

mercury droplets to be formed and dislodged from the dropping mercury electrode at a steady and consistent rate. Measure height of current peak above base line at an applied potential of about -1.1 V relative to the saturated calomel electrode.

b. Internal standard additions: Add 0.05 mL 25 μ M standard iodate solution to cell. Purge solution, with stirring, with oxygen-free argon gas for 0.5 min.

Record a polarogram under conditions described in ¶ 4a and determine height of current peak again. Repeat this procedure two additional times.

c. Blank determination: Determine method reagent blank by treating reagent water as a sample.

5. Calculation

Follow calculations given in Section 4500-I⁻.D.5, with substitution of appropriate compounds in the definitions of terms.

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

6. Precision

In one laboratory, analyzing seawater samples with a concentration of iodate of 60 μ g I/L, the precision was about $\pm 3\%$. 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.
TAKAYANAGI, K. & G.T.F. WONG. 1986. The oxidation of iodide to iodate for the polarographic determination of total iodine in natural waters. *Talanta* 33:451.
WONG, G.T.F. & L.S. ZHANG. 1992. Chemical removal of oxygen with sulfite for the polarographic or voltammetric determination of iodate or iodide in seawater. *Mar. Chem.* 38:109.

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 μ g 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.

Joint Task Group: (4500-N. D)—Thomas R. Holm (chair); 20th Edition (4500-N. B)—Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

Chloride ions do not interfere with persulfate oxidation, but the rate of reduction of nitrate to nitrite (during subsequent nitrate analysis by cadmium reduction) is significantly decreased by chlorides. Ammonium and nitrate ions adsorbed on suspended pure clay or silt particles should give a quantitative yield from persulfate digestion. If suspended matter remains after digestion, remove it before the reduction step.

If suspended organic matter is dissolved by the persulfate digestion reagent, yields comparable to those from true solutions

are obtained; if it is not dissolved, the results are unreliable and probably reflect a negative interference. The persulfate method is not effective in wastes with high organic loadings. Dilute such samples and re-analyze until results from two dilutions agree.

A conductimetric method (4500-N.D) for the determination of inorganic nitrogen also is presented.

4500-N B. In-Line UV/Persulfate Digestion and Oxidation with Flow Injection Analysis

1. General Discussion

a. Principle: Nitrogen compounds are digested and oxidized in-line to nitrate by use of heated alkaline persulfate and ultraviolet radiation. The digested sample is injected onto the manifold where its nitrate is reduced to nitrite by a cadmium granule column. The nitrite then is determined by diazotization with sulfanilamide under acidic conditions to form a diazonium ion. The diazonium ion is coupled with *N*-(1-naphthyl)ethylenediamine dihydrochloride. The resulting pink dye absorbs at 540 nm and is proportional to total nitrogen.

This method recovers nearly all forms of organic and inorganic nitrogen, reduced and oxidized, including ammonia, nitrate, and nitrite. It differs from the total kjeldahl nitrogen method described in Section 4500-N_{org}.D, which does not recover the oxidized forms of nitrogen. This method recovers nitrogen components of biological origin such as amino acids, proteins, and peptides as ammonia, but may not recover the nitrogenous compounds of some industrial wastes such as amines, nitro-compounds, hydrazones, oximes, semicarbazones, and some refractory tertiary amines.

See Section 4500-N.A for a discussion of the various forms of nitrogen found in waters and wastewaters, Sections 4500-N_{org}.A and B for a discussion of total nitrogen methods, and Section 4130, Flow Injection Analysis (FIA). Also see Section 4500-N.C for a similar, batch total nitrogen method that uses only persulfate.

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

2. Apparatus

Flow injection analysis equipment consisting of:

- FIA injection valve with sample loop or equivalent.
- Multichannel proportioning pump.
- FIA manifold (Figure 4500-N:1) with tubing heater, in-line ultraviolet digestion fluidics including a debubbler consisting of a gas-permeable TFE membrane and its holder, and flow cell. In Figure 4500-N:1, relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down

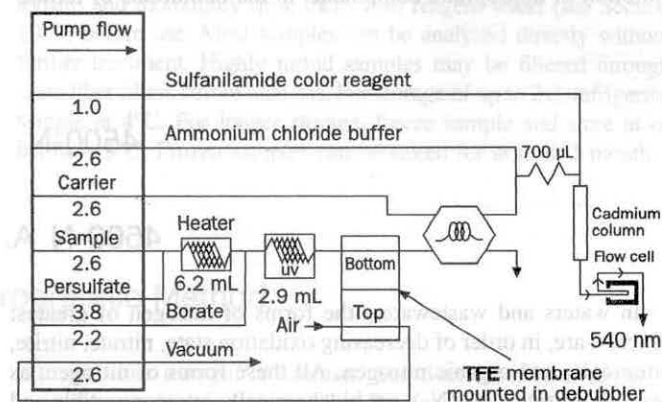


Figure 4500-N:1. FIA in-line total nitrogen manifold.

proportionally. Use manifold tubing of an inert material such as TFE. The block marked "UV" should consist of TFE tubing irradiated by a mercury discharge ultraviolet lamp emitting radiation at 254 nm.

d. Absorbance detector, 540 nm, 10-nm bandpass.

e. Injection valve control and data acquisition system.

3. Reagents

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

a. Borate solution, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$: In a 1-L volumetric flask dissolve 38.0 g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ and 3.0 g sodium hydroxide, NaOH, in approximately 900 mL water, using a magnetic stirring bar. Gentle heating will speed dissolution. Adjust to pH 9.0 with NaOH or conc hydrochloric acid (HCl). Dilute to mark and invert to mix.

b. Persulfate solution, $\text{K}_2\text{S}_2\text{O}_8$: Potassium persulfate solid reagent usually contains nitrogen contamination. Higher contamination levels result in larger blank peaks.

To a tared 1-L container, add 975 g water and 49 g $K_2S_2O_8$. Add a magnetic stirring bar, dissolve persulfate, and dilute to mark. Invert to mix.

c. *Ammonium chloride buffer*: CAUTION: *Fumes. Use a hood.* To a 1-L volumetric flask add 500 mL water, 105 mL conc HCl, and 95 mL conc ammonium hydroxide, NH_4OH . Dissolve, dilute to mark, and invert to mix. Adjust to pH 8.5 with 1N HCl or 1N NaOH solution.

d. *Sulfanilamide color reagent*: To a tared, dark, 1-L container add 876 g water, 170 g 85% phosphoric acid, H_3PO_4 , 40.0 g sulfanilamide, and 1.0 g *N*-(1-naphthyl)ethylenediamine dihydrochloride (NED). Shake to wet solids and stir for 30 min to dissolve. Store in a dark bottle and discard when solution turns dark pink.

e. *Cadmium column*: See Section 4500- NO_3^- .I.3c, d, and e.

f. *Stock nitrate standard*, 1000 mg N/L: In a 1-L volumetric flask dissolve 7.221 g potassium nitrate, KNO_3 (dried at 60°C for 1 h), or 4.93 g sodium nitrite, $NaNO_2$, in about 800 mL water. Dilute to mark and invert to mix. When refrigerated the standard may be stored for up to 3 months.

g. *Standard solutions*: Prepare nitrate standards in the desired concentration range, using stock nitrate standards (§ 3f), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-N:1 and follow method supplied by manufacturer, or laboratory standard operating procedure for this method.

Carry both standards and samples through this procedure. If samples have been preserved with sulfuric acid, preserve standards similarly. Samples may be homogenized. Turbid samples may be filtered, since digestion effectiveness on nitrogen-containing particles is unknown; however, organic nitrogen may be lost in the filtration.

5. Calculation

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

Verify digestion efficiency by determining urea, glutamic acid, or nicotinic acid standards (4500-N.C.3d) at regular intervals. In the concentration range of the method, the recovery of these compounds should be >95%.

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4500-N C. Persulfate Method

1. General Discussion

The persulfate method determines total nitrogen by oxidation of all nitrogenous compounds to nitrate. Should ammonia, nitrate, and nitrite be determined individually, "organic nitrogen" can be obtained by difference.

