 490
7, 2, 0.1	Chlorine	70 906	2	35 453
2, 0.1	Chlorite	67 452	4	16 863
0.1	Chlorate	83 451	6	13 909

NOTE: The 15-mL sample volume can be adjusted to provide an appropriate dilution, but maintain the ratio of sample to HCl.

5. Calculations

Because the combining power of the titrants is pH-dependent, all calculations are based on the equivalents of reducing titrant required to react with equivalents of oxidant present. Use Table 4500-ClO_2:I to obtain the equivalent weights to be used in the calculations.

In the following equations, N is the normality of the titrant used in equivalents per liter and A through E are as defined previously.

$$\text{Chlorite, mg } ClO_2^-/L = D \times N \times 16\,863$$

$$\text{Chlorate, mg } ClO_3^-/L = [E - (A + B)] \times N \times 13\,909$$

$$\text{Chlorine dioxide, mg } ClO_2/L = (5/4) \times (B - D) \times N \times 13\,490$$

$$\text{Chlorine, mg } Cl_2/L = |A - [(B - D)/4]| \times N \times 35\,453$$

6. References

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3. WHITE, G. 1972. Handbook of Chlorination. Van Nostrand Reinhold Co., New York, N.Y.
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7. Bibliography

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GORDON, G. 1982. Improved methods of analysis for chlorate, chlorite, and hypochlorite ions. Presented at American Water Works Assoc. Water Quality Technology Conf., Nashville, Tenn.

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4500-F^- FLUORIDE*

4500-F^- A. Introduction

A fluoride concentration of approximately 1.0 mg/L in drinking water effectively reduces dental caries without harmful effects on health. Fluoride may occur naturally in water or it may be added in controlled amounts. Some fluorosis may occur when the fluoride level exceeds the recommended limits. In rare instances the naturally occurring fluoride concentration may approach 10 mg/L; such waters should be defluoridated.

Accurate determination of fluoride has increased in importance with the growth of the practice of fluoridation of water supplies as a public health measure. Maintenance of an optimal fluoride concentration is essential in maintaining effectiveness and safety of the fluoridation procedure.

1. Preliminary Treatment

Among the methods suggested for determining fluoride ion (F^-) in water, the electrode and colorimetric methods are the most satisfactory. Because both methods are subject to errors due to interfering ions (Table 4500:F^-:I), it may be necessary to distill the sample as directed in Section 4500-F^-.B before making the determination. When interfering ions are not present in excess of the tolerance of the method, the fluoride determination may be made directly without distillation.

2. Selection of Method

The electrode methods (C and G) are suitable for fluoride concentrations from 0.1 to more than 10 mg/L. Adding the prescribed buffer frees the electrode method from most interferences that adversely effect the SPADNS colorimetric method and necessitate preliminary distillation. Some substances in industrial wastes, such as fluoborates, may be sufficiently concentrated to present problems in electrode measurements and will not be measured without a preliminary distillation. Fluoride measurements can be made with an ion-selective electrode and either an expanded-scale pH meter or a specific ion meter, usually without distillation, in the time necessary for electrode equilibration.

The SPADNs method (D) has a linear analytical range of 0 to 1.40 mg F^-/L. Use of a nonlinear calibration can extend the range to 3.5 mg F^-/L. Color development is virtually instantaneous. Color determinations are made photometrically, using either a filter photometer or a spectrophotometer. A curve developed from standards is used for determining the fluoride concentration of a sample.

TABLE 4500F^-:I. CONCENTRATION OF SOME SUBSTANCES CAUSING 0.1-MG/L ERROR AT 1.0 MG F^-/L IN FLUORIDE METHODS

	Method C (Electrode)		Method D (SPADNS)	
Substance	Conc *mg/L*	Type of Error*	Conc *mg/L*	Type of Error*
Alkalinity ($CaCO_3$)	7 000	+	5 000	−
Aluminum (Al^{3+})	3.0	−	0.1†	−
Chloride (Cl^-)	20 000		7 000	+
Chlorine	5 000			Remove completely with arsenite
Color & turbidity				Remove or compensate for
Iron	200	−	10	−
Hexametaphosphate ($[NaPO_3]_6$)	50 000		1.0	+
Phosphate (PO_4^{3-})	50 000		16	+
Sulfate (SO_4^{2-})	50 000	−	200	−

* + denotes positive error
− denotes negative error
Blank denotes no measurable error.
† On immediate reading. Tolerance increases with time: after 2 h, 3.0; after 4 h, 30.

Fluoride also may be determined by the automated complexone method, Method E.

Ion chromatography (Section 4110) is an acceptable method if weaker eluents are used to separate fluoride from interfering peaks or fluoride can be determined by capillary ion electrophoresis (Section 4140).

The flow injection method (G) is a convenient automated technique for analyzing large numbers of samples.

3. Sampling and Storage

Preferably use polyethylene bottles for collecting and storing samples for fluoride analysis. Glass bottles are satisfactory if previously they have not contained high-fluoride solutions. Always rinse bottle with a portion of sample.

For the SPADNs method, never use an excess of dechlorinating agent. Dechlorinate with sodium arsenite rather than sodium thiosulfate when using the SPADNS method because the latter may produce turbidity that causes erroneous readings.

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* Approved by Standard Methods Committee, 1997.
Joint Task Group: 20th Edition (4500-F^-.G)—Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

4500-F⁻ B. Preliminary Distillation Step

1. Discussion

Fluoride can be separated from other nonvolatile constituents in water by conversion to hydrofluoric or fluosilicic acid and subsequent distillation. The conversion is accomplished by using a strong, high-boiling acid. To protect against glassware etching, hydrofluoric acid is converted to fluosilicic acid by using soft glass beads. Quantitative fluoride recovery is approached by using a relatively large sample. Acid and sulfate carryover are minimized by distilling over a controlled temperature range.

Distillation will separate fluoride from most water samples. Some tightly bound fluoride, such as that in biological materials, may require digestion before distillation, but water samples seldom require such drastic treatment. Distillation produces a distillate volume equal to that of the original water sample so that usually it is not necessary to incorporate a dilution factor when expressing analytical results. The distillate will be essentially free of substances that might interfere with the fluoride determination if the apparatus used is adequate and distillation has been carried out properly. The only common volatile constituent likely to cause interference with colorimetric analysis of the distillate is chloride. When the concentration of chloride is high enough to interfere, add silver sulfate to the sulfuric acid distilling mixture to minimize the volatilization of hydrogen chloride.

Heating an acid-water mixture can be hazardous if precautions are not taken: *Mix acid and water thoroughly before heating.* Use of a quartz heating mantle and a magnetic stirrer in the distillation apparatus simplifies the mixing step.

2. Apparatus

a. Distillation apparatus consisting of a 1-L round-bottom long-neck borosilicate glass boiling flask, a connecting tube, an efficient condenser, a thermometer adapter, and a thermometer that can be read to 200°C. Use standard taper joints for all connections in the direct vapor path. Position the thermometer so that the bulb always is immersed in boiling mixture. The apparatus should be disassembled easily to permit adding sample. Substituting a thermoregulator and necessary circuitry for the thermometer is acceptable and provides some automation.

Alternative types of distillation apparatus may be used. Carefully evaluate any apparatus for fluoride recovery and sulfate carryover. The critical points are obstructions in the vapor path and trapping of liquid in the adapter and condenser. (The condenser should have a vapor path with minimum obstruction. A double-jacketed condenser, with cooling water in the outer jacket and the inner spiral tube, is ideal, but other condensers are acceptable if they have minimum obstructions. Avoid using Graham-type condensers.) Avoid using an open flame as a heat source if possible, because heat applied to the boiling flask above the liquid level causes superheating of vapor and subsequent sulfate carryover.

CAUTION: *Regardless of apparatus used, provide for thorough mixing of sample and acid; heating a non-homogenous acid-water mixture will result in bumping or possibly a violent explosion.*

The preferred apparatus is illustrated in Figure 4500-F⁻:1.

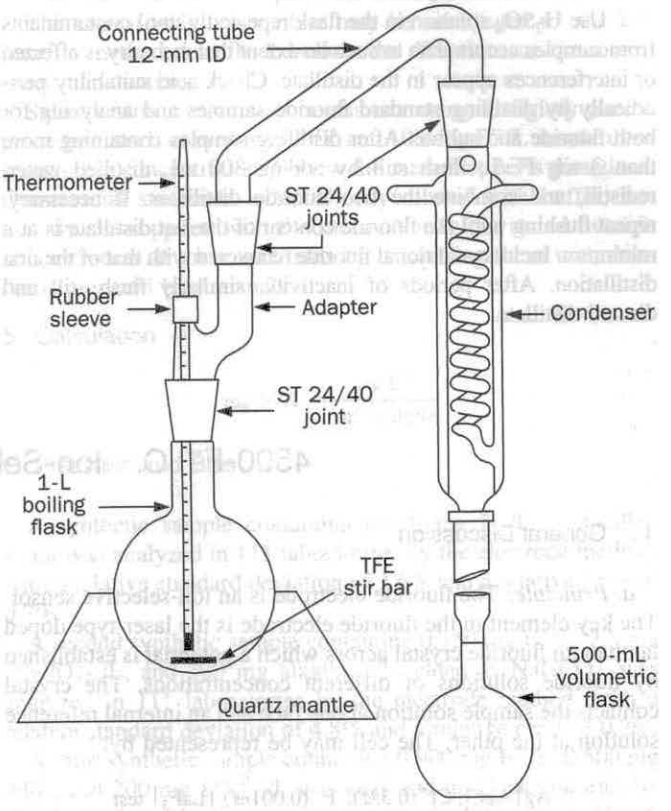

Figure 4500-F⁻:1. Direct distillation apparatus for fluoride.

b. Quartz hemispherical heating mantle, for full-voltage operation.

c. Magnetic stirrer, with TFE-coated stirring bar.

d. Soft glass beads.

3. Reagents

a. Sulfuric acid, H_2SO_4, conc, reagent grade.

b. Silver sulfate, Ag_2SO_4, crystals, reagent grade.

4. Procedure

a. Place 400 mL distilled water in the distilling flask and, with the magnetic stirrer operating, carefully add 200 mL conc H_2SO_4. Keep stirrer in operation throughout distillation. Add a few glass beads and connect the apparatus as shown in Figure 4500-F⁻:1, making sure all joints are tight. Begin heating and continue until flask contents reach 180°C (because of heat retention by the mantle, it is necessary to discontinue heating when the temperature reaches 178°C to prevent overheating). Discard distillate. This process removes fluoride contamination and adjusts the acid-water ratio for subsequent distillations.

b. After the acid mixture remaining in the steps outlined in ¶ 4*a*, or previous distillations, has cooled to 80°C or below, add 300 mL sample, with stirrer operating, and distill until the temperature reaches 180°C. To prevent sulfate carryover, turn off heat before 178°C. Retain the distillate for analysis.

c. Add Ag_2SO_4 to the distilling flask at the rate of 5 mg/mg Cl^- when the chloride concentration is high enough to interfere (see Table 4500-F^-:I).

d. Use H_2SO_4 solution in the flask repeatedly until contaminants from samples accumulate to such an extent that recovery is affected or interferences appear in the distillate. Check acid suitability periodically by distilling standard fluoride samples and analyzing for both fluoride and sulfate. After distilling samples containing more than 3 mg F^-/L, flush still by adding 300 mL distilled water, redistill, and combine the two fluoride distillates. If necessary, repeat flushing until the fluoride content of the last distillate is at a minimum. Include additional fluoride recovered with that of the first distillation. After periods of inactivity, similarly flush still and discard distillate.

5. Interpretation of Results

The recovery of fluoride is quantitative within the accuracy of the methods used for its measurement.

6. Bibliography

BELLACK, E. 1958. Simplified fluoride distillation method. *J. Amer. Water Works Assoc.* 50:530.

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4500-F^- C. Ion-Selective Electrode Method

1. General Discussion

a. Principle: The fluoride electrode is an ion-selective sensor. The key element in the fluoride electrode is the laser-type doped lanthanum fluoride crystal across which a potential is established by fluoride solutions of different concentrations. The crystal contacts the sample solution at one face and an internal reference solution at the other. The cell may be represented by:

$$Ag|AgCl, Cl^-(0.3M), F^-(0.001M)|LaF_3|\ \text{test solution}\ |\ \text{reference electrode}$$

The fluoride electrode can be used with a standard calomel reference electrode and almost any modern pH meter having an expanded millivolt scale. Calomel electrodes contain both metallic and dissolved mercury; therefore, dispose of them only in approved sites or recycle. For this reason, the Ag/AgCl reference electrode is preferred.