TABLE 4500-N:I. RECOVERIES OF TOTAL NITROGEN

Compound	Mean Recovery %	
	10 mg N/L	4 mg N/L
Ammonium chloride	98.1	99.7
Sodium nitrite	100.5	101.8
Glycine	101.0	100.8
Glutamic acid	99.7	99.2
Ammonium <i>p</i> -toluenesulfonate	99.6	97.4
Glycine <i>p</i> -toluenesulfonate	101.4	102.3
Nicotinic acid	98.6	102.0
Urea	94.9	98.0
EDTA	89.4	89.4

6. Quality Control

See Section 4130B.

7. Precision and Bias

a. *MDL*: Using a 70- μ L sample loop and a published MDL method,¹ analysts ran 21 replicates of a 0.20-mg N/L standard. These gave a mean of 0.18 mg N/L, a standard deviation of 0.008 mg N/L, and MDL of 0.020 mg N/L.

b. *Precision study*: Ten injections each of a 4.00-mg N/L standard and of a 10.0-mg N/L standard both gave a relative standard deviation of 0.6%.

c. *Recovery of total nitrogen*: Table 4500-N:I shows recoveries for various nitrogen compounds determined at 10 mg N/L and 4.0 mg N/L. All compounds were determined in triplicate.

d. *Ammonia recoveries from wastewater treatment plant effluent with known additions*: To a sample of wastewater treatment plant effluent, ammonium chloride was added at two concentrations, 2.50 and 5.00 mg N/L, and analyses were made in triplicate to give mean recoveries of 96% and 95%, respectively. A sample with no additions also was diluted twofold in triplicate to give a mean recovery of 99%.

8. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430, October 26, 1984.

Verify digestion efficiency by determining urea, glutamic acid, or nicotinic acid standards (4500-N.C.3d) at regular intervals. In the concentration range of the method, the recovery of these compounds should be >95%.

Verify digestion efficiency by determining urea, glutamic acid, or nicotinic acid standards (4500-N.C.3d) at regular intervals. In the concentration range of the method, the recovery of these compounds should be >95%.

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Verify digestion efficiency by determining urea, glutamic acid, or nicotinic acid standards (4500-N.C.3d) at regular intervals. In the concentration range of the method, the recovery of these compounds should be >95%.

a. *Principle*: Alkaline oxidation at 100 to 110°C converts organic and inorganic nitrogen to nitrate. Total nitrogen is determined by analyzing the nitrate in the digestate.

b. *Selection of nitrate measurement method*: Automated or manual cadmium reduction may be used to determine total nitrogen levels below 2.9 mg N/L. Results summarized in

TABLE 4500-N:II. PRECISION DATA FOR TOTAL NITROGEN, PERSULFATE METHOD, BASED ON TRIPPLICATE ANALYSES OF NICOTINIC ACID

Lab/ Analyst	Nicotinic Acid mg N/L	Recovery of N %	Standard Deviation mg/L	Relative Standard Deviation %
1/1	0.5	104	0.019	3.82
1/2	0.5	99.7	0.012	2.44
2/1	0.5	97.7	0.035	7.02
2/2	0.5	87.8	0.024	4.89
3/1	0.5	105	0.072	13.7
3/2	0.5	95.3	0.015	3.20
1/1	1.0	98.5	0.023	2.32
1/2	1.0	99.3	0.022	2.21
2/1	1.0	113	0.053	5.31
2/2	1.0	97.1	0.031	3.10
3/1	1.0	96.2	0.019	1.97
3/2	1.0	102	0.025	2.46
1/1	2.0	100	0.030	1.50
1/2	2.0	97.8	0.014	0.7
2/1	2.0	104	0.069	3.45
2/2	2.0	100	0.080	3.98
3/1	2.0	95.3	0.078	4.12
3/2	2.0	98.3	0.015	0.75

Table 4500-N:II were obtained using automated cadmium reduction.

2. Apparatus

a. *Autoclave, or hotplate and pressure cooker* capable of developing 100 to 110°C for 30 min.

b. *Glass culture tubes*:* 30-mL screw-capped (polypropylene linerless caps), 20 mm OD × 150 mm long. Clean before initial use by autoclaving with digestion reagent.

c. *Apparatus for nitrate determination*: See Section 4500-NO₃⁻.E or F.

d. *Automated analytical equipment*: An example of the continuous-flow analytical instrument consists of components shown in Figure 4500-NO₃⁻:2.

3. Reagents

a. *Ammonia-free and nitrate-free water*: Prepare by ion exchange or distillation methods as directed in 4500-NH₃.B.3a and 4500-NO₃⁻.B.3a.

b. *Stock nitrate solution*: Prepare as directed in 4500-NO₃⁻.B.3b.

c. *Intermediate nitrate solution*: Prepare as directed in 4500-NO₃⁻.B.3c.

d. *Stock glutamic acid solution*: Dry glutamic acid, C₃H₅NH₂(COOH)₂, in an oven at 105°C for 24 h. Dissolve 1.051 g in water and dilute to 1000 mL; 1.00 mL = 100 µg N. Preserve with 2 mL CHCl₃/L.

e. *Intermediate glutamic acid solution*: Dilute 100 mL stock glutamic acid solution to 1000 mL with water; 1.00 mL = 10.0 µg N. Preserve with 2 mL CHCl₃/L.

f. *Digestion reagent*: Dissolve 20.1 g low nitrogen (<0.001% N) potassium persulfate, K₂S₂O₈, and 3.0 g NaOH in water and dilute to 1000 mL just before use.

g. *Borate buffer solution*: Dissolve 61.8 g boric acid, H₃BO₃, and 8.0 g NaOH in water and dilute to 1000 mL.

h. *Copper sulfate solution*: Dissolve 2.0 g CuSO₄ · 5H₂O in 90 mL water and dilute to 100 mL.

i. *Ammonium chloride solution*: Dissolve 10.0 g NH₄Cl in 1 L water. Adjust to pH 8.5 by adding three or four NaOH pellets as necessary or NaOH solution before bringing to volume. This reagent is stable for 2 weeks when refrigerated.

j. *Color reagent*: Combine 1500 mL water, 200.0 mL conc phosphoric acid, H₃PO₄, 20.0 g sulfanilamide, and 1.0 g N-(1-naphthyl)-ethylenediamine dihydrochloride. Dilute to 2000 mL. Add 2.0 mL polyoxyethylene 23 lauryl ether.† Store at 4°C in the dark. Prepare fresh reagent every 6 weeks. Alternatively, prepare proportionally smaller volumes to minimize waste.

4. Procedure

a. *Calibration curve*: Prepare NO₃⁻ calibration standards in the range 0 to 2.9 mg NO₃⁻-N/L by diluting to 100 mL the following volumes of intermediate nitrate solution: 0, 1.00, 2.00, 4.00 ... 29.0 mL. Treat standards in the same manner as samples.

b. *Digestion check standard*: Prepare glutamic acid digestion check standard of 2.9 mg N/L by diluting, to 100 mL, a 29.0-mL volume of intermediate glutamic acid solution. Treat digestion check standard in the same manner as samples.

c. *Digestion*: Samples preserved with acid cannot be analyzed by this method. To a culture tube, add 10.0 mL sample or standard or a portion diluted to 10.0 mL. Add 5.0 mL digestion reagent. Cap tightly. Mix by inverting twice. Heat for 30 min in an autoclave or pressure cooker at 100 to 110°C. Slowly cool to room temperature. Add 1.0 mL borate buffer solution. Mix by inverting at least twice.

d. *Blank*: Carry a reagent blank through all steps of the procedure and apply necessary corrections to the results.

e. *Nitrate measurement*: Determine nitrate by cadmium reduction. Set up manifold as shown in Figure 4500-NO₃⁻:2, but use ammonium chloride and color reagents specified in ¶s 3i and j above.

5. Calculation

Prepare the standard curve by plotting the absorbances or peak heights of the nitrate calibration standards carried through the digestion procedure against their nitrogen concentrations. Compute organic N sample concentration by comparing sample absorbance or peak height with the standard curve.

6. Precision and Bias

See Table 4500-N:II.

7. Bibliography

D'ELIA, C.F., P.A. STEUDLER & N. CORWIN. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnol. Oceanogr.* 22:760.

*18-415, Comar, Inc., Vineland, NJ, or equivalent.

† Brij-35, available from ICI Americas, Inc., Wilmington, DE, or equivalent.

- NYDAHL, F. 1978. On the peroxodisulphate oxidation of total nitrogen in waters to nitrate. *Water Res.* 12:1123.
- VALDERRAMA, J.C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar. Chem.* 10:109.
- EBINA, J., T. TSUTSUI & T. SHIRAI. 1983. Simultaneous determination of total nitrogen and total phosphorus in water using peroxodisulphate oxidation. *Water Res.* 17:1721.

4500-N D. Conductimetric Determination of Inorganic Nitrogen

1. General Discussion

a. Principle: Aqueous ammonia nitrogen in samples is converted to dissolved ammonia gas by raising sample pH above 11 with alkaline solution. The sample/alkaline solution is placed in one cell of a two-cell diffusion chamber with a buffered absorbing solution in the second cell. A hydrophobic membrane, through which the dissolved ammonia gas diffuses, separates the cells. The pH of the absorbing solution is neutral, facilitating conversion of the dissolved ammonia gas to the ammonium ion form. Change in conductivity of the absorbing solution is determined in a conductivity cell. The method is best applied using a continuous-flow conductimetric system such as shown in Figure 4500-N:2. In the continuous-flow system, samples are injected into a continuously fed stream of alkaline solution at selected time intervals, usually 1 to 2 min, and pass through the system as plugs. Response time usually is less than 1 min.

Total oxidized inorganic nitrogen (nitrate and/or nitrite) is measured by reducing to ammonia with zinc after mixing sample with the alkaline solution.¹ The reaction is



In the continuous-flow system, a zinc reduction cartridge is placed in the flow stream, as indicated in Figure 4500-N:2. The reduction reaction is catalyzed by copper; addition of copper sulfate to the alkaline solution is beneficial.

When both ammonia and oxidized forms of nitrogen are present in a sample, the total nitrogen and the ammonia nitrogen concentrations are determined separately and the oxidized nitrogen is calculated by difference. Because the reaction with zinc

- AMEEL, J.J., R.P. AXLER & C.J. OWEN. 1993. Persulfate digestion for determination of total nitrogen and phosphorus in low-nutrient waters. *Amer. Environ. Lab.* 10/93:1.

reduces both nitrite and nitrate to ammonia, differentiation between the two ions is not possible with this method.

b. Interferences: Remove particulate matter by filtration before analysis to prevent plugging of analyzer lines.

Calcium and magnesium precipitate formation can clog analyzer lines. Prevent precipitate formation by adding diethylenetriaminepentaacetic acid (DTPA) to the alkaline feed at a concentration up to 10 g/L.

Volatile amines, chloramines, or the amides of carboxylic acids can diffuse through the hydrophobic membrane to produce a conductivity response or react in the diffusion cell to form ammonia. Reduce or eliminate this response for volatile amines or the amides of carboxylic acids by adjusting pH of the alkaline solution below pK_a of the amine or amide but above pK_a of ammonia. Reduce or eliminate this response for chloramines by not reducing the chloramines over zinc to ammonia.

c. Minimum detectable concentration: The estimated minimum detectable concentration of $\text{NH}_3\text{-N}$ or combined $\text{NO}_3^- \text{-N}$ and $\text{NO}_2^- \text{-N}$ is 0.01 mg/L.²

d. Storage of samples: Optional storage conditions for samples depend on the forms of nitrogen and interferences present. Refer to 4500-NH₃.A.3, 4500-NO₂⁻.B.1c, or 4500-NO₃⁻.A.2 for specific conditions.

2. Apparatus

Automated analytical equipment: An example of the automated, continuous-flow analytical equipment required is shown in Figure 4500-N:2. Details of the requirements and current manufacturers of commercial apparatus* can be found in the literature.³

3. Reagents

Use ammonia-free, deionized water for making all reagents.

a. Ammonia standards: Prepare at least three standard ammonia nitrogen standards in the desired analysis range between 0.1 and 75 mg N/L by dissolving an appropriate quantity of ammonium sulfate, $(\text{NH}_4)_2\text{SO}_4$, in water and diluting to 1 L.

b. Nitrate standards: Prepare at least three standard nitrate-nitrogen standards in analysis range between 0.1 mg and 75 mg N/L by dissolving an appropriate quantity of sodium nitrate, NaNO_3 , in water and diluting to 1 L.

* Timberline Instruments, 1800 S. Flatiron Court, Boulder, CO 80301 (www.timberlineinstruments.com).

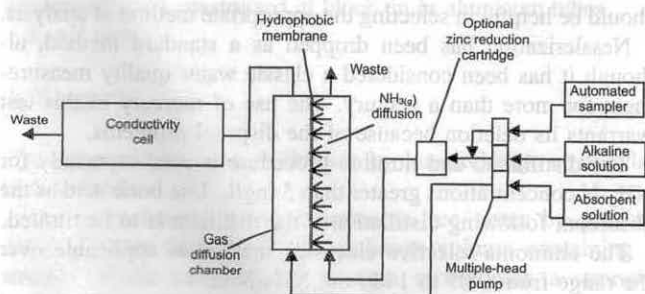


Figure 4500-N:2. Continuous-flow conductimetric analyzer system.

c. *Alkaline solution I* (for determination of ammonia nitrogen): Dissolve 25 g potassium hydroxide, KOH, and 10 g diethylenetriaminepentaacetic acid, DTPA, precipitate inhibitor in 1 L water.

d. *Copper catalyst solution*: Dissolve 200 mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 1 L water.

e. *Alkaline solution II* (for determination of nitrate and nitrite nitrogen): Dissolve 25 g potassium hydroxide, KOH, 10 g diethylenetriaminepentaacetic acid, DTPA, precipitate inhibitor, and 10 mL copper catalyst solution in 1 L water.

f. *Absorbent solution*: Dissolve 100 mg boric acid, H_3BO_3 , in 1 L water. Adjust to pH 7 with ammonium hydroxide, NH_4OH .

g. *Zinc reduction cartridges*, containing 20/30 mesh zinc metal.

4. Procedure

Set up instrument equivalent to that in Figure 4500-N:2 and follow manufacturer's operating procedure. Calibrate instrument using appropriate standard solutions and insert a standard every tenth tube in the autosampler tray. Filter turbid samples before analysis.

5. Calculation

Prepare standard curves by plotting nitrogen concentration of standards versus conductivity of absorbent solution. The calibration is linear. Compute sample nitrogen concentration by comparing sample conductivity with standard curve.

6. Quality Control

See Section 4020 for minimum quality assurance/quality control (QA/QC) guidelines. It is the analyst's responsibility to

demonstrate method proficiency and to evaluate method bias. Certain regulatory programs may have more extensive QC requirements.

7. Precision and Bias

Ammonia standard concentrations of 0.1, 0.8, 5, 15, 30, 55, and 75 mg/L $\text{NH}_3\text{-N}$ were analyzed. Recovery ranged from 99.31 and 101.64% at concentrations of 5 and 0.1 mg/L $\text{NH}_3\text{-N}$, respectively. Precision ranged from 0.33 to 3.57% for concentrations of 15 and 0.1 mg/L $\text{NH}_3\text{-N}$, respectively.

Nitrate standard concentrations of 0.1, 0.8, 5, 15, 30, 55, and 75 mg/L $\text{NO}_3^-\text{-N}$ were analyzed. Recovery ranged from 99.93 to 101.84% at concentrations of 75 and 0.8 mg/L $\text{NO}_3^-\text{-N}$, respectively. Precision ranged from 0.64 to 4% at concentrations of 75 and 0.1 mg/L $\text{NO}_3^-\text{-N}$, respectively.