The fluoride electrode measures the ion activity of fluoride in solution rather than concentration. Fluoride ion activity depends on the solution total ionic strength and pH, and on fluoride complexing species. Adding an appropriate buffer provides a nearly uniform ionic strength background, adjusts pH, and breaks up complexes so that, in effect, the electrode measures concentration.

b. Interference: Table 4500-F^-:I lists common interferences. Fluoride forms complexes with several polyvalent cations, notably aluminum and iron. The extent to which complexation takes place depends on solution pH, relative levels of fluoride, and complexing species. However, CDTA (cyclohexylenediaminetetraacetic acid), a component of the buffer, preferentially will complex interfering cations and release free fluoride ions. Concentrations of aluminum, the most common interference, up to 3.0 mg/L can be complexed preferentially. In acid solution, F^- forms a poorly ionized HF · HF complex but the buffer maintains a pH above 5 to minimize hydrogen fluoride complex formation. In alkaline solution hydroxide ion also can interfere with electrode response to fluoride ion whenever the hydroxide ion concentration is greater than one-tenth the concentration of fluoride ion. At the pH maintained by the buffer, no hydroxide interference occurs.

Fluoborates are widely used in industrial processes. Dilute solutions of fluoborate or fluoboric acid hydrolyze to liberate fluoride ion but in concentrated solutions, as in electroplating wastes, hydrolysis does not occur completely. Distill such samples or measure fluoborate with a fluoborate-selective electrode. Also distill the sample if the dissolved solids concentration exceeds 10 000 mg/L.

2. Apparatus

a. Expanded-scale or digital pH meter or ion-selective meter.

b. Sleeve-type reference electrode: Do not use fiber-tip reference electrodes because they exhibit erratic behavior in very dilute solutions.

c. Fluoride electrode.

d. Magnetic stirrer, with TFE-coated stirring bar.

e. Timer.

3. Reagents

a. Stock fluoride solution: Dissolve 221.0 mg anhydrous sodium fluoride, NaF, in distilled water and dilute to 1000 mL; 1.00 mL = 100 μg F^-.

b. Standard fluoride solution: Dilute 100 mL stock fluoride solution to 1000 mL with distilled water; 1.00 mL = 10.0 μg F^-.

c. Fluoride buffer: Place approximately 500 mL distilled water in a 1-L beaker and add 57 mL glacial acetic acid, 58 g NaCl, and 4.0 g 1,2 cyclohexylenediaminetetraacetic acid (CDTA).* Stir to dissolve. Place beaker in a cool water bath and add slowly 6*N* NaOH (about 125 mL) with stirring, until pH is between 5.3 and 5.5. Transfer to a 1-L volumetric flask and add distilled water to the mark. This buffer, as well as a more concentrated version, is available commercially. In using the concentrated buffer follow the manufacturer's directions.

* Also known as 1,2 cyclohexylenedinitrilotetraacetic acid.

4. Procedure

a. Instrument calibration: No major adjustment of any instrument normally is required to use electrodes in the range of 0.2 to 2.0 mg F^-/L. For those instruments with zero at center scale adjust calibration control so that the 1.0 mg F^-/L standard reads at the center zero (100 mV) when the meter is in the expanded-scale position. This cannot be done on some meters that do not have a millivolt calibration control. To use a selective-ion meter follow the manufacturer's instructions.

b. Preparation of fluoride standards: Prepare a series of standards by diluting with distilled water 5.0, 10.0, and 20.0 mL of standard fluoride solution to 100 mL with distilled water. These standards are equivalent to 0.5, 1.0, and 2.0 mg F^-/L.

c. Treatment of standards and sample: In 100-mL beakers or other convenient containers add by volumetric pipet from 10 to 25 mL standard or sample. Bring standards and sample to same temperature, preferably room temperature. Add an equal volume of buffer. The total volume should be sufficient to immerse the electrodes and permit operation of the stirring bar.

d. Measurement with electrode: Immerse electrodes in each of the fluoride standard solutions and measure developed potential while stirring on a magnetic stirrer. Avoid stirring before immersing electrodes because entrapped air around the crystal can produce erroneous readings or needle fluctuations. Let electrodes remain in the solution 3 min (or until reading is constant) before taking a final millivolt reading. A layer of insulating material between stirrer and beaker minimizes solution heating. Withdraw electrodes, rinse with distilled water, and *blot dry* between readings. (CAUTION: Blotting may poison electrode if not done gently.) Repeat measurements with samples.

When using an expanded-scale pH meter or selective-ion meter, frequently recalibrate the electrode by checking potential reading of the 1.00-mg F^-/L standard and adjusting the calibration control, if necessary, until meter reads as before.

If a direct-reading instrument is not used, plot potential measurement of fluoride standards against concentration on two-cycle semilogarithmic graph paper. Plot milligrams F^- per liter on the logarithmic axis (ordinate), with the lowest concentration at the bottom of the graph. Plot millivolts on the abscissa. From the potential measurement for each sample, read the corresponding fluoride concentration from the standard curve.

The known-additions method may be substituted for the calibration method described. Follow the directions of the instrument manufacturer.

Selective-ion meters may necessitate using a slightly altered procedure, such as preparing 1.00 and 10.0 mg F^-/L standards or some other concentration. Follow the manufacturer's directions. Commercial standards, often already diluted with buffer, frequently are supplied with the meter. Verify the stated fluoride concentration of these standards by comparing them with standards prepared by the analyst.

5. Calculation

$$\text{mg } F^-/\text{L} = \frac{\mu\text{g } F^-}{\text{mL sample}}$$

6. Precision and Bias

A synthetic sample containing 0.850 mg F^-/L in distilled water was analyzed in 111 laboratories by the electrode method, with a relative standard deviation of 3.6% and a relative error of 0.7%.

A second synthetic sample containing 0.750 mg F^-/L, 2.5 mg $(NaPO_3)_6$/L, and 300 mg alkalinity/L added as $NaHCO_3$, was analyzed in 111 laboratories by the electrode method, with a relative standard deviation of 4.8% and a relative error of 0.2%.

A third synthetic sample containing 0.900 mg F^-/L, 0.500 mg Al/L, and 200 mg SO_4^{2-}/L was analyzed in 13 laboratories by the electrode method, with a relative standard deviation of 2.9% and a relative error of 4.9%.

7. Bibliography

FRANT, M.S. & J.W. ROSS, JR. 1968. Use of total ionic strength adjustment buffer for electrode determination of fluoride in water supplies. *Anal. Chem.* 40:1169.

HARWOOD, J.E. 1969. The use of an ion-selective electrode for routine analysis of water samples. *Water Res.* 3:273.

4500-F^- D. SPADNS Method

1. General Discussion

a. Principle: The SPADNS colorimetric method is based on the reaction between fluoride and a zirconium-dye lake. Fluoride reacts with the dye lake, dissociating a portion of it into a colorless complex anion (ZrF_6^{2-}); and the dye. As the amount of fluoride increases, the color produced becomes progressively lighter.

The reaction rate between fluoride and zirconium ions is influenced greatly by the acidity of the reaction mixture. If the proportion of acid in the reagent is increased, the reaction can be made almost instantaneous. Under such conditions, however, the effect of various ions differs from that in the conventional alizarin methods. The selection of dye for this rapid fluoride method is governed largely by the resulting tolerance to these ions.

b. Interference: Table 4500-F^-:I lists common interferences. Because these are neither linear in effect nor algebraically additive, mathematical compensation is impossible. Whenever any one substance is present in sufficient quantity to produce an error of 0.1 mg/L or whenever the total interfering effect is in doubt, distill the sample. Also distill colored or turbid samples. In some instances, sample dilution or adding appropriate amounts of interfering substances to the standards may be used to compensate for the interference effect. If alkalinity is the only significant

interference, neutralize it with either hydrochloric or nitric acid. Chlorine interferes and provision for its removal is made.

Volumetric measurement of sample and reagent is extremely important to analytical accuracy. Use samples and standards at the same temperature or at least within 2°C. Maintain constant temperature throughout the color development period. Prepare different calibration curves for different temperature ranges.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 570 nm, providing a light path of at least 1 cm.

b. Filter photometer, providing a light path of at least 1 cm and equipped with a greenish yellow filter having maximum transmittance at 550 to 580 nm.

3. Reagents

a. Standard fluoride solution: Prepare as directed in the electrode method, Section 4500-F^-.C.3*b*.

b. SPADNS solution: Dissolve 958 mg SPADNS, sodium 2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalene disulfonate, also called 4,5-dihydroxy-3-(parasulfophenylazo)-2,7-naphthalenedisulfonic acid trisodium salt, in distilled water and dilute to 500 mL. This solution is stable for at least 1 year if protected from direct sunlight.

c. Zirconyl-acid reagent: Dissolve 133 mg zirconyl chloride octahydrate, $ZrOCl_2 \cdot 8H_2O$, in about 25 mL distilled water. Add 350 mL conc HCl and dilute to 500 mL with distilled water.

d. Acid zirconyl-SPADNS reagent: Mix equal volumes of SPADNS solution and zirconyl-acid reagent. The combined reagent is stable for at least 2 years.

e. Reference solution: Add 10 mL SPADNS solution to 100 mL distilled water. Dilute 7 mL conc HCl to 10 mL and add to the diluted SPADNS solution. The resulting solution, used for setting the instrument reference point (zero), is stable for at least 1 year. Alternatively, use a prepared standard of 0 mg F^-/L as a reference.

f. Sodium arsenite solution: Dissolve 5.0 g $NaAsO_2$ and dilute to 1 L with distilled water. (CAUTION: *Toxic—avoid ingestion.*)

4. Procedure

a. Preparation of standard curve: Prepare fluoride standards in the range of 0 to 1.40 mg F^-/L by diluting appropriate quantities of standard fluoride solution to 50 mL with distilled water. Pipet 5.00 mL each of SPADNS solution and zirconyl-acid reagent, or 10.00 mL mixed acid-zirconyl-SPADNS reagent, to each standard and mix well. Avoid contamination. Set photometer to zero absorbance with the reference solution and obtain absorbance readings of standards. Plot a curve of the milligrams fluoride-absorbance relationship. Prepare a new standard curve whenever a fresh reagent is made or a different standard temperature is desired. As an alternative to using a reference, set photometer at some convenient point (0.300 or 0.500 absorbance) with the prepared 0 mg F^-/L standard.

b. Sample pretreatment: If the sample contains residual chlorine, remove it by adding 1 drop (0.05 mL) $NaAsO_2$ solution/ 0.1 mg residual chlorine and mix. (Sodium arsenite concentrations of 1300 mg/L produce an error of 0.1 mg/L at 1.0 mg F^-/L.)

c. Color development: Use a 50.0-mL sample or a portion diluted to 50 mL with distilled water. Adjust sample temperature to that used for the standard curve. Add 5.00 mL each of SPADNS solution and zirconyl-acid reagent, or 10.00 mL acid-zirconyl-SPADNS reagent; mix well and read absorbance, first setting the reference point of the photometer as above. If the absorbance falls beyond the range of the standard curve, repeat using a diluted sample.

5. Calculation

$$\text{mg } F^-/\text{L} = \frac{A}{\text{mL sample}} \times \frac{B}{C}$$

where:

A = μg F^- determined from plotted curve,
B = final volume of diluted sample, mL, and
C = volume of diluted sample used for color development, mL.

When the prepared 0 mg F^-/L standard is used to set the photometer, alternatively calculate fluoride concentration as follows:

$$\text{mg } F^-/\text{L} = \frac{A_0 - A_x}{A_0 - A_1}$$

where:

A_0 = absorbance of the prepared 0 mg F^-/L standard,
A_1 = absorbance of a prepared 1.0 mg F^-/L standard, and
A_x = absorbance of the prepared sample.

6. Precision and Bias

A synthetic sample containing 0.830 mg F^-/L and no interference in distilled water was analyzed in 53 laboratories by the SPADNS method, with a relative standard deviation of 8.0% and a relative error of 1.2%. After direct distillation of the sample, the relative standard deviation was 11.0% and the relative error 2.4%.

A synthetic sample containing 0.570 mg F^-/L, 10 mg Al/L, 200 mg SO_4^{2-}/L, and 300 mg total alkalinity/L was analyzed in 53 laboratories by the SPADNS method without distillation, with a relative standard deviation of 16.2% and a relative error of 7.0%. After direct distillation of the sample, the relative standard deviation was 17.2% and the relative error 5.3%.

A synthetic sample containing 0.680 mg F^-/L, 2 mg Al/L, 2.5 mg $(NaPO_3)_6$/L, 200 mg SO_4^{2-}/L, and 300 mg total alkalinity/L was analyzed in 53 laboratories by direct distillation and SPADNS methods with a relative standard deviation of 2.8% and a relative error of 5.9%.

7. Bibliography

BELLACK, E. & P.J. SCHOUBOE. 1968. Rapid photometric determination of fluoride with SPADNS-zirconium lake. *Anal. Chem.* 30:2032.

4500-F⁻ E. Complexone Method

1. General Discussion

a. Principle: The sample is distilled in the automated system, and the distillate is reacted with alizarin fluorine blue-lanthanum reagent to form a blue complex that is measured colorimetrically at 620 nm.

b. Interferences: Interferences normally associated with the determination of fluoride are removed by distillation.

c. Application: This method is applicable to potable, surface, and saline waters as well as domestic and industrial wastewaters. The range of the method, which can be modified by using the adjustable colorimeter, is 0.1 to 2.0 mg F^-/L.

2. Apparatus

An example of the required continuous-flow analytical instrument consists of the interchangeable components in the number and manner indicated in Figure 4500-F⁻:2.

3. Reagents

a. Standard fluoride solution: Prepare in appropriate concentrations from 0.10 to 2.0 mg F^-/L using the stock fluoride solution (see Section 4500-F⁻.C.3*a*).

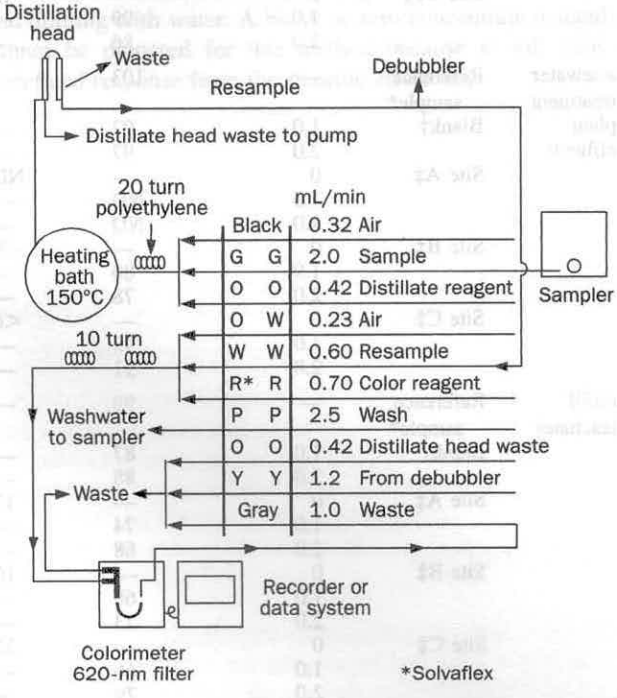

Figure 4500-F⁻:2. Fluoride manifold.

b. Distillation reagent: Add 50 mL conc H_2SO_4 to about 600 mL distilled water. Add 10.00 mL stock fluoride solution (see Section 4500-F⁻.C.3*a*; 1.00 mL = 100 μg F^-) and dilute to 1000 mL.

c. Acetate buffer solution: Dissolve 60 g anhydrous sodium acetate, $NaC_2H_3O_2$, in about 600 mL distilled water. Add 100 mL conc (glacial) acetic acid and dilute to 1 L.

d. Alizarin fluorine blue stock solution: Add 960 mg alizarin fluorine,* $C_{14}H_7O_4 \cdot CH_2N(CH_2 \cdot COOH)_2$, to 100 mL distilled water. Add 2 mL conc NH_4OH and mix until dye is dissolved. Add 2 mL conc (glacial) acetic acid, dilute to 250 mL and store in an amber bottle in the refrigerator.

e. Lanthanum nitrate stock solution: Dissolve 1.08 g $La(NO_3)_3$ in about 100 mL distilled water, dilute to 250 mL, and store in refrigerator.

f. Working color reagent: Mix in the following order: 300 mL acetate buffer solution, 150 mL acetone, 50 mL tertiary butanol, 36 mL alizarin fluorine blue stock solution, 40 mL lanthanum nitrate stock solution, and 2 mL polyoxyethylene 23 lauryl ether.† Dilute to 1 L with distilled water. This reagent is stable for 2 to 4 d.

4. Procedure

No special handling or preparation of sample is required.

Set up manifold as shown in Figure 4500-F⁻:2 and follow the manufacturer's instructions.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against constituent concentrations in standards. Compute sample concentrations by comparing sample response with standard curve.

6. Precision and Bias

In a single laboratory four samples of natural water containing from 0.40 to 0.82 mg F^-/L were analyzed in septuplicate. Average precision was ± 0.03 mg F^-/L. To two of the samples, additions of 0.20 and 0.80 mg F^-/L were made. Average recovery of the additions was 98%.

7. Bibliography

WEINSTEIN, L.H., R.H. MANDL, D.C. MCCUNE, J.S. JACOBSON & A.E. HITCHCOCK. 1963. A semi-automated method for the determination of fluorine in air and plant tissues. *Boyce Thompson Inst.* 22:207.

* J.T. Baker Catalog number J-112 or equivalent.

† Brij-35, available from ICI Americas, Wilmington, DE, or equivalent.

4500-F⁻ F. (Reserved)

Section 4500-F⁻ G. Ion-Selective Electrode Flow Injection Analysis

1. General Discussion

a. Principle: Fluoride is determined potentiometrically by using a combination fluoride-selective electrode in a flow cell. The fluoride electrode consists of a lanthanum fluoride crystal across which a potential is developed by fluoride ions. The reference cell is a $Ag/AgCl/Cl^-$ cell. The reference junction is of the annular liquid-junction type and encloses the fluoride-sensitive crystal.

Also see Section 4500-F⁻.C and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

The polyvalent cations Si^{4+}, Al^{3+}, and Fe^{3+} interfere by forming complexes with fluoride. As part of the buffer reagent, 1,2-cyclohexyldiaminetetraacetic acid (CDTA) is added to preferentially complex these cations and eliminate this interference when these concentrations do not exceed 3.0 mg Al^{3+}/L and 20 mg Fe^{3+}/L.

Some interferents are removed by distillation; see Section 4500-F⁻.B. Drinking water samples generally do not require sample distillation.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold (Figure 4500-F⁻:3) with tubing heater and ion-selective electrode flow cell. In Figure 4500-F⁻:3, relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

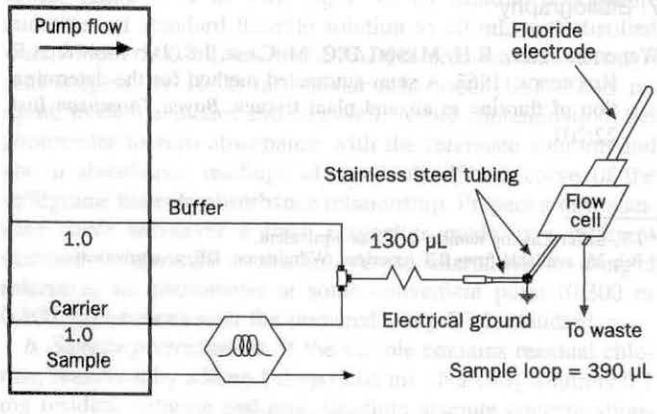

Figure 4500-F⁻:3. FIA fluoride manifold.

TABLE 4500-F⁻:II. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg F⁻/L	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	101	—
	Blank†	1.0	91	—
		2.0	97	—
	Site A‡	0	—	4.8
		1.0	93	—
		2.0	82	—
	Site B‡	0	—	6.4
		1.0	96	—
		2.0	86	—
	Site C‡§	0	—	15
		1.0	99	—
		2.0	86	—
Wastewater treatment plant effluent	Reference sample*	—	103	—
	Blank†	1.0	97	—
		2.0	97	—
	Site A‡	0	—	ND
		1.0	ND	—
		2.0	ND	—
	Site B‡	0	—	<0.1
		1.0	80	—
		2.0	78	—
	Site C‡	0	—	<0.1
		1.0	93	—
		2.0	91	—
Landfill leachate‖	Reference sample*	—	99	—
	Blank†	1.0	87	—
		2.0	88	—
	Site A‡	0	—	13
		1.0	74	—
		2.0	68	—
	Site B‡	0	—	10
		1.0	68	—
		2.0	73	—
	Site C‡	0	—	32
		1.0	66	—
		2.0	79	—

ND = not detectable.

* U.S. EPA QC sample, 1.81 mg F⁻/L.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate. Typical difference between duplicates for influent 5%, for effluent 6%.

§ Mean concentration 0.18 mg F⁻/L.

‖ All sites had mean concentration of <0.2 mg F⁻/L.

d. Combination ion-selective electrode.

e. Injection valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min.

a. Carrier, 1.0 mg F^-/L: Add 10 mL or 10 g stock fluoride standard (¶ 3*d*) to 990 mL water and mix well.

b. Buffer: To a tared 1-L polyethylene container add 929.5 g water, 59.8 g glacial acetic acid, 30.0 g sodium hydroxide, NaOH, 58.0 g sodium chloride, NaCl, 0.5 g stock fluoride standard (¶ 3*d*), and 4.0 g 1,2-cyclohexyldiaminetetraacetic acid (CDTA) (also called *trans*-1,2-diaminocyclohexane). Stir on a magnetic stir plate until all material has dissolved.

c. Electrode conditioning solution: To a tared 1-L container, add 534 g buffer (¶ 3*b*) and 500 g carrier (¶ 3*a*). Shake or stir to mix thoroughly. Store fluoride electrode in this solution when it is not in use.

d. Stock fluoride standard, 100.0 mg F^-/L: In a 1-L volumetric flask, dissolve 0.2210 g sodium fluoride, NaF, in approximately 950 mL water. Dilute to mark with water and mix well. Store in a polyethylene bottle.

e. Standard fluoride solutions: Prepare fluoride standards in the desired concentration range, using the stock standard (¶ 3*d*), and diluting with water. A blank or zero concentration standard cannot be prepared for this method because it will give an undefined response from the fluoride electrode.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-F^-:3 and follow method supplied by manufacturer or laboratory standard operating procedure for this method. Follow quality control procedures outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting the electrode response to standards processed through the manifold vs. fluoride concentration. Standards greater than 1.0 mg F^-/L will give positive peaks, standards less than 1.0 mg F^-/L will give negative peaks, and the 1.0 mg F^-/L standard having the same concentration as the carrier will give no peak. The calibration curve gives a good fit to a second-order polynomial.

It is not necessary to plot the response versus log[F^-]; if this is done the calibration curve will still be a second-order polynomial because there is a concentration-dependent kinetic effect in the flowing stream electrode system.

6. Precision and Bias

The samples used in the studies described below were not distilled.

a. Recovery and relative standard deviation: The results of single-laboratory studies with various matrices are given in Table 4500-F^-:II.

b. MDL: A 390-μL sample loop was used in the method described above. Ten replicates of a 1.0-mg F^-/L standard were run to obtain an MDL of 0.02 mg F^-/L.

c. Precision: Ten replicate standards of 2.0 mg F^-/L gave a % RSD of 0.5%.