Combined nitrogen standards were prepared using 50% portions of $\text{NH}_3\text{-N}$ and $\text{NO}_3^-\text{-N}$. Concentrations analyzed were 0.1, 0.8, 5, 15, 30, 50, and 75 mg N/L. Ranges of recovery and precision of the combined nitrogen analysis were 99.83 to 101.58% and 0.45 and 4.22%. Ranges of recovery and precision of the ammonia nitrogen analysis were 97.68 to 106.19% and 2.58 to 12.1%. Ranges of recovery and precision for the calculated nitrate nitrogen were 95.08 to 104.08% and 0.84 and 11%.²

8. References

- CARLSON, R.M. 1986. Continuous flow reduction of nitrate to ammonia with granular zinc. *Anal. Chem.* 58:1590.
- MANSSELL, B.O. & E.D. SCHROEDER. 2000. Automated separation and conductimetric determination of inorganic nitrogen. *J. Environ. Eng.* 126:778.
- CARLSON, R.M. 1978. Automated separation and conductimetric determination of ammonia and dissolved carbon dioxide. *Anal. Chem.* 50:1528.

4500-NH₃ NITROGEN (AMMONIA)*

4500-NH₃ A. Introduction

1. Selection of Method

The two major factors that influence selection of the method to determine ammonia are concentration and presence of interferences. In general, direct manual determination of low concentrations of ammonia is confined to drinking waters, clean surface or groundwater, and good-quality nitrified wastewater effluent. In other instances, and where interferences are present or greater precision is necessary, a preliminary distillation step (B) is required.

A titrimetric method (C), an ammonia-selective electrode method (D), an ammonia-selective electrode method using

known addition (E), a phenate method (F), and two automated versions of the phenate method (G and H) are presented. Methods D, E, F, G, and H may be used either with or without sample distillation. The data presented in Tables 4500-NH₃:I and III should be helpful in selecting the appropriate method of analysis.

Nesslerization has been dropped as a standard method, although it has been considered a classic water quality measurement for more than a century. The use of mercury in this test warrants its deletion because of the disposal problems.

The distillation and titration procedure is used especially for $\text{NH}_3\text{-N}$ concentrations greater than 5 mg/L. Use boric acid as the absorbent following distillation if the distillate is to be titrated.

The ammonia-selective electrode method is applicable over the range from 0.03 to 1400 mg $\text{NH}_3\text{-N/L}$.

The manual phenate method is applicable to both fresh water and seawater and is linear to 0.6 mg $\text{NH}_3\text{-N/L}$. Distill into

* Approved by Standard Methods Committee, 1997.

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sulfuric acid (H₂SO₄) absorbent for the phenate method when interferences are present.

The automated phenate method is applicable over the range of 0.02 to 2.0 mg NH₃-N/L.

2. Interferences

Glycine, urea, glutamic acid, cyanates, and acetamide hydrolyze very slowly in solution on standing but, of these, only urea and cyanates will hydrolyze on distillation at pH of 9.5. Hydrolysis amounts to about 7% at this pH for urea and about 5% for cyanates. Volatile alkaline compounds such as hydrazine and amines will influence titrimetric results. Residual chlorine reacts with ammonia; remove by sample pretreatment. If a sample is likely to contain residual chlorine, immediately upon collection, treat with dechlorinating agent as in 4500-NH₃.B.3d.

3. Storage of Samples

Most reliable results are obtained on fresh samples. If samples are to be analyzed within 24 h of collection, refrigerate unacidified.

4500-NH₃. B. Preliminary Distillation Step

1. General Discussion

The sample is buffered at pH 9.5 with a borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds. It is distilled into a solution of boric acid when titration is to be used or into H₂SO₄ when the phenate method is used. The ammonia in the distillate can be determined either colorimetrically by the phenate method or titrimetrically with standard H₂SO₄ and a mixed indicator or a pH meter. The choice between the colorimetric and the acidimetric methods depends on the concentration of ammonia. Ammonia in the distillate also can be determined by the ammonia-selective electrode method, using 0.04N H₂SO₄ to trap the ammonia.

2. Apparatus

a. Distillation apparatus: Arrange a borosilicate glass flask of 800- to 2000-mL capacity attached to a vertical condenser so that the outlet tip may be submerged below the surface of the receiving acid solution. Use an all-borosilicate-glass apparatus or one with condensing units constructed of block tin or aluminum tubes.

b. pH meter.

3. Reagents

a. Ammonia-free water: Prepare by ion-exchange or distillation methods:

1) Ion exchange—Prepare ammonia-free water by passing distilled water through an ion-exchange column containing a strongly acidic cation-exchange resin mixed with a strongly basic anion-exchange resin. Select resins that will remove organic compounds that interfere with the ammonia determination.

fied at 4°C. For preservation for up to 28 d, freeze at -20°C unacidified, or preserve samples by acidifying to pH <2 and storing at 4°C. If acid preservation is used, neutralize samples with NaOH or KOH immediately before making the determination. CAUTION: Although acidification is suitable for certain types of samples, it produces interferences when exchangeable ammonium is present in unfiltered solids.

4. Bibliography

- THAYER, G.W. 1970. Comparison of two storage methods for the analysis of nitrogen and phosphorus fractions in estuarine water. *Chesapeake Sci.* 11:155.
- SALLEY, B.A., J.G. BRADSHAW & B.J. NELSON. 1986. Results of Comparative Studies of Preservation Techniques for Nutrient Analysis on Water Samples. Virginia Institute of Marine Science, Gloucester Point.

Some anion-exchange resins tend to release ammonia. If this occurs, prepare ammonia-free water with a strongly acidic cation-exchange resin. Regenerate the column according to the manufacturer's instructions. Check ammonia-free water for the possibility of a high blank value.

2) Distillation—Eliminate traces of ammonia in distilled water by adding 0.1 mL conc H₂SO₄ to 1 L distilled water and redistilling. Alternatively, treat distilled water with sufficient bromine or chlorine water to produce a free halogen residual of 2 to 5 mg/L and redistill after standing at least 1 h. Discard the first 100 mL distillate. Check redistilled water for the possibility of a high blank.

It is very difficult to store ammonia-free water in the laboratory without contamination from gaseous ammonia. However, if storage is necessary, store in a tightly stoppered glass container to which is added about 10 g ion-exchange resin (preferably a strongly acidic cation-exchange resin)/L ammonia-free water. For use, let resin settle and decant ammonia-free water. If a high blank value is produced, replace the resin or prepare fresh ammonia-free water.

Use ammonia-free distilled water for preparing all reagents, rinsing, and sample dilution.

b. Borate buffer solution: Add 88 mL 0.1N NaOH solution to 500 mL approximately 0.025M sodium tetraborate (Na₂B₄O₇) solution (9.5 g Na₂B₄O₇ · 10 H₂O/L) and dilute to 1 L.

c. Sodium hydroxide, 6N.

d. Dechlorinating reagent: Dissolve 3.5 g sodium thiosulfate (Na₂S₂O₃ · 5H₂O) in water and dilute to 1 L. Prepare fresh weekly. Use 1 mL reagent to remove 1 mg/L residual chlorine in 500-mL sample.

e. Neutralization agent.

1) Sodium hydroxide, NaOH, 1N.

- 2) Sulfuric acid, H_2SO_4 , 1N.
 f. Absorbent solution, plain boric acid: Dissolve 20 g H_3BO_3 in water and dilute to 1 L.
 g. Indicating boric acid solution: See Section 4500-NH₃.C.3a and b.
 h. Sulfuric acid, 0.04N: Dilute 1.0 mL conc H_2SO_4 to 1 L.

4. Procedure

a. *Preparation of equipment:* Add 500 mL water and 20 mL borate buffer, adjust pH to 9.5 with 6N NaOH solution, and add to a distillation flask. Add a few glass beads or boiling chips and use this mixture to steam out the distillation apparatus until distillate shows no traces of ammonia.

b. *Sample preparation:* Use 500 mL dechlorinated sample or a known portion diluted to 500 mL with water. When $\text{NH}_3\text{-N}$ concentration is less than 100 $\mu\text{g/L}$, use a sample volume of 1000 mL. Remove residual chlorine by adding, at the time of collection, dechlorinating agent equivalent to the chlorine residual. If necessary, neutralize to approximately pH 7 with dilute acid or base, using a pH meter.

Add 25 mL borate buffer solution and adjust to pH 9.5 with 6N NaOH using a pH meter.

c. *Distillation:* To minimize contamination, leave distillation apparatus assembled after steaming out and until just before starting sample distillation. Disconnect steaming-out flask and

immediately transfer sample flask to distillation apparatus. Distill at a rate of 6 to 10 mL/min with the tip of the delivery tube below the surface of acid receiving solution. Collect distillate in a 500-mL erlenmeyer flask containing 50 mL indicating boric acid solution for titrimetric method. Distill ammonia into 50 mL 0.04N H_2SO_4 for the ammonia-selective electrode method and for the phenate method. Collect at least 200 mL distillate. Lower distillation receiver so that the end of the delivery tube is free of contact with the liquid and continue distillation during the last minute or two to cleanse condenser and delivery tube. Dilute to 500 mL with water.

When the phenate method is used for determining $\text{NH}_3\text{-N}$, neutralize distillate with 1N NaOH solution.

d. *Ammonia determination:* Determine ammonia by the titrimetric method (C), the ammonia-selective electrode methods (D and E), or the phenate methods (F and G).

5. Bibliography

- NICHOLS, M.S. & M.E. FOOTE. 1931. Distillation of free ammonia from buffered solutions. *Ind. Eng. Chem., Anal. Ed.* 3:311.
 GRIFFIN, A.E. & N.S. CHAMBERLIN. 1941. Relation of ammonia nitrogen to breakpoint chlorination. *Amer. J. Pub. Health* 31:803.
 PALIN, A.T. 1950. Symposium on the sterilization of water, Chemical aspects of chlorination. *J. Inst. Water Eng.* 4:565.
 TARAS, M.J. 1953. Effect of free residual chlorination of nitrogen compounds in water. *J. Amer. Water Works Assoc.* 45:47.

4500-NH₃ C. Titrimetric Method

1. General Discussion

The titrimetric method is used only on samples that have been carried through preliminary distillation (see Section 4500-NH₃.B). The following table is useful in selecting sample volume for the distillation and titration method.

Ammonia Nitrogen in Sample mg/L	Sample Volume mL
5-10	250
10-20	100
20-50	50.0
50-100	25.0

2. Apparatus

Distillation apparatus: See Section 4500-NH₃.B.2a and b.

3. Reagents

Use ammonia-free water in making all reagents and dilutions.

a. *Mixed indicator solution:* Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol. Combine solutions. Prepare monthly.

b. *Indicating boric acid solution:* Dissolve 20 g H_3BO_3 in water, add 10 mL mixed indicator solution, and dilute to 1 L. Prepare monthly.

c. *Standard sulfuric acid titrant, 0.02N:* Prepare and standardize as directed in Alkalinity, Section 2320B.3c. For greatest accuracy, standardize titrant against an amount of Na_2CO_3 that has been incorporated in the indicating boric acid solution to reproduce the actual conditions of sample titration; 1.00 mL = $14 \times \text{normality} \times 1000 \mu\text{g N}$. (For 0.02N, 1.00 mL = 280 $\mu\text{g N}$.)

4. Procedure

a. Proceed as described in Section 4500-NH₃.B using indicating boric acid solution as absorbent for the distillate.

b. *Sludge or sediment samples:* Rapidly weigh to within $\pm 1\%$ an amount of wet sample, equivalent to approximately 1 g dry weight, in a weighing bottle or crucible. Wash sample into a 500-mL kjeldahl flask with water and dilute to 250 mL. Proceed as in ¶ 4a but add a piece of paraffin wax to distillation flask and collect only 100 mL distillate.

c. Titrate ammonia in distillate with standard 0.02N H_2SO_4 titrant until indicator turns a pale lavender.

d. *Blank:* Carry a blank through all steps of the procedure and apply the necessary correction to the results.

5. Calculation

a. Liquid samples:

$$\text{mg NH}_3\text{-N/L} = \frac{(A - B) \times 280}{\text{mL sample}}$$

b. Sludge or sediment samples:

$$\text{mg NH}_3\text{-N/kg} = \frac{(A - B) \times 280}{\text{g dry wt sample}}$$

where:

A = volume of H₂SO₄ titrated for sample, mL, and

B = volume of H₂SO₄ titrated for blank, mL.

6. Precision and Bias

Three synthetic samples containing ammonia and other constituents dissolved in distilled water were distilled and analyzed by titration.

4500-NH₃ D. Ammonia-Selective Electrode Method

1. General Discussion

a. *Principle:* The ammonia-selective electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an electrode internal solution of ammonium chloride. Dissolved ammonia (NH_{3(aq)} and NH₄⁺) is converted to NH_{3(aq)} by raising pH to above 11 with a strong base. NH_{3(aq)} diffuses through the membrane and changes the internal solution pH that is sensed by a pH electrode. The fixed level of chloride in the internal solution is sensed by a chloride ion-selective electrode that serves as the reference electrode. Potentiometric measurements are made with a pH meter having an expanded millivolt scale or with a specific ion meter.

b. *Scope and application:* This method is applicable to the measurement of 0.03 to 1400 mg NH₃-N/L in potable and surface waters and domestic and industrial wastes. High concentrations of dissolved ions affect the measurement, but color and turbidity do not. Sample distillation is unnecessary. Use standard solutions and samples that have the same temperature and contain about the same total level of dissolved species. The ammonia-selective electrode responds slowly below 1 mg NH₃-N/L; hence, use longer times of electrode immersion (2 to 3 min) to obtain stable readings.

c. *Interference:* Amines are a positive interference. This may be enhanced by acidification. Mercury and silver interfere by complexing with ammonia, unless the NaOH/EDTA solution (3c) is used.

d. *Sample preservation:* Refrigerate at 4°C for samples to be analyzed within 24 h. Preserve samples high in organic and nitrogenous matter, and any other samples for longer storage, by lowering pH to 2 or less with conc H₂SO₄.

Sample 1 contained 200 µg NH₃-N/L, 10 mg Cl⁻/L, 1.0 mg NO₃⁻-N/L, 1.5 mg organic N/L, 10.0 mg PO₄³⁻/L, and 5.0 mg silica/L. The relative standard deviation and relative error for the 21 participating laboratories were 69.8% and 20%, respectively.

Sample 2 contained 800 µg NH₃-N/L, 200 mg Cl⁻/L, 1.0 mg NO₃⁻-N/L, 0.8 mg organic N/L, 5.0 mg PO₄³⁻/L, and 15.0 mg silica/L. The relative standard deviation and relative error for the 20 participating laboratories were 28.6% and 5%, respectively.

Sample 3 contained 1500 µg NH₃-N/L, 400 mg Cl⁻/L, 1.0 mg NO₃⁻-N/L, 0.2 mg organic N/L, 0.5 mg PO₄³⁻/L, and 30.0 mg silica/L. The relative standard deviation and relative error for the 21 participating laboratories were 21.6%, and 2.6%, respectively.

7. Bibliography

- MEEKER, E.W. & E.C. WAGNER. 1933. Titration of ammonia in the presence of boric acid. *Ind. Eng. Chem., Anal. Ed.* 5:396.
WAGNER, E.C. 1940. Titration of ammonia in the presence of boric acid. *Ind. Eng. Chem., Anal. Ed.* 12:711.

2. Apparatus

a. *Electrometer:* A pH meter with expanded millivolt scale capable of 0.1 mV resolution between -700 mV and +700 mV or a specific ion meter.

b. *Ammonia-selective electrode.**

c. *Magnetic stirrer*, thermally insulated, with TFE-coated stirring bar.

3. Reagents

a. *Ammonia-free water:* See Section 4500-NH₃.B.3a. Use for making all reagents.

b. *Sodium hydroxide*, 10N.

c. *NaOH/EDTA solution*, 10N: Dissolve 400 g NaOH in 800 mL water. Add 45.2 g ethylenediaminetetraacetic acid, tetrasodium salt, tetrahydrate (Na₄EDTA · 4 H₂O) and stir to dissolve. Cool and dilute to 1000 mL.

d. *Stock ammonium chloride solution:* Dissolve 3.819 g anhydrous NH₄Cl (dried at 100°C) in water, and dilute to 1000 mL; 1.00 mL = 1.00 mg N = 1.22 mg NH₃.

e. *Standard ammonium chloride solutions:* See ¶ 4a below.