4500-H$^+$ pH VALUE*

4500-H$^+$ A. Introduction

1. Principles

Measurement of pH is one of the most important and frequently used tests in water chemistry. Practically every phase of water supply and wastewater treatment, e.g., acid-base neutralization, water softening, precipitation, coagulation, disinfection, and corrosion control, is pH-dependent. pH is used in alkalinity and carbon dioxide measurements and many other acid-base equilibria. At a given temperature the *intensity* of the acidic or basic character of a solution is indicated by pH or hydrogen ion activity. Alkalinity and acidity are the acid- and base-neutralizing capacities of a water and usually are expressed as milligrams $CaCO_3$ per liter. Buffer capacity is the amount of strong acid or base, usually expressed in moles per liter, needed to change the pH value of a 1-L sample by 1 unit. pH as defined by Sorenson[1] is $-\log [H^+]$; it is the "intensity" factor of acidity. Pure water is very slightly ionized and at equilibrium the ion product is

$$[H^+][OH^-] = K_w = 1.01 \times 10^{-14} \text{ at } 25°C \quad (1)$$

and

$$[H^+] = [OH^-] = 1.005 \times 10^{-7}$$

where:

$[H^+]$ = activity of hydrogen ions, moles/L,
$[OH^-]$ = activity of hydroxyl ions, moles/L, and
K_w = ion product of water.

Because of ionic interactions in all but very dilute solutions, it is necessary to use the "activity" of an ion and not its molar concentration. Use of the term pH assumes that the activity of the hydrogen ion, a_{H^+}, is being considered. The *approximate* equivalence to molarity, $[H^+]$ can be presumed only in very dilute solutions (ionic strength <0.1).

A logarithmic scale is convenient for expressing a wide range of ionic activities. Equation 1 in logarithmic form and corrected to reflect activity is:

$$(-\log_{10} a_{H^+}) + (-\log_{10} a_{OH^-}) = 14 \quad (2)$$

or

$$pH + pOH = pK_w$$

where:

pH† = $\log_{10} a_{H+}$ and
pOH = $\log_{10} a_{OH^-}$.

Equation 2 states that as pH increases pOH decreases correspondingly and vice versa because pK_w is constant for a given temperature. At 25°C, pH 7.0 is neutral, the activities of the hydrogen and hydroxyl ions are equal, and each corresponds to an approximate activity of 10^{-7} moles/L. The neutral point is temperature-dependent and is pH 7.5 at 0°C and pH 6.5 at 60°C.

The pH value of a highly dilute solution is approximately the same as the negative common logarithm of the hydrogen ion concentration. Natural waters usually have pH values in the range of 4 to 9, and most are slightly basic because of the presence of bicarbonates and carbonates of the alkali and alkaline earth metals.

2. Reference

1. SORENSON, S. 1909. Über die Messung und die Bedeutung der Wasserstoff ionen Konzentration bei Enzymatischen Prozessen. *Biochem. Z.* 21:131.

* Approved by Standard Methods Committee, 2000.

† p designates $-\log_{10}$ of a number.

4500-H$^+$ B. Electrometric Method

1. General Discussion

a. Principle: The basic principle of electrometric pH measurement is determination of the activity of the hydrogen ions by potentiometric measurement using a standard hydrogen electrode and a reference electrode. The hydrogen electrode consists of a platinum electrode across which hydrogen gas is bubbled at a pressure of 101 kPa. Because of difficulty in its use and the potential for poisoning the hydrogen electrode, the glass electrode commonly is used. The electromotive force (emf) produced in the glass electrode system varies linearly with pH. This linear relationship is described by plotting the measured emf against the pH of different buffers. Sample pH is determined by extrapolation.

Because single ion activities such as a_{H^+} cannot be measured, pH is defined operationally on a potentiometric scale. The pH measuring instrument is calibrated potentiometrically with an indicating (glass) electrode and a reference electrode using National Institute of Standards and Technology (NIST) buffers having assigned values so that:

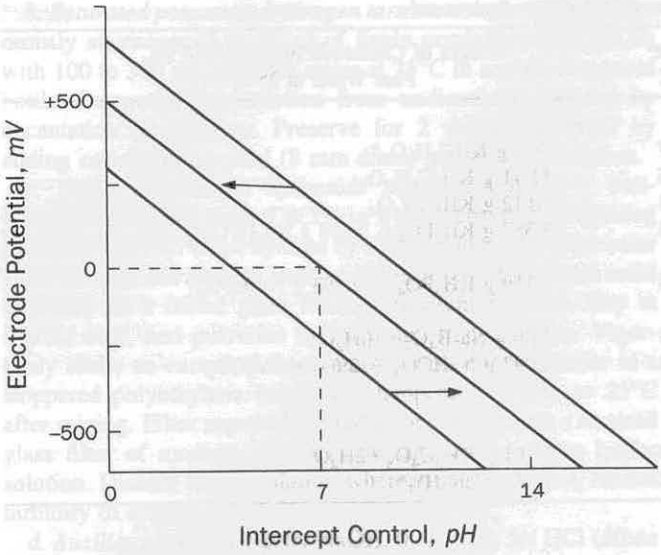

Figure 4500-H+:1. Electrode potential vs. pH. Intercept control shifts response curve laterally.

$$pH_B = -\log_{10} a_{H^+}$$

where:

pH_B = assigned pH of NIST buffer.

The operational pH scale is used to measure sample pH and is defined as:

$$pH_x = pH_B \pm \frac{F(E_x - E_s)}{2.303\,RT}$$

where:

pH_x = potentiometrically measured sample pH,
F = Faraday: 9.649×10^4 coulomb/mole,
E_x = sample emf, V,
E_s = buffer emf, V,
R = gas constant; 8.314 joule/(mole °K), and
T = absolute temperature, °K.

NOTE: Although the equation for pH_x appears in the literature with a plus sign, the sign of emf readings in millivolts for most pH meters manufactured in the U.S. is negative. The choice of negative sign is consistent with the IUPAC Stockholm convention concerning the sign of electrode potential.[1,2]

The activity scale gives values that are higher than those on Sorenson's scale by 0.04 units:

$$\text{pH (activity)} = \text{pH (Sorenson)} + 0.04$$

The equation for pH_x assumes that the emf of the cells containing the sample and buffer is due solely to hydrogen ion activity unaffected by sample composition. In practice, samples will have varying ionic species and ionic strengths, both affecting H^+ activity. This imposes an experimental limitation on pH measurement; thus, to obtain meaningful results, the differences between E_x and E_s should be minimal. Samples must be dilute aqueous solutions of simple solutes (<0.2*M*). (Choose buffers to bracket the sample.) Determination of pH cannot be made accurately in nonaqueous media, suspensions, colloids, or high-ionic-strength solutions.

b. Interferences: The glass electrode is relatively free from interference from color, turbidity, colloidal matter, oxidants, reductants, or high salinity, except for a sodium error at pH > 10. Reduce this error by using special "low sodium error" electrodes.

pH measurements are affected by temperature in two ways: mechanical effects that are caused by changes in the properties of the electrodes and chemical effects caused by equilibrium changes. In the first instance, the Nernstian slope increases with increasing temperature and electrodes take time to achieve thermal equilibrium. This can cause long-term drift in pH. Because chemical equilibrium affects pH, standard pH buffers have a specified pH at indicated temperatures.

Always report temperature at which pH is measured.

2. Apparatus

a. pH meter consisting of potentiometer, a glass electrode, a reference electrode, and a temperature-compensating device. A circuit is completed through the potentiometer when the electrodes are immersed in the test solution. Many pH meters are capable of reading pH or millivolts and some have scale expansion that permits reading to 0. 001 pH unit, but most instruments are not that precise.

For routine work use a pH meter accurate and reproducible to 0.1 pH unit with a range of 0 to 14 and equipped with a temperature-compensation adjustment.

Although manufacturers provide operating instructions, the use of different descriptive terms may be confusing. For most instruments, there are two controls: intercept (set buffer, asymmetry, standardize) and slope (temperature, offset); their functions are shown diagramatically in Figures 4500-H+:1 and 2. The intercept control shifts the response curve laterally to pass through the isopotential point with no change in slope. This

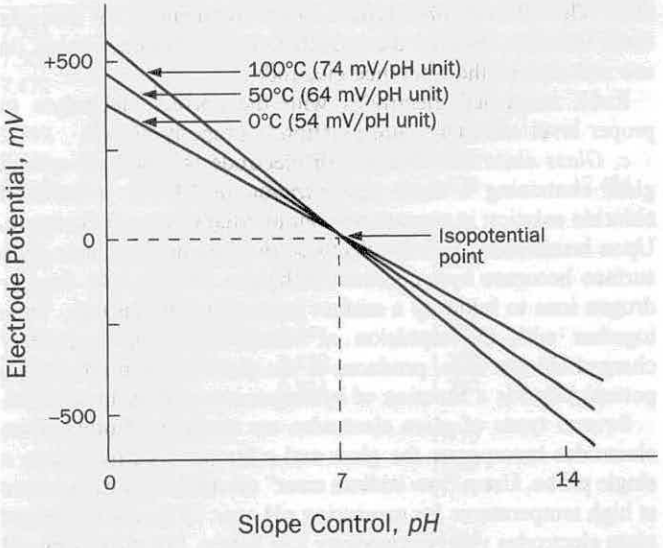

Figure 4500-H+:2. Typical pH electrode response as a function of temperature.

TABLE 4500-H^+:I. PREPARATION OF pH STANDARD SOLUTIONS[3]

Standard Solution (molality)	pH at 25°C	Weight of Chemicals Needed/1000 mL Pure Water at 25°C
Primary standards:		
Potassium hydrogen tartrate (saturated at 25°C)	3.557	> 7 g $KHC_4H_4O_6$*
0.05 potassium dihydrogen citrate	3.776	11.41 g $KH_2C_6H_5O_7$
0.05 potassium hydrogen phthalate	4.004	10.12 g $KHC_8H_4O_4$
0.025 potassium dihydrogen phosphate + 0.025 disodium hydrogen phosphate	6.863	3.387 g KH_2PO_4 + 3.533 g Na_2HPO_4†
0.008 695 potassium dihydrogen phosphate + 0.030 43 disodium hydrogen phosphate	7.415	1.179 g KH_2PO_4 + 4.303 g Na_2HPO_4†
0.01 sodium borate decahydrate (borax)	9.183	3.80 g $Na_2B_4O_7 \cdot 10H_2O$†
0.025 sodium bicarbonate + 0.025 sodium carbonate	10.014	2.092 g $NaHCO_3$ + 2.640 g Na_2CO_3
Secondary standards:		
0.05 potassium tetroxalate dihydrate	1.679	12.61 g $KH_3C_4O_8 \cdot 2H_2O$
Calcium hydroxide (saturated at 25°C)	12.454	> 2 g $Ca(OH)_2$*

* Approximate solubility.
† Prepare with freshly boiled and cooled distilled water (carbon-dioxide-free).

permits bringing the instrument on scale (0 mV) with a pH 7 buffer that has no change in potential with temperature.

The slope control rotates the emf/pH slope about the isopotential point (0 mV/pH 7). To adjust slope for temperature without disturbing the intercept, select a buffer that brackets the sample with pH 7 buffer and adjust slope control to pH of this buffer. The instrument will indicate correct millivolt change per unit pH at the test temperature.

b. Reference electrode consisting of a half cell that provides a constant electrode potential. Commonly used are calomel and silver: silver-chloride electrodes. Either is available with several types of liquid junctions.

The liquid junction of the reference electrode is critical because at this point the electrode forms a salt bridge with the sample or buffer and a liquid junction potential is generated that in turn affects the potential produced by the reference electrode. Reference electrode junctions may be annular ceramic, quartz, or asbestos fiber, or the sleeve type. The quartz type is most widely used. The asbestos fiber type is not recommended for strongly basic solutions. Follow the manufacturer's recommendation on use and care of the reference electrode.

Refill nonsealed electrodes with the correct electrolyte to proper level and make sure junction is properly wetted.

c. Glass electrode: The sensor electrode is a bulb of special glass containing a fixed concentration of HCl or a buffered chloride solution in contact with an internal reference electrode. Upon immersion of a new electrode in a solution the outer bulb surface becomes hydrated and exchanges sodium ions for hydrogen ions to build up a surface layer of hydrogen ions. This, together with the repulsion of anions by fixed, negatively charged silicate sites, produces at the glass-solution interface a potential that is a function of hydrogen ion activity in solution.

Several types of glass electrodes are available. Combination electrodes incorporate the glass and reference electrodes into a single probe. Use a "low sodium error" electrode that can operate at high temperatures for measuring pH over 10 because standard glass electrodes yield erroneously low values. For measuring pH below 1 standard glass electrodes yield erroneously high values; use liquid membrane electrodes instead.

d. Beakers: Preferably use polyethylene or TFE* beakers.

e. Stirrer: Use either a magnetic, TFE-coated stirring bar or a mechanical stirrer with inert plastic-coated impeller.

f. Flow chamber: Use for continuous flow measurements or for poorly buffered solutions.

3. Reagents

a. General preparation: Calibrate the electrode system against standard buffer solutions of known pH. Because buffer solutions may deteriorate as a result of mold growth or contamination, prepare fresh as needed for accurate work by weighing the amounts of chemicals specified in Table 4500-H^+:I, dissolving in distilled water at 25°C, and diluting to 1000 mL. This is particularly important for borate and carbonate buffers.

Boil and cool distilled water having a conductivity of less than 2 μmhos/cm. To 50 mL add 1 drop of saturated KCl solution suitable for reference electrode use. If the pH of this test solution is between 6.0 and 7.0, use it to prepare all standard solutions.

Dry KH_2PO_4 at 110 to 130°C for 2 h before weighing but do not heat unstable hydrated potassium tetroxalate above 60°C nor dry the other specified buffer salts.

Although ACS-grade chemicals generally are satisfactory for preparing buffer solutions, use certified materials available from the National Institute of Standards and Technology when the greatest accuracy is required. For routine analysis, use commercially available buffer tablets, powders, or solutions of tested quality. In preparing buffer solutions from solid salts, insure complete solution.

As a rule, select and prepare buffer solutions classed as primary standards in Table 4500-H^+:I; reserve secondary standards for extreme situations encountered in wastewater measurements. Consult Table 4500- H^+:II for accepted pH of standard buffer solutions at temperatures other than 25°C. In routine use, store buffer solutions and samples in polyethylene bottles. Replace buffer solutions every 4 weeks.

* Teflon or equivalent

b. Saturated potassium hydrogen tartrate solution: Shake vigorously an excess (5 to 10 g) of finely crystalline $KHC_4H_4O_6$ with 100 to 300 mL distilled water at 25°C in a glass-stoppered bottle. Separate clear solution from undissolved material by decantation or filtration. Preserve for 2 months or more by adding one thymol crystal (8 mm diam) per 200 mL solution.

c. Saturated calcium hydroxide solution: Calcine a well-washed, low-alkali grade $CaCO_3$ in a platinum dish by igniting for 1 h at 1000°C. Cool, hydrate by slowly adding distilled water with stirring, and heat to boiling. Cool, filter, and collect solid $Ca(OH)_2$ on a fritted glass filter of medium porosity. Dry at 110°C, cool, and pulverize to uniformly fine granules. Vigorously shake an excess of fine granules with distilled water in a stoppered polyethylene bottle. Let temperature come to 25°C after mixing. Filter supernatant under suction through a sintered glass filter of medium porosity and use filtrate as the buffer solution. Discard buffer solution when atmospheric CO_2 causes turbidity to appear.

d. Auxiliary solutions: 0.1*N* NaOH, 0.1*N* HCl, 5*N* HCl (dilute five volumes 6*N* HCl with one volume distilled water), and acid potassium fluoride solution (dissolve 2 g KF in 2 mL conc H_2SO_4 and dilute to 100 mL with distilled water).

4. Procedure

a. Instrument calibration: In each case follow manufacturer's instructions for pH meter and for storage and preparation of electrodes for use. Recommended solutions for short-term storage of electrodes vary with type of electrode and manufacturer, but generally have a conductivity greater than 4000 μmhos/cm. Tap water is a better substitute than distilled water, but pH 4 buffer is best for the single glass electrode and saturated KCl is preferred for a calomel and Ag/AgCl reference electrode. Saturated KCl is the preferred solution for a combination electrode. Keep electrodes wet by returning them to storage solution whenever pH meter is not in use.

Before use, remove electrodes from storage solution, rinse, blot dry with a soft tissue, place in initial buffer solution, and set the isopotential point (¶ 2*a* above). Select a second buffer within 2 pH units of sample pH and bring sample and buffer to same temperature, which may be the room temperature, a fixed temperature such as 25°C, or the temperature of a fresh sample. Remove electrodes from first buffer, rinse thoroughly with distilled water, blot dry, and immerse in second buffer. Record temperature of measurement and adjust temperature dial on meter so that meter indicates pH value of buffer at test temperature (this is a slope adjustment).

Use the pH value listed in the tables for the buffer used at the test temperature. Remove electrodes from second buffer, rinse thoroughly with distilled water and dry electrodes as indicated above. Immerse in a third buffer below pH 10, approximately 3 pH units different from the second; the reading should be within 0.1 unit for the pH of the third buffer. If the meter response shows a difference greater than 0.1 pH unit from expected value, look for trouble with the electrodes or potentiometer (see ¶s 5*a* and *b* below).

The purpose of standardization is to adjust the response of the glass electrode to the instrument. When only occasional pH measurements are made standardize instrument before each measurement. When frequent measurements are made and the instrument is stable, standardize less frequently. If sample pH

TABLE 4500-H$^+$:II. STANDARD pH VALUES[3]

	Primary Standards							Secondary Standards	
Temperature °C	Tartrate (Saturated)	Citrate (0.05*M*)	Phthalate (0.05*M*)	Phosphate (1:1)	Phosphate (1:3.5)	Borax (0.01*M*)	Bicarbonate-Carbonate (0.025*M*)	Tetroxalate (0.05*M*)	Calcium Hydroxide (Saturated)
0			4.003	6.982	7.534	9.460	10.321	1.666	
5			3.998	6.949	7.501	9.392	10.248	1.668	
10			3.996	6.921	7.472	9.331	10.181	1.670	
15			3.996	6.898	7.449	9.276	10.120	1.672	
20			3.999	6.878	7.430	9.227	10.064	1.675	
25	3.557	3.776	4.004	6.863	7.415	9.183	10.014	1.679	12.454
30	3.552		4.011	6.851	7.403	9.143	9.968	1.683	
35	3.549		4.020	6.842	7.394	9.107	9.928	1.688	
37			4.024	6.839	7.392	9.093			
40	3.547		4.030	6.836	7.388	9.074	9.891	1.694	
45	3.547		4.042	6.832	7.385	9.044	9.859	1.700	
50	3.549		4.055	6.831	7.384	9.017	9.831	1.707	
55	3.554		4.070					1.715	
60	3.560		4.085					1.723	
70	3.580		4.12					1.743	
80	3.609		4.16					1.766	
90	3.650		4.19					1.792	
95	3.674		4.21					1.806	

values vary widely, standardize for each sample with a buffer having a pH within 1 to 2 pH units of the sample.

b. Sample analysis: Establish equilibrium between electrodes and sample by stirring sample to insure homogeneity; stir gently to minimize carbon dioxide entrainment. For buffered samples or those of high ionic strength, condition electrodes after cleaning by dipping them into sample for 1 min. Blot dry, immerse in a fresh portion of the same sample, and read pH.

With dilute, poorly buffered solutions, equilibrate electrodes by immersing in three or four successive portions of sample. Take a fresh sample to measure pH.

5. Trouble Shooting

a. Potentiometer: To locate trouble source disconnect electrodes and, using a short-circuit strap, connect reference electrode terminal to glass electrode terminal. Observe change in pH when instrument calibration knob is adjusted. If potentiometer is operating properly, it will respond rapidly and evenly to changes in calibration over a wide scale range. A faulty potentiometer will fail to respond, will react erratically, or will show a drift upon adjustment. Switch to the millivolt scale on which the meter should read zero. If inexperienced, do not attempt potentiometer repair other than maintenance as described in instrument manual.

b. Electrodes: If potentiometer is functioning properly, look for the instrument fault in the electrode pair. Substitute one electrode at a time and cross-check with two buffers that are about 4 pH units apart. A deviation greater than 0.1 pH unit indicates a faulty electrode. Glass electrodes fail because of scratches, deterioration, or accumulation of debris on the glass surface. Rejuvenate electrode by alternately immersing it three times each in 0.1*N* HCl and 0.1*N* NaOH. If this fails, immerse tip in KF solution for 30 s. After rejuvenation, soak in pH 7.0 buffer overnight. Rinse and store in pH 7.0 buffer. Rinse again with distilled water before use. Protein coatings can be removed by soaking glass electrodes in a 10% pepsin solution adjusted to pH 1 to 2.

To check reference electrode, oppose the emf of a questionable reference electrode against another one of the same type that is known to be good. Using an adapter, plug good reference electrode into glass electrode jack of potentiometer; then plug questioned electrode into reference electrode jack. Set meter to read millivolts and take readings with both electrodes immersed in the same electrolyte (KCl) solution and then in the same buffer solution. The millivolt readings should be 0 ± 5 mV for both solutions. If different electrodes are used, i.e., silver: silver-chloride against calomel or vice versa, the reading will be 44 ± 5 mV for a good reference electrode.

Reference electrode troubles generally are traceable to a clogged junction. Interruption of the continuous trickle of electrolyte through the junction causes increase in response time and drift in reading. Clear a clogged junction by applying suction to the tip or by boiling tip in distilled water until the electrolyte flows freely when suction is applied to tip or pressure is applied to the fill hole. Replaceable junctions are available commercially.

6. Precision and Bias

By careful use of a laboratory pH meter with good electrodes, a precision of ±0.02 pH unit and an accuracy of ±0.05 pH unit can be achieved. However, ±0.1 pH unit represents the limit of accuracy under normal conditions, especially for measurement of water and poorly buffered solutions. For this reason, report pH values to the nearest 0.1 pH unit. A synthetic sample of a Clark and Lubs buffer solution of pH 7.3 was analyzed electrometrically by 30 laboratories with a standard deviation of ±0.13 pH unit.

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4500-I IODINE*

4500-I A. Introduction

1. Uses and Forms

Elemental iodine is not a natural constituent of natural waters. Iodine may be added to potable and swimming pool waters as a disinfectant. For wastewaters, iodine has had limited application. Use of iodine generally is restricted to personal or remote water supplies where ease of application, storage stability, and an inertness toward organic matter are important considerations. Some swimming pool waters are treated with iodine to lessen eye burn among swimmers and to provide a stable disinfectant residual less affected by adverse environmental conditions.

Iodine is applied in the elemental form or produced in situ by the simultaneous addition of an iodide salt and a suitable oxidant. In the latter case, an excess of iodide may be maintained to serve as a reservoir for iodine production; the determination of iodide is desirable for disinfectant control (see Iodide, Section 4500-I^-).

Elemental I_2 can undergo hydrolysis to form hypoiodous acid (HOI), which can dissociate to form hypoiodite (OI^-) under strongly basic conditions. Hypoiodous acid/hypoiodite ion may further disproportionate to form iodate. In the presence of excess iodide, iodine may react with iodide to form tri-iodide ion (I_3^-). The rate and the extent to which these reactions may occur depend on pH and the concentration of iodide in the solution. Basic conditions favor formation of hypoiodite and iodate. Acidic conditions and the presence of iodide favor formation of iodine and tri-iodide ion. Thus, the relative concentrations of these iodine species in the resulting solution can be quite variable. Hypoiodous acid/hypoiodite also can act as an iodinating agent, reacting with organic compounds to form iodinated organic compounds. Elemental I_2, hypoiodous acid, hypoiodite ion, and tri-iodide ion are considered active iodine. There is no generally accepted method for the determination of each of these species individually. Most analytical methods use the oxidizing power of all forms of active iodine for its determination and the results usually are expressed as an equivalent concentration of elemental iodine. The effects of iodate or dissolved organic iodine on these methods have not been thoroughly investigated.

2. Selection of Method

For potable and swimming pool waters treated with elemental iodine, both the amperometric titration and leuco crystal violet colorimetric methods give acceptable results. However, oxidized forms of manganese interfere with the leuco crystal violet method. Where the iodide and chloride ion concentrations are above 50 mg/L and 200 mg/L, respectively, interference in color production may occur in the leuco crystal violet method and the amperometric method is preferred. However, because of the extreme sensitivity of the leuco crystal violet method, this interference may be eliminated by sample dilution to obtain halogen ion concentrations less than 50 mg/L.