4. Procedure

a. *Preparation of standards:* Prepare a series of standard solutions covering the concentrations of 1000, 100, 10, 1, and 0.1

* Orion Model 95-10, EIL Model 8002-2, Beckman Model 39565, or equivalent.

mg $\text{NH}_3\text{-N/L}$ by making decimal dilutions of stock NH_4Cl solution with water.

b. Electrometer calibration: Place 100 mL of each standard solution in a 150-mL beaker. Immerse electrode in standard of lowest concentration and mix with a magnetic stirrer. Limit stirring speed to minimize possible loss of ammonia from the solution. Maintain the same stirring rate and a temperature of about 25°C throughout calibration and testing procedures. Add a sufficient volume of 10N NaOH solution (1 mL usually is sufficient) to raise pH above 11. If the presence of silver or mercury is possible, use NaOH/EDTA solution in place of NaOH solution. If it is necessary to add more than 1 mL of either NaOH or NaOH/EDTA solution, note volume used, because it is required for subsequent calculations. Keep electrode in solution until a stable millivolt reading is obtained. Do not add NaOH solution before immersing electrode, because ammonia may be lost from a basic solution. Repeat procedure with remaining standards, proceeding from lowest to highest concentration. Wait until the reading has stabilized (at least 2 to 3 min) before recording millivolts for standards and samples containing ≤ 1 mg $\text{NH}_3\text{-N/L}$.

c. Preparation of standard curve: Using semilogarithmic graph paper, plot ammonia concentration in milligrams $\text{NH}_3\text{-N}$ per liter on the log axis vs. potential in millivolts on the linear axis starting with the lowest concentration at the bottom of the scale. If the electrode is functioning properly a tenfold change of $\text{NH}_3\text{-N}$ concentration produces a potential change of about 59 mV.

d. Calibration of specific ion meter: Refer to manufacturer's instructions and proceed as in ¶s 4a and b.

e. Measurement of samples: Dilute if necessary to bring $\text{NH}_3\text{-N}$ concentration to within calibration curve range. Place 100 mL sample in 150-mL beaker and follow procedure in ¶ 4b above. Record volume of 10N NaOH added. Read $\text{NH}_3\text{-N}$ concentration from standard curve.

5. Calculation

$$\text{mg } \text{NH}_3\text{-N/L} = A \times B \times \left[\frac{100 + D}{100 + C} \right]$$

where:

A = dilution factor,

B = concentration of $\text{NH}_3\text{-N/L}$, mg/L, from calibration curve,

C = volume of 10N NaOH added to calibration standards, mL, and

D = volume of 10N NaOH added to sample, mL.

4500-NH₃ E. Ammonia-Selective Electrode Method Using Known Addition

1. General Discussion

a. Principle: When a linear relationship exists between concentration and response, known addition is convenient for mea-

4500-NH₃:I. PRECISION AND BIAS OF AMMONIA-SELECTIVE ELECTRODE

Level mg/L	Matrix	Mean Recovery %	Precision	
			Overall % RSD	Single Operator % RSD
0.04	Distilled water	200	125	25
	Effluent water	100	75	0
0.10	Distilled water	180	50	10
	Effluent water	470	610	10
0.80	Distilled water	105	14	5
	Effluent water	105	38	7.5
20	Distilled water	95	10	5
	Effluent water	95	15	10
100	Distilled water	98	5	2
	Effluent water	97	—	—
750	Distilled water	97	10.4	1.6
	Effluent water	99	14.1	1.3

Source: AMERICAN SOCIETY FOR TESTING AND MATERIALS. Method 1426-79. American Soc. Testing & Materials, Philadelphia, Pa.

6. Precision and Bias

For the ammonia-selective electrode in a single laboratory using surface water samples at concentrations of 1.00, 0.77, 0.19, and 0.13 mg $\text{NH}_3\text{-N/L}$, standard deviations were ± 0.038 , ± 0.017 , ± 0.007 , and ± 0.003 , respectively. In a single laboratory using surface water samples at concentrations of 0.10 and 0.13 mg $\text{NH}_3\text{-N/L}$, recoveries were 96% and 91%, respectively. The results of an interlaboratory study involving 12 laboratories using the ammonia-selective electrode on distilled water and effluents are summarized in Table 4500-NH₃:I.

7. Bibliography

- BANWART, W.L., J.M. BREMNER & M.A. TABATABAI. 1972. Determination of ammonium in soil extracts and water samples by an ammonia electrode. *Comm. Soil Sci. Plant Anal.* 3:449.
- MIDGLEY, C. & K. TERRANCE. 1972. The determination of ammonia in condensed steam and boiler feed-water with a potentiometric ammonia probe. *Analyst* 97:626.
- BOOTH, R.L. & R.F. THOMAS. 1973. Selective electrode determination of ammonia in water and wastes. *Environ. Sci. Technol.* 7:523.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020, National Environmental Research Center, Cincinnati, Ohio.
- AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1979. Method 1426-79. American Soc. Testing & Materials, Philadelphia, Pa.

suring occasional samples because no calibration is needed. Because an accurate measurement requires that the concentration at least double as a result of the addition, sample concentration must be known within a factor of three. Total concentration of

ammonia can be measured in the absence of complexing agents down to 0.8 mg NH₃-N/L or in the presence of a large excess (50 to 100 times) of complexing agent. Known addition is a convenient check on the results of direct measurement.

b. See Section 4500-NH₃.D.1 for further discussion.

2. Apparatus

Use apparatus specified in Section 4500-NH₃.D.2.

3. Reagents

Use reagents specified in Section 4500-NH₃.D.3.

Add standard ammonium chloride solution approximately 10 times as concentrated as samples being measured.

4. Procedure

a. Dilute 1000 mg/L stock solution to make a standard solution about 10 times as concentrated as the sample concentrate.

b. Add 1 mL 10N NaOH to each 100 mL sample and immediately immerse electrode. When checking a direct measurement, leave electrode in 100 mL of sample solution. Use magnetic stirring throughout. Measure mV reading and record as E_1 .

c. Pipet 10 mL of standard solution into sample. Thoroughly stir and immediately record new mV reading as E_2 .

5. Calculation

a. $\Delta E = E_1 - E_2$.

b. From Table 4500-NH₃:II find the concentration ratio, Q , corresponding to change in potential, ΔE . To determine original total sample concentration, multiply Q by the concentration of the added standard:

$$C_o = Q C_s$$

where:

C_o = total sample concentration, mg/L,

Q = reading from known-addition table, and

C_s = concentration of added standard, mg/L.

c. To check a direct measurement, compare results of the two methods. If they agree within $\pm 4\%$, the measurements probably are good. If the known-addition result is much larger than the direct measurement, the sample may contain complexing agents.

6. Precision and Bias

In 38 water samples analyzed by both the phenate and the known-addition ammonia-selective electrode method, the electrode method yielded a mean recovery of 102% of the values obtained by the phenate method when the NH₃-N concentrations varied between 0.30 and 0.78 mg/L. In 57 wastewater samples similarly compared, the electrode method yielded a mean recovery of 108% of the values obtained by the phenate method using distillation when the NH₃-N concentrations varied between 10.2 and 34.7 mg N/L. In 20 instances in which two to four replicates