For wastewaters or highly polluted waters, organic constituents normally do not interfere with either the amperometric or leuco crystal violet procedures. Determine which of the methods yields the more acceptable results, because specific substances present in these waters may interfere in one method but not in the other. Certain metallic cations such as copper and silver interfere in the amperometric titration procedure. Iodate, which is a naturally occurring species of iodine in marine waters, will also interfere in the amperometric titration by reacting with excess iodide under acidic conditions to form I_2 and/or I_3^-. The rate of the reaction is most pH-dependent between pH 3 and 5. Thus, the magnitude of this interference may depend on the concentration of iodate present and the analytical conditions. The leuco crystal violet method is relatively free of interference from these and other cations and anions with the exceptions noted previously.

For waters containing iodine coexisting with free chlorine, combined chlorine, or other excess oxidants, of the methods described only the leuco crystal violet method can determine iodine specifically. This condition occurs in the in-situ production of iodine by the reaction of iodide and excess oxidant. Under these conditions, the amperometric method would continue to titrate the iodine produced in a cyclic reaction until exhaustion of the oxidant.

* Approved by Standard Methods Committee, 1997.
Joint Task Group: 20th Edition—George T.F. Wong (chair), Ling-Su Zhang.

4500-I B. Leuco Crystal Violet Method

1. General Discussion

The leuco crystal violet method determines aqueous iodine present as elemental iodine and hypoiodous acid. Excess common oxidants do not interfere. While the method utilizes the sum of the oxidative power of all forms of active iodine residuals, the results are expressed as the equivalent concentration of iodine. The method also is capable of determining the sum of iodine and free iodide concentrations; the free iodide concentration can be determined by difference (see Iodide, Section 4500-I^-).

a. Principle: Mercuric chloride added to aqueous elemental iodine solutions causes essentially complete hydrolysis of iodine and the stoichiometric production of hypoiodous acid. The compound 4,4′,4″-methylidynetris (*N,N*-dimethylani-

line), also known by the common name of leuco crystal violet, reacts instantaneously with the hypoiodous acid to form crystal violet dye. The absorbance of this dye is highly pH-dependent. The maximum absorbance is produced in the pH range of 3.5 to 4.0 and is measured at a wavelength of 592 nm. Below a pH of 3.5, the absorbance drops precipitously. Above a pH of about 4.7, the excess leuco crystal violet in the sample precipitates and masks the absorbance of the crystal violet dye. Accurate pH control is essential to maximize precision. The absorbance follows Beer's law over a wide range of iodine concentrations and the developed color is stable for several hours.

In the presence of certain excess oxidants such as free chlorine or chloramines, the iodine residual will exist exclusively in the form of hypoiodous acid. The leuco crystal violet is relatively insensitive to the combined forms of chlorine while any free chlorine is converted to chloramine by reaction with an ammonium salt incorporated in the test reagents. All the hypoiodous acid is determined. As hypoiodous acid, the weight concentration value found, expressed as an equivalent elemental I_2 concentration, is equal to twice that of an elemental I_2 solution of the same weight concentration.

b. Interference: Oxidized forms of manganese interfere by oxidizing the indicator to crystal violet dye and yield apparent high iodine concentrations.

Iodide and chloride ion concentrations above 50 mg/L and 200 mg/L, respectively, interfere by inhibiting full color production. Dilute the sample to eliminate this interference.

Combined chlorine residuals normally do not interfere provided that the test is completed within 5 min after adding the indicator solution. Eliminate interference from free chlorine by adding an ammonium salt buffer to form combined chlorine.

c. Minimum detectable concentration: 10 μg I as I_2/L.

2. Apparatus

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

1) *Filter photometer,* with a light path of 1 cm or longer, equipped with an orange filter having maximum transmittance near 592 nm.

2) *Spectrophotometer,* for use at 592 nm, with a light path of 1 cm or longer.

b. Volumetric flasks, 100-mL, with plastic caps or ground-glass stoppers.

c. Glassware: Completely remove reducing substances from glassware or plastic containers, including containers for storage of reagent solutions (see Section 4500-Cl.D.2*d*).

3. Reagents

a. Iodine-demand-free water: See Section 4500-I^-.B.3*a*. Prepare all stock iodine and reagent solutions with iodine-demand-free water.

b. Stock iodine solution: Prepare a saturated iodine solution by dissolving 20 g elemental iodine in 300 mL water. Let stand several hours. Decant iodine solution and dilute 170 mL to 2000 mL. Standardize solution by titrating with standard sodium thiosulfate ($Na_2S_2O_3$) titrant as described in Section 4500-Cl.B.3*b* and *c* or amperometrically as in Section 4500-I.C.

Calculate iodine concentration:

$$\text{mg I as } I_2\text{/mL} = \text{normality of iodine solution} \times 126.9$$

Prepare a working solution of 10 μg I as I_2/mL by appropriate dilution of the standardized stock solution.

c. Citric buffer solution, pH 3.8: See Section 4500-I^-.B.3*c*.

d. Leuco crystal violet indicator: See Section 4500-I^-.B.3*d*.

e. Sodium thiosulfate solution: See Section 4500-I^-.B.3*f*.

4. Procedure

a. Preparation of temporary iodine standards: For greater accuracy, standardize working solution immediately before use by the amperometric titration method (Method C). Prepare standards in the range of 0.1 to 6.0 mg I as I_2/L by adding 1 to 60 mL working solution to 100 mL glass-stoppered volumetric flasks, in increments of 1 mL or larger. Adjust these volumes if the measured iodine concentration of working solution varies by 5% or more from 10 μg I as I_2/mL.

Measure 50.0 mL of each diluted iodine working solution into a 100-mL glass-stoppered volumetric flask. Add 1.0 mL citric buffer solution, gently swirl to mix, and let stand for at least 30 s. Add 1.0 mL leuco crystal violet indicator and swirl to develop color. Dilute to 100 mL and mix.

b. Photometric calibration: Transfer colored temporary standards of known iodine concentrations to cells of 1-cm light path and read absorbance in a photometer or spectrophotometer at a wavelength of 592 nm against a distilled water reference. Plot absorbance values against iodine concentrations to construct a curve that follows Beer's law.

c. Color development of iodine sample: Measure 50.0 mL sample into a 100-mL volumetric flask and treat as described for preparation of temporary iodine standards, ¶ 4*a*. Match test sample visually with temporary standards or read absorbance photometrically and refer to standard calibration curve for the iodine equivalent.

d. Samples containing >6.0 mg I as I_2/L: Place approximately 25 mL water in a 100-mL volumetric flask. Add 1.0 mL citric buffer solution and a measured volume of 25 mL or less of sample. Mix and let stand for at least 30 s. Add 1.0 mL leuco crystal violet indicator, mix, and dilute to mark. Match visually with standards or read absorbance photometrically and compare with calibration curve from which the initial iodine is obtained by applying the dilution factor. Select one of the following sample volumes to remain within optimum iodine range:

Iodine *mg/L*	Sample Volume Required *mL*
6.0–12.0	25.0
12.0–30	10.0
30–60	5.0

e. Samples containing both chlorine and iodine: For samples containing free or combined chlorine and iodine, follow procedure given in ¶ 4*c* or *d* above but read absorbance within 5 min after adding leuco crystal violet indicator.

f. Compensation for turbidity and color: Compensate for natural color or turbidity by adding 5 mL $Na_2S_2O_3$ solution to a

50-mL sample. Add reagents to sample as described previously and use as blank to set zero absorbance on the photometer. Measure all samples in relation to this blank and, from calibration curve, determine concentrations of iodine.

4500-I C. Amperometric Titration Method

1. General Discussion

The amperometric titration method for iodine is a modification of the amperometric method for residual chlorine (see Section 4500-Cl.D). Iodine residuals over 7 mg/L are best measured with smaller samples or by dilution. In most cases the titration results represent free iodine because combined iodine rarely is encountered.

a. Principle: The principle of the amperometric method as described for the determination of total residual chlorine is applicable to the determination of residual iodine. Iodine is determined using buffer solution, pH 4.0, and potassium iodide (KI) solution. Maintain pH at 4.0 because at pH values less than 3.5 substances such as oxidized forms of manganese interfere, while at pH values greater than 4.5, the reaction is not quantitative. Adding KI improves the sharpness of the end point.

b. Interference: Free chlorine and the interferences described in Section 4500-Cl.D.1*b* also interfere in the iodine determination.

2. Apparatus

See Section 4500-Cl.D.2*a* through *d*.

3. Reagents

With the exception of phosphate buffer solution, pH 7.0, all reagents listed for the determination of residual chlorine in Section 4500-Cl.D.3 are required. Standardized phenylarsine oxide solution (1 mL = 1 mg chlorine/L for a 200-mL sample) is equivalent to 3.58 mg I as I_2/mL for a 200-mL sample.

4. Procedure

a. Sample volume: Select a sample volume that will require no more than 2 mL phenylarsine oxide titrant. For iodine concentrations of 7 mg/L or less, take a 200-mL volume; for iodine levels above 7 mg/L, use 100 mL or proportionately less diluted to 200 mL with water.

b. Free iodine: To the sample add 1 mL KI solution and 1 mL acetate buffer, pH 4.0 solution. Titrate with phenylarsine oxide titrant to the end point described in Section 4500-Cl.D.4.

5. Calculation

Calculate the iodine concentration by the following equation:

$$\text{mg I as } I_2/\text{L} = \frac{A \times 3.58 \times 200}{\text{mL sample}}$$

where:

A = mL phenylarsine oxide titration to the end point.

6. Bibliography

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5. Bibliography

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4500-I⁻ IODIDE*

4500-I⁻ A. Introduction

1. Occurrence

Iodide is found in natural waters at concentrations ranging from 40 μg I⁻/L in coastal surface seawater to <1 μg I⁻/L in deep ocean water and fresh water. Higher concentrations may be found in brines, certain industrial wastes, and waters treated with iodine. Iodide is thermodynamically unstable relative to iodate in oxygenated waters.

2. Selection of Method

The leuco crystal violet method (B) is applicable to iodide concentrations of 50 to 6000 μg/L. The catalytic reduction method (C) is applicable to iodide concentrations of 80 μg I⁻/L

* Approved by Standard Methods Committee, 1997.
Joint Task Group: 20th Edition—George T.F. Wong (chair), Ling-Su Zhang.

or less. The voltammetric method (D) is the most sensitive method. It can be used for samples with iodide concentrations of 0.13 to 10.2 μg I^-/L. It is also species-specific. It is insensitive to iodate, iodine, and most organic iodine compounds. It requires minimal sample manipulation, aside from an occasional dilution for samples with high concentrations of iodide. Thus, the concentrations of iodide in many types of water samples may be determined directly with the voltammetric method.

The choice of method depends on the sample and concentration to be determined. The high chloride concentrations of brines, seawater, and many estuarine waters will interfere with color development in the leuco crystal violet method. In the presence of iodine, the leuco crystal violet method gives the sum of iodine and iodide. Iodide may be determined by the difference after concentration of iodine has been estimated independently (see Section 4500-I). In the catalytic reduction method, As(III), under acidic conditions, is a strong reducing agent and will reduce the oxidized forms of iodine to iodide. Thus, this method measures not only iodide, but also the sum of all the inorganic iodine species including iodide, iodate, hypoiodous acid, hypoiodite ion, and elemental iodine. Because iodate is the thermodynamically stable form of dissolved iodine in oxygenated natural waters and is frequently the dominant species of dissolved iodine, the catalytic reduction method is likely to overestimate the concentration of iodide. This method works well only under exactly reproducible conditions.

4500-I^- B. Leuco Crystal Violet Method

1. General Discussion

a. Principle: Iodide is selectively oxidized to iodine by the addition of potassium peroxymonosulfate, $KHSO_5$. The iodine produced reacts instantaneously with the colorless indicator reagent containing 4,4′,4″-methylidynetris (*N,N*-dimethylaniline), also known as leuco crystal violet, to produce the highly colored crystal violet dye. The developed color is sufficiently stable for the determination of an absorbance value and adheres to Beer's law over a wide range of iodine concentrations. Absorbance is highly pH-dependent, and must be measured within the pH range of 3.5 to 4.0 at a wavelength of 592 nm. Accurate control of pH is essential for maximum precision. (See Section 4500-I.B.1*a*.) Follow the general principles for quality control (Section 4020).

b. Interference: Chloride concentrations greater than 200 mg/L may interfere with color development. Reduce these interferences by diluting sample to contain less than 200 mg Cl^-/L.

2. Apparatus

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

1) *Filter photometer,* providing a light path of 1 cm or longer, equipped with an orange filter having maximum transmittance near 592 nm.

2) *Spectrophotometer,* for use at 592 nm, providing a light path of 1 cm or longer.

b. Volumetric flasks: 100-mL with plastic caps or ground-glass stoppers.

c. Glassware: Completely remove any reducing substances from all glassware or plastic containers, including containers for storing reagent solutions (see Section 4500-Cl.D.2*d*).

3. Reagents

a. Iodine-demand-free water: Prepare a 1-m ion-exchange column of 2.5 to 5 cm diam, containing strongly acid cation and strong basic anion exchange resins. If a commercial analytical-grade mixed-bed resin is used, verify that compounds that react with iodine are removed. Pass distilled water at a slow rate through the resin bed and collect in clean container that will protect the treated water from undue exposure to the atmosphere.

Prepare all stock iodide and reagent solutions with iodine-demand-free water.

b. Stock iodide solution: Dissolve 1.3081 g KI in water and dilute to 1000 mL; 1 mL = 1 mg I^-.

c. Citric buffer solution, pH 3.8:

1) *Citric acid:* Dissolve 192.2 g $C_6H_8O_7$ or 210.2 g $C_6H_8O_7 \cdot H_2O$ and dilute to 1 L with water.

2) *Ammonium hydroxide, 2N:* Add 131 mL conc NH_4OH to about 700 mL water and dilute to 1 L. Store in a polyethylene bottle.

3) *Final buffer solution:* Slowly add, with mixing, 350 mL 2*N* NH_4OH solution to 670 mL citric acid. Add 80 g ammonium dihydrogen phosphate ($NH_4H_2PO_4$) and stir to dissolve.

d. Leuco crystal violet indicator: Measure 200 mL water and 3.2 mL conc sulfuric acid (H_2SO_4) into a brown glass container of at least 1-L capacity. Introduce a magnetic stirring bar and mix at moderate speed. Add 1.5 g 4,4′,4″-methylidynetris (*N,N*-dimethylaniline)* and with a small amount of water wash down any reagent adhering to neck or sides of container. Mix until dissolved.

To 800 mL water, add 2.5 g mercuric chloride ($HgCl_2$) and stir to dissolve. With mixing, add $HgCl_2$ solution to leuco crystal violet solution. For maximum stability, adjust pH of final solution to 1.5 or less, adding, if necessary, conc H_2SO_4 dropwise. Store in a brown glass bottle away from direct sunlight. Discard after 6 months. Do not use a rubber stopper.

e. Potassium peroxymonosulfate solution: Obtain $KHSO_5$ as a commercial product,† which is a stable powdered mixture containing 42.8% $KHSO_5$ by weight and a mixture of $KHSO_4$ and K_2SO_4. Dissolve 1.5 g powder in water and dilute to 1 L.

f. Sodium thiosulfate solution: Dissolve 5.0 g $Na_2S_2O_3 \cdot 5H_2O$ in water and dilute to 1 L.

* Eastman chemical No. 3651 or equivalent.

† Oxone, E.I. duPont de Nemours and Co., Inc., Wilmington, DE, or equivalent.

4. Procedure

a. Preparation of temporary iodine standards: Add suitable portions of stock iodide solution, or of dilutions of stock iodide solution, to water to prepare a series of 0.1 to 6.0 mg I^-/L in increments of 0.1 mg/L or larger.

Measure 50.0 mL dilute KI standard solution into a 100-mL glass-stoppered volumetric flask. Add 1.0 mL citric buffer and 0.5 mL $KHSO_5$ solution. Swirl to mix and let stand approximately 1 min. Add 1.0 mL leuco crystal violet indicator, mix, and dilute to 100 mL. For best results, read absorbance as described below within 5 min after adding indicator solution.

b. Photometric calibration: Transfer colored temporary standards of known iodide concentrations to cells of 1-cm light path and read absorbance in a photometer or spectrophotometer at a wavelength of 592 nm against a water reference. Plot absorbance values against iodide concentrations to construct a curve that follows Beer's law.

c. Color development of sample: Measure a 50.0-mL sample into a 100-mL volumetric flask and treat as described for preparation of temporary iodine standards, ¶ 4*a*. Read absorbance photometrically and refer to standard calibration curve for iodide equivalent.

d. Samples containing >6.0 mg I^-/L: Place approximately 25 mL water in a 100-mL volumetric flask. Add 1.0 mL citric buffer and a measured volume of 25 mL or less of sample. Add 0.5 mL $KHSO_5$ solution. Swirl to mix and let stand approximately 1 min. Add 1.0 mL leuco crystal violet indicator, mix, and dilute to 100 mL.

Read absorbance photometrically and compare with calibration curve from which the initial iodide concentration is obtained by applying the dilution factor. Select one of the following sample volumes to remain within the optimum iodide range.

Iodide *mg/L*	Sample Volume Required *mL*
6.0–12.0	25.0
12.0–30	10.0
30–60	5.0

e. Determination of iodide in the presence of iodine: On separate samples determine (1) total iodide and iodine, and (2) iodine. The iodide concentration is the difference between the iodine determined and the total iodine-iodide obtained. Determine iodine by not adding $KHSO_5$ solution in the iodide method and by comparing the absorbance value to the calibration curve developed for iodide.

f. Compensation for turbidity and color: Compensate for natural color or turbidity by adding 5 mL $Na_2S_2O_3$ solution to a 50-mL sample. Add reagents to sample as described previously and use as the blank to set zero absorbance on photometer. Measure all samples in relation to this blank and, from the calibration curve, determine concentrations of iodide or total iodine-iodide.

5. Bibliography

BLACK, A.P. & G.P. WHITTLE. 1967. New methods for the colorimetric determination of halogen residuals. Part I. Iodine, iodide, and iodate. *J. Amer. Water Works Assoc.* 59:471.

4500-I⁻ C. Catalytic Reduction Method

1. General Discussion

a. Principle: Iodide can be determined by using its ability to catalyze the reduction of ceric ions by arsenious acid. The effect is nonlinearly proportional to the amount of iodide present. The reaction is stopped after a specific time interval by the addition of ferrous ammonium sulfate. The resulting ferric ions are directly proportional to the remaining ceric ions and develop a relatively stable color complex with potassium thiocyanate.

Pretreatment by digestion with chromic acid and distillation is necessary to estimate the nonsusceptible bound forms of iodine.

b. Interferences: The formation of noncatalytic forms of iodine and the inhibitory effects of silver and mercury are reduced by adding an excess of sodium chloride (NaCl) that sensitizes the reaction. Iodate, hypoiodous acid/hypoiodite ion, and elemental iodine interfere. Under acidic conditions, As(III) may reduce these forms of inorganic iodine to iodide and include them as iodide in the subsequent detection of iodide.

2. Apparatus

a. Water bath, capable of temperature control to 30 ± 0.5°C.

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

1) *Spectrophotometer,* for use at wavelengths of 510 or 525 nm and providing a light path of 1 cm.

2) *Filter photometer,* providing a light path of 1 cm and equipped with a green filter having maximum transmittance near 525 nm.

c. Test tubes, 2 × 15 cm.

d. Stopwatch.

3. Reagents

Store all stock solutions in tightly stoppered containers in the dark. Prepare all reagent solutions in distilled water.

a. Distilled water, containing less than 0.3 µg total I/L.

b. Sodium chloride solution: Dissolve 200.0 g NaCl in water and dilute to 1 L. Recrystallize the NaCl if an interfering amount of iodine is present, using a water-ethanol mixture.

c. Arsenious acid: Dissolve 4.946 g As_2O_3 in water, add 0.20 mL conc H_2SO_4, and dilute to 1000 mL.

d. Sulfuric acid, H_2SO_4, conc.

e. Ceric ammonium sulfate: Dissolve 13.38 g $Ce(NH_4)_4(SO_4)_4 \cdot 4H_2O$ in water, add 44 mL conc H_2SO_4, and make up to 1 L.

f. Ferrous ammonium sulfate reagent: Dissolve 1.50 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 100 mL distilled water containing 0.6 mL conc H_2SO_4. Prepare daily.

g. Potassium thiocyanate solution: Dissolve 4.00 g KSCN in 100 mL water.

h. Stock iodide solution: Dissolve 261.6 mg anhydrous KI in water and dilute to 1000 mL; 1.00 mL = 200 μg I^-.

i. Intermediate iodide solution: Dilute 20.00 mL stock iodide solution to 1000 mL with water; 1.00 mL = 4.00 μg I^-.

j. Standard iodide solution: Dilute 25.00 mL intermediate iodide solution to 1000 mL with water; 1.00 mL = 0.100 μg I^-.

4. Procedure

a. Sample size: Add 10.00 mL sample, or a portion made up to 10.00 mL with water, to a 2- × 15-cm test tube. If possible, keep iodide content in the range 0.2 to 0.6 μg. Use thoroughly clean glassware and apparatus.

b. Color measurement: Add reagents in the following order: 1.00 mL NaCl solution, 0.50 mL As_2O_3 solution, and 0.50 mL conc H_2SO_4.

Place reaction mixture and ceric ammonium sulfate solution in 30°C water bath and let come to temperature equilibrium. Add 1.0 mL ceric ammonium sulfate solution, mix by inversion, and start stopwatch to time reaction. Use an inert clean test tube stopper when mixing. After 15 ± 0.1 min remove sample from water bath and add immediately 1.00 mL ferrous ammonium sulfate reagent with mixing, whereupon the yellow ceric ion color should disappear. Then add, with mixing, 1.00 mL KSCN solution. Replace sample in water bath. Within 1 h after adding thiocyanate read absorbance in a photometric instrument. Maintain temperature of solution and cell compartment at 30 ± 0.5°C until absorbance is determined. If several samples are run, start reactions at 1-min intervals to allow time for additions of ferrous ammonium sulfate and thiocyanate. (If temperature control of cell compartment is not possible, let final solution come to room temperature and measure absorbance with cell compartment at room temperature.)

c. Calibration standards: Treat standards containing 0, 0.2, 0.4, 0.6, and 0.8 μg I^-/10.00 mL of solution as in ¶ 4*b* above. Run with each set of samples to establish a calibration curve.

5. Calculation

$$\text{mg } I^-/\text{L} = \frac{\mu\text{g I (in 15 mL final volume)}}{\text{mL sample}}$$

6. Precision and Bias

Results obtained by this method are reproducible on samples of Los Angeles source waters, and have been reported to be accurate to ±0.3 μg I^-/L on samples of Yugoslavian water containing from 0 to 14.0 μg I^-/L. Follow general principles for quality control (Section 4020).

7. Bibliography

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4500-I^- D. Voltammetric Method

1. General Discussion

a. Principle: Iodide is deposited onto the surface of a static mercury drop electrode (SMDE) as mercurous iodide under an applied potential for a specified period of time. The deposited mercurous iodide is reduced by a cathodic potential scan. This reaction gives rise to a current peak at about −0.33 V relative to the saturated calomel electrode. The height of the current peak is directly proportional to the concentration of iodide in solution, which is quantified by the method of internal standard additions.

b. Interferences: Sulfide can interfere. Remove it as hydrogen sulfide by acidifying the sample and then purging it with air. Adjust pH of sample back to about pH 8 before analysis.

2. Apparatus

a. Voltammetric analyzer system, consisting of a potentiostat, static mercury drop electrode (SMDE), stirrer, and plotter, that can be operated in the cathodic stripping square wave voltammetry-SMDE mode with adjustable deposition potential, deposition time, equilibration time, scan rate, scan range, scan increment, pulse height, frequency, and drop size.

A saturated calomel electrode is used as the reference electrode through a salt bridge.

b. Glassware: Wash glassware and other surfaces contacting the sample or reagents with 10% (v/v) HCl (low in iodide); thoroughly rinse with reagent water (see Section 1080) before use.