TABLE 4500-NH₃:II.* VALUES OF Q VS. ΔE (59 mV SLOPE) FOR 10% VOLUME CHANGE

ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q
5.0	0.297	9.0	0.178	16.0	0.0952	24.0	0.0556	32.0	0.0354
5.1	0.293	9.1	0.176	16.2	0.0938	24.2	0.0549	32.2	0.0351
5.2	0.288	9.2	0.174	16.4	0.0924	24.4	0.0543	32.4	0.0347
5.3	0.284	9.3	0.173	16.6	0.0910	24.6	0.0536	32.6	0.0343
5.4	0.280	9.4	0.171	16.8	0.0897	24.8	0.0530	32.8	0.0340
5.5	0.276	9.5	0.169	17.0	0.0884	25.0	0.0523	33.0	0.0335
5.6	0.272	9.6	0.167	17.2	0.0871	25.2	0.0517	33.2	0.0333
5.7	0.268	9.7	0.165	17.4	0.0858	25.4	0.0511	33.4	0.0329
5.8	0.264	9.8	0.164	17.6	0.0846	25.6	0.0505	33.6	0.0326
5.9	0.260	9.9	0.162	17.8	0.0834	25.8	0.0499	33.8	0.0323
6.0	0.257	10.0	0.160	18.0	0.0822	26.0	0.0494	34.0	0.0319
6.1	0.253	10.2	0.157	18.2	0.0811	26.2	0.0488	34.2	0.0316
6.2	0.250	10.4	0.154	18.4	0.0799	26.4	0.0482	34.4	0.0313
6.3	0.247	10.6	0.151	18.6	0.0788	26.6	0.0477	34.6	0.0310
6.4	0.243	10.8	0.148	18.8	0.0777	26.8	0.0471	34.8	0.0307
6.5	0.240	11.0	0.145	19.0	0.0767	27.0	0.0466	35.0	0.0304
6.6	0.237	11.2	0.143	19.2	0.0756	27.2	0.0461	36.0	0.0289
6.7	0.234	11.4	0.140	19.4	0.0746	27.4	0.0456	37.0	0.0275
6.8	0.231	11.6	0.137	19.6	0.0736	27.6	0.0450	38.0	0.0261
6.9	0.228	11.8	0.135	19.8	0.0726	27.8	0.0445	39.0	0.0249
7.0	0.225	12.0	0.133	20.0	0.0716	28.0	0.0440	40.0	0.0237
7.1	0.222	12.2	0.130	20.2	0.0707	28.2	0.0435	41.0	0.0226
7.2	0.219	12.4	0.128	20.4	0.0698	28.4	0.0431	42.0	0.0216
7.3	0.217	12.6	0.126	20.6	0.0689	28.6	0.0426	43.0	0.0206
7.4	0.214	12.8	0.123	20.8	0.0680	28.8	0.0421	44.0	0.0196
7.5	0.212	13.0	0.121	21.0	0.0671	29.0	0.0417	45.0	0.0187
7.6	0.209	13.2	0.119	21.2	0.0662	29.2	0.0412	46.0	0.0179
7.7	0.207	13.4	0.117	21.4	0.0654	29.4	0.0408	47.0	0.0171
7.8	0.204	13.6	0.115	21.6	0.0645	29.6	0.0403	48.0	0.0163
7.9	0.202	13.8	0.113	21.8	0.0637	29.8	0.0399	49.0	0.0156
8.0	0.199	14.0	0.112	22.0	0.0629	30.0	0.0394	50.0	0.0149
8.1	0.197	14.2	0.110	22.2	0.0621	30.2	0.0390	51.0	0.0143
8.2	0.195	14.4	0.108	22.4	0.0613	30.4	0.0386	52.0	0.0137
8.3	0.193	14.6	0.106	22.6	0.0606	30.6	0.0382	53.0	0.0131
8.4	0.190	14.8	0.105	22.8	0.0598	30.8	0.0378	54.0	0.0125
8.5	0.188	15.0	0.103	23.0	0.0591	31.0	0.0374	55.0	0.0120
8.6	0.186	15.2	0.1013	23.2	0.0584	31.2	0.0370	56.0	0.0115
8.7	0.184	15.4	0.0997	23.4	0.0576	31.4	0.0366	57.0	0.0110
8.8	0.182	15.6	0.0982	23.6	0.0569	31.6	0.0362	58.0	0.0105
8.9	0.180	15.8	0.0967	23.8	0.0563	31.8	0.0358	59.0	0.0101

* Orion Research Inc. Instruction Manual, Ammonia Electrode, Model 95-12, Boston, MA. 02129.

of these samples were analyzed, the mean standard deviation was 1.32 mg N/L. In three measurements at a sewer outfall, distillation did not change statistically the value obtained by the electrode method. In 12 studies using standards in the 2.5- to 30-mg N/L range, average recovery by the phenate method was 97% and by the electrode method 101%.

d. Typically, use a 60/h, 6:1 cam with a common wash.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against $\text{NH}_3\text{-N}$ concentrations in standards. Compute sample $\text{NH}_3\text{-N}$ concentration by comparing sample response with standard curve.

6. Precision and Bias

For an automated phenate system in a single laboratory using surface water samples at concentrations of 1.41, 0.77, 0.59, and 0.43 mg $\text{NH}_3\text{-N/L}$, the standard deviation was ± 0.005 mg/L, and

at concentrations of 0.16 and 1.44 mg $\text{NH}_3\text{-N/L}$, recoveries were 107 and 99%, respectively.

7. Bibliography

- HILLER, A. & D. VAN SLYKE. 1933. Determination of ammonia in blood. *J. Biol. Chem.* 102:499.
- FIGURE, J. & J.E. O'BRIEN. 1962. Ammonia determination by automatic analysis. *Wastes Eng.* 33:352.
- AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1966. Manual on Industrial Water and Industrial Waste Water, 2nd ed. American Soc. Testing & Materials, Philadelphia, Pa.
- O'CONNOR, B., R. DOBBS, B. VILLIERS & R. DEAN. 1967. Laboratory distillation of municipal waste effluents. *J. Water Pollut. Control Fed.* 39:25.

4500-NH₃ H. Flow Injection Analysis

1. General Discussion

a. **Principle:** A water sample containing ammonia or ammonium cation is injected into an FIA carrier stream to which a complexing buffer, alkaline phenol, and hypochlorite are added. This reaction, the Berthelot reaction, produces the blue indophenol dye. The blue color is intensified by the addition of nitro-ferricyanide. The resulting peak's absorbance is measured at 630 nm. The peak area is proportional to the concentration of ammonia in the original sample.

Also see Section 4500-NH₃.F and Section 4130, Flow Injection Analysis (FIA).

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

Also see Section 4500-NH₃.A. Some interferents are removed by distillation; see Section 4500-NH₃.B.

2. Apparatus

Flow injection analysis equipment consisting of:

- FIA injection valve with sample loop or equivalent.
- Multichannel proportioning pump.
- FIA manifold (Figure 4500-NH₃.2) with tubing heater and flow cell. In Figure 4500-NH₃.2, relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*
- Absorbance detector, 630 nm, 10-nm bandpass.
- Injection valve control and data acquisition system.

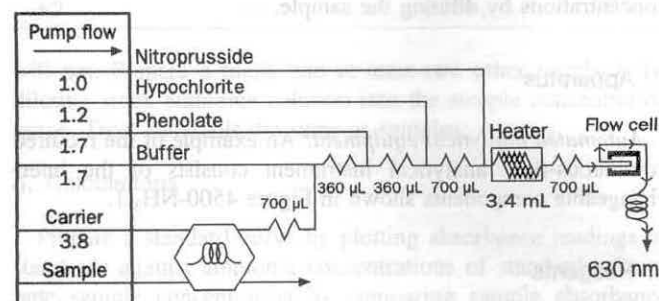


Figure 4500-NH₃.2. FIA ammonia manifold.

3. Reagents

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

a. **Buffer:** To a 1-L tared container add 50.0 g disodium ethylenediamine tetraacetate and 5.5 g sodium hydroxide, NaOH. Add 968 mL water. Mix with a magnetic stirrer until dissolved.

b. **Phenolate:** CAUTION: Wear gloves. Phenol causes severe burns and is rapidly absorbed into the body through the skin. To a tared 1-L container, add 888 g water. Add 94.2 g 88% liquefied phenol or 83 g crystalline phenol, $\text{C}_6\text{H}_5\text{OH}$. While stirring, slowly add 32 g NaOH. Cool and invert to mix thoroughly. Do not degas.

c. **Hypochlorite:** To a tared 500-mL container add 250 g 5.25% sodium hypochlorite, NaOCl bleach solution† and 250 g water. Stir or shake to mix.