3. Reagents

Use chemicals low in iodide whenever available.

a. Oxygen-free water: Remove oxygen in reagent water (see Section 1080) by bubbling it with argon gas while boiling it for 20 min in an erlenmeyer flask. Let water cool while argon bubbling continues. Tightly stopper flask and store water under nitrogen. Prepare water immediately before use.

b. Alkaline pyrogallol solution: Dissolve 30 g pyrogallol in 200 mL oxygen-free water. Dissolve 120 g potassium hydroxide (KOH) in 400 mL oxygen-free water. Mix 300 mL KOH solution with 100 mL pyrogallol solution.

c. Sodium sulfite solution, 1M: Dissolve 1.26 g sodium sulfite, Na_2SO_3, in oxygen-free water and dilute to 10 mL.

d. Sodium sulfite solution, 0.1M: Dilute 5.0 mL 1*M* sodium sulfite solution to 50 mL. Prepare fresh daily.

e. Oxygen-free argon gas: Bubble argon gas (at least 99.99% pure) through a series of three traps containing, respectively, alkaline pyrogallol solution, 0.1*M* sodium sulfite solution, and oxygen-free water.

f. Standard iodide solution: Dry several grams potassium iodide, KI, in an oven at 80°C overnight. Dissolve 1.660 g dried KI in reagent water (see Section 1080) and dilute to 500 mL. Dilute 5 mL solution to 500 mL, and dilute 5 mL of the latter solution to 500 mL.

g. Polyethylene glycol p-*isooctylphenyl ether (PEG-IOPE) solution,* 0.2%: Dilute 0.2 mL commercially available reagent* to 100 mL in reagent water (see Section 1080).

4. Procedure

a. Sample measurement: Transfer 10 mL sample, 0.05 mL PEG-IOPE solution, and 0.2 mL 1*M* Na_2SO_3 solution (which also acts as the supporting electrolyte in fresh-water samples) to polarographic cell containing a magnetic stirrer. Purge solution with oxygen-free argon gas for 1 min. Set electrode at SMDE mode. Record a voltammogram in the cathodic stripping square wave voltammetry mode under the following conditions: deposition potential, −0.15 V; deposition time, 60 s; equilibration time, 5 s; scan rate, 200 mV/s; scan range, 0.15 to −0.6 V; scan increment, 2 mV; pulse height, 20 mV; frequency, 100 Hz; and the largest drop size. Measure magnitude of current peak above baseline at center of peak at an applied potential of about −0.33 V relative to saturated calomel electrode in the voltammogram.

b. Internal standard additions: Add 0.1 mL 2 μM standard KI solution to the cell. Purge solution with oxygen-free argon gas for 0.5 min. Record a voltammogram under conditions described in ¶ 4*a* and again determine magnitude of current peak. Repeat procedure twice, for a total of three additions.

c. Blank determination: Determine method reagent blank by treating reagent water as a sample.

5. Calculation

For the *j*th addition of the standard KI ($j = 0, 1, 2, 3$), compute the following variables:

$$Y_j = I_j (V_x + jV_s + V_c)$$

$$X_j = jV_sC_s$$

where:

I_j = height of *j*th peak, nA,
V_x = sample volume, mL,
V_s = volume of standard KI added during each internal addition, mL,
V_c = total volume of PEG-IOPE solution and sodium sulfite added during analysis, mL, and
C_s = concentration of iodide in standard KI solution, n*M*.

Determine slope, *B*, and intercept, *A*, of line relating Y_j to X_j by linear least squares method. Calculate concentration of iodide in sample as:

$$C_x = \frac{A}{B \times V_x}$$

where:

C_x = concentration of iodide, n*M*, and other terms are as defined above.

If there is a reagent blank, subtract the reagent blank from C_x to get true concentration in sample.

Multiply C_x (or blank-corrected C_x) by 0.1269 to obtain concentration in μg/L.

6. Precision

In one laboratory, using seawater samples with a concentration of iodide of about 6 μg I/L, the precision was about ±5%. Follow general principles of quality control as in Section 4020.

7. Bibliography

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* Triton X-100, Catalog No. T9284, Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178.

4500-IO_3^- IODATE*

4500-IO_3^- A. Introduction

1. Occurrence

Iodate is found in natural waters at concentrations ranging from 60 μg I/L in deep ocean water to undetectable (3 μg I/L) in estuarine water and fresh water. Iodate is the thermodynamically stable form of dissolved inorganic iodine in waters containing dissolved oxygen; it is absent in anoxic waters.

2. Selection of Method

The differential pulse polarographic method is species-specific and highly sensitive. It is applicable to iodate concentrations of 3 to at least 130 μg I/L and can determine iodate in the presence of other iodine species such as iodide and organic iodine. It can be used for the direct determination of iodate in many types of water samples.

3. Sampling and Storage

Collect representative samples in clean glass or plastic bottles. Clean sample bottles with 10% (v/v) hydrochloric acid (low in iodate) and thoroughly rinse them with reagent water (see Section 1080) before use. Most samples can be analyzed directly without further treatment. Highly turbid samples may be filtered through glass fiber filters before analysis. For storage of up to 2 d, refrigerate sample at 4°C. For longer storage, freeze sample and store at or below −5°C. Frozen samples can be stored for at least 1 month.

* Approved by Standard Methods Committee, 1997.
Joint Task Group: 20th Edition—George T.F. Wong (chair), Ling-Su Zhang.

4500-IO_3^- B. Polarographic Method

1. General Discussion

a. Principle: Under mildly basic conditions, iodate is reduced to iodide at a dropping mercury electrode by a cathodic potential scan. This reaction gives rise to a current peak centered around −1.1 V relative to the saturated calomel electrode. The height of the current peak is directly proportional to the concentration of iodate, which is quantified by the method of standard additions.

b. Interferences: Dissolved oxygen and zinc interfere. Remove dissolved oxygen by bubbling oxygen-free argon gas through sample and by reacting oxygen with added sodium sulfite. Remove interference from zinc by complexing with EDTA (ethylene diaminetetraacetate).

2. Apparatus

a. Polarographic analyzer system: A polarographic analyzer system, consisting of a potentiostat, a static mercury drop electrode (SMDE), a stirrer, and a plotter, that can be operated in the difference pulse polarography-SMDE mode with adjustable drop time, scan increment, pulse height, scan range, and drop size.

Use a saturated calomel electrode as the reference electrode.

b. Glassware: Acid-wash glassware and other surfaces contacting sample or reagents with 10% (v/v) HCl (low in iodate); thoroughly rinse with reagent water (see Section 1080) before use.

3. Reagents

Use chemicals low in iodate whenever available.

a. Oxygen-free water: See Section 4500-I^-.D.3*a*.

b. Alkaline pyrogallol solution: See Section 4500-I^-.D.3*b*.

c. Oxygen-free argon gas: See Section 4500-I^-.D.3*e*.

d. Sodium sulfite solution: See Section 4500-I^-.D.3*c*.

e. Standard iodate solution, 25 μM: Dry several grams potassium iodate, KIO_3, in an oven at 80°C overnight. Dissolve 1.070 g dried KIO_3 in reagent water (see Section 1080) and dilute to 1000 mL. Dilute 5 mL of this solution to 1000 mL.

f. Na_2EDTA solution, 0.1M: Dissolve 3.722 g $Na_2EDTA \cdot 2H_2O$ (disodium ethylenediaminetetraacetate) in reagent water (see Section 1080) and dilute to 100 mL.

g. Supporting electrolyte: Dissolve 54.8 g sodium chloride, 0.30 g potassium bromide, and 1.05 g sodium bicarbonate in reagent water (see Section 1080) to form a final volume of 250 mL.

4. Procedure

a. Sample measurement: Transfer 5 mL sample and 0.5 mL supporting electrolyte to polarographic cell containing a magnetic stirrer. Check pH of solution to make sure it is about 8. (For marine waters with salinities above 15, the supporting electrolyte is not needed.) Remove dissolved oxygen by bubbling sample rigorously with oxygen-free argon gas for 0.5 min with stirring. Add 0.1 mL 1*M* sodium sulfite solution to sample and purge, with stirring, with oxygen-free argon gas for one additional minute. Add 0.01 mL 0.1*M* disodium EDTA and purge, with stirring, with oxygen-free argon for another 0.5 min. Set electrode in the SMDE mode. Record a polarogram in the differential pulse polarography mode under the following conditions: drop time, 1 s; scan increment, 6 mV; pulse height, 0.06 V; and scan range, −0.65 to −1.35 V. Use a medium drop size that allows

mercury droplets to be formed and dislodged from the dropping mercury electrode at a steady and consistent rate. Measure height of current peak above base line at an applied potential of about −1.1 V relative to the saturated calomel electrode.

b. Internal standard additions: Add 0.05 mL 25 μM standard iodate solution to cell. Purge solution, with stirring, with oxygen-free argon gas for 0.5 min.

Record a polarogram under conditions described in ¶ 4*a* and determine height of current peak again. Repeat this procedure two additional times.

c. Blank determination: Determine method reagent blank by treating reagent water as a sample.

5. Calculation

Follow calculations given in Section 4500-I^-.D.5, with substitution of appropriate compounds in the definitions of terms.

6. Precision

In one laboratory, analyzing seawater samples with a concentration of iodate of 60 μg I/L, the precision was about ±3%. Follow general principles for quality control (see Section 4020).

7. Bibliography

HERRING, J.R. & P.S. LISS. 1974. A new method for the determination of iodine species in seawater. *Deep-Sea Res.* 21:777.

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4500-N NITROGEN*

4500-N A. Introduction

In waters and wastewaters the forms of nitrogen of greatest interest are, in order of decreasing oxidation state, nitrate, nitrite, ammonia, and organic nitrogen. All these forms of nitrogen, as well as nitrogen gas (N_2), are biochemically interconvertible and are components of the nitrogen cycle. They are of interest for many reasons.

Organic nitrogen is defined functionally as organically bound nitrogen in the trinegative oxidation state. It does not include all organic nitrogen compounds. Analytically, organic nitrogen and ammonia can be determined together and have been referred to as "kjeldahl nitrogen," a term that reflects the technique used in their determination. Organic nitrogen includes such natural materials as proteins and peptides, nucleic acids and urea, and numerous synthetic organic materials. Typical organic nitrogen concentrations vary from a few hundred micrograms per liter in some lakes to more than 20 mg/L in raw sewage.

Total oxidized nitrogen is the sum of nitrate and nitrite nitrogen. Nitrate generally occurs in trace quantities in surface water but may attain high levels in some groundwater. In excessive amounts, it contributes to the illness known as methemoglobinemia in infants. A limit of 10 mg nitrate as nitrogen/L has been imposed on drinking water to prevent this disorder. Nitrate is found only in small amounts in fresh domestic wastewater but in the effluent of nitrifying biological treatment plants nitrate may be found in concentrations of up to 30 mg nitrate as nitrogen/ L. It is an essential nutrient for many photosynthetic autotrophs and in some cases has been identified as the growth-limiting nutrient.

Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate and in the reduction of nitrate. Such oxidation and reduction may occur in wastewater treatment plants, water distribution systems, and natural waters. Nitrite can enter a water supply system through its use as a corrosion inhibitor in industrial process water. Nitrite is the actual etiologic agent of methemoglobinemia. Nitrous acid, which is formed from nitrite in acidic solution, can react with secondary amines (RR′NH) to form nitrosamines (RR′N-NO), many of which are known to be carcinogens. The toxicologic significance of nitrosation reactions in vivo and in the natural environment is the subject of much current concern and research.

Ammonia is present naturally in surface and wastewaters. Its concentration generally is low in groundwaters because it adsorbs to soil particles and clays and is not leached readily from soils. It is produced largely by deamination of organic nitrogen-containing compounds and by hydrolysis of urea. At some water treatment plants ammonia is added to react with chlorine to form a combined chlorine residual. Ammonia concentrations encountered in water vary from less than 10 μg ammonia nitrogen/L in some natural surface and groundwaters to more than 30 mg/L in some wastewaters.

In this manual, organic nitrogen is referred to and reported as organic N, nitrate nitrogen as NO_3^--N, nitrite nitrogen as NO_2^--N, and ammonia nitrogen as NH_3-N.

Total nitrogen can be determined through oxidative digestion of all digestible nitrogen forms to nitrate, followed by quantitation of the nitrate. Two procedures, one using a persulfate/UV digestion (4500-N.B), and the other using persulfate digestion (4500-N.C) are presented. The procedures give good results for total nitrogen, composed of organic nitrogen (including some aromatic nitrogen-containing compounds), ammonia, nitrite, and nitrate. Molecular nitrogen is not determined and recovery of some industrial nitrogen-containing compounds is low.

* Approved by Standard Methods Committee, A,B,C, 1997; D, 2001.
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