* Teflon or equivalent.

d. *Nitroprusside*: To a tared 1-L container add 3.50 g sodium nitroprusside (sodium nitroferrocyanide), Na₂Fe(CN)₅NO · 2H₂O, and 1000 g water. Invert to mix.

e. *Stock ammonia standard*, 1000 mg N/L: In a 1-L volumetric flask dissolve 3.819 g ammonium chloride, NH₄Cl, that has been dried for 2 h at 110°C, in about 800 mL water. Dilute to mark and invert to mix.

f. *Standard ammonia solutions*: Prepare ammonia standards in desired concentration range, using the stock standard (§ 3e), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-NH₃:2 and follow method supplied by manufacturer or laboratory standard operating procedure for this method. Follow quality control procedures described in Section 4020.

5. Calculations

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

6. Precision and Bias

a. *Recovery and relative standard deviation*: The results of single-laboratory studies with various matrices are given in Table 4500-NH₃:IV.

b. *MDL*: A 650-μL sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 0.020-mg N/L standard. These gave a mean of 0.0204 mg N/L, a standard deviation of 0.0007 mg N/L, and an MDL of 0.002 mg N/L.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and Procedure for the Determination of Method Detection Limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

TABLE 4500-NH₃:IV. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg NH ₃ -N/L	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	104	—
	Blank†	0.4	100	—
		0.8	102	—
	Site A‡§	0	—	0.5
		0.4	108	—
		0.8	105	—
		0	—	0.5
	Site B‡§	0.4	105	—
		0.8	105	—
		0	—	1.1
		0.4	109	—
		0.8	107	—
Wastewater treatment plant effluent	Reference sample*	—	106	—
	Blank†	0.4	105	—
		0.8	105	—
	Site A‡	0	—	ND
		0.4	90	—
		0.8	88	—
		0	—	<0.1
	Site B‡	0.4	93	—
		0.8	94	—
		0	—	<0.1
		0.4	89	—
		0.8	91	—
Landfill leachate	Reference sample*	—	106	—
	Blank†	0.4	105	—
		0.8	106	—
	Site A‡#	0	—	1.9
		0.4	125	—
		0.8	114	—
	Site B‡#	0	—	0.4
		0.4	96	—
		0.8	106	—
	Site C‡#	0	—	0.2
		0.4	102	—
		0.8	107	—

ND = not detectable.

* U.S. EPA QC sample, 1.98 mg N/L.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate.

§ Site A and C samples diluted 20-fold; Site B sample diluted 100-fold. Typical relative difference between duplicates 0.2%.

|| Samples not diluted. Typical relative difference between duplicates <1%.

Site A and C samples diluted 50-fold; Site B sample diluted 150-fold. Typical relative difference between duplicates 0.3%.

4500-NO₂⁻ NITROGEN (NITRITE)*4500-NO₂⁻ A. Introduction

1. Occurrence and Significance

For a discussion of the chemical characteristics, sources, and effects of nitrite nitrogen, see Section 4500-N.

* Approved by Standard Methods Committee, 2000.

2. Selection of Method

The colorimetric method (B) is suitable for concentrations of 5 to 1000 µg NO₂⁻-N/L (See ¶ B.1a). Nitrite values can be obtained by the automated method given in Section 4500-NO₃⁻-E with the Cu-Cd reduction step omitted. Additionally, nitrite nitrogen can be determined by ion chromatography (Section 4110), and by flow injection analysis (see Sections 4130 and 4500-NO₃⁻-I).

4500-NO₂⁻ B. Colorimetric Method

1. General Discussion

a. Principle: Nitrite (NO₂⁻) is determined through formation of a reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulfanilamide with *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride). The applicable range of the method for spectrophotometric measurements is 10 to 1000 µg NO₂⁻-N/L. Photometric measurements can be made in the range 5 to 50 µg N/L if a 5-cm light path and a green color filter are used. The color system obeys Beer's law up to 180 µg N/L with a 1-cm light path at 543 nm. Higher NO₂⁻ concentrations can be determined by diluting a sample.

b. Interferences: Chemical incompatibility makes it unlikely that NO₂⁻, free chlorine, and nitrogen trichloride (NCl₃) will coexist. NCl₃ imparts a false red color when color reagent is added. The following ions interfere because of precipitation under test conditions and should be absent: Sb³⁺, Au³⁺, Bi³⁺, Fe³⁺, Pb²⁺, Hg²⁺, Ag⁺, chloroplatinate (PtCl₆²⁻), and metavanadate (VO₃²⁻). Cupric ion may cause low results by catalyzing decomposition of the diazonium salt. Colored ions that alter the color system also should be absent. Remove suspended solids by filtration.

c. Storage of sample: Never use acid preservation for samples to be analyzed for NO₂⁻. Make the determination promptly on fresh samples to prevent bacterial conversion of NO₂⁻ to NO₃⁻ or NH₃. For short-term preservation for 1 to 2 d, freeze at -20°C or store at 4°C.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 543 nm, providing a light path of 1 cm or longer.

b. Filter photometer, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 540 nm.

3. Reagents

a. Nitrite-free water: If it is not known that the distilled or demineralized water is free from NO₂⁻, use either of the following procedures to prepare nitrite-free water:

1) Add to 1 L distilled water one small crystal each of KMnO₄ and either Ba(OH)₂ or Ca(OH)₂. Redistill in an all-borosilicate-glass apparatus and discard the initial 50 mL of distillate. Collect the distillate fraction that is free of permanganate; a red color with DPD reagent (Section 4500-Cl.F.2b) indicates the presence of permanganate.

2) Add 1 mL conc H₂SO₄ and 0.2 mL MnSO₄ solution (36.4 g MnSO₄ · H₂O/100 mL distilled water) to each 1 L distilled water, and make pink with 1 to 3 mL KMnO₄ solution (400 mg KMnO₄/L distilled water). Redistill as described in the preceding paragraph.

Use nitrite-free water in making all reagents and dilutions.

b. Color reagent: To 800 mL water add 100 mL 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1 L with water. Solution is stable for about a month when stored in a dark bottle in refrigerator.

c. Sodium oxalate, 0.025M (0.05N): Dissolve 3.350 g Na₂C₂O₄, primary standard grade, in water and dilute to 1000 mL.

d. Ferrous ammonium sulfate, 0.05M (0.05N): Dissolve 19.607 g Fe(NH₄)₂(SO₄)₂ · 6H₂O plus 20 mL conc H₂SO₄ in water and dilute to 1000 mL. Standardize as in Section 5220B.3d.

e. Stock nitrite solution: Commercial reagent-grade NaNO₂ assays at less than 99%. Because NO₂⁻ is oxidized readily in the presence of moisture, use a fresh bottle of reagent for preparing the stock solution and keep bottles tightly stoppered against the free access of air when not in use. To determine NaNO₂ content, add a known excess of standard 0.01M (0.05N) KMnO₄ solution (see ¶ h below), discharge permanganate color with a known

quantity of standard reductant such as 0.025M Na₂C₂O₄ or 0.05M Fe(NH₄)₂(SO₄)₂, and back-titrate with standard permanganate solution.

1) Preparation of stock solution—Dissolve 1.232 g NaNO₂ in water and dilute to 1000 mL; 1.00 mL = 250 µg N. Preserve with 1 mL CHCl₃.

2) Standardization of stock nitrite solution—Pipet, in order, 50.00 mL standard 0.01M (0.05N) KMnO₄, 5 mL conc H₂SO₄, and 50.00 mL stock NO₂⁻ solution into a glass-stoppered flask or bottle. Submerge pipet tip well below surface of permanganate-acid solution while adding stock NO₂⁻ solution. Shake gently and warm to 70 to 80°C on a hot plate. Discharge permanganate color by adding sufficient 10-mL portions of standard 0.025M Na₂C₂O₄. Titrate excess Na₂C₂O₄ with 0.01M (0.05N) KMnO₄ to the faint pink end point. Carry a water blank through the entire procedure and make the necessary corrections in the final calculation as shown in the equation below.

If standard 0.05M ferrous ammonium sulfate solution is substituted for Na₂C₂O₄, omit heating and extend reaction period between KMnO₄ and Fe²⁺ to 5 min before making final KMnO₄ titration.

Calculate NO₂⁻-N content of stock solution by the following equation:

$$A = \frac{[(B \times C) - (D \times E)] \times 7}{F}$$

where:

- A = mg NO₂⁻-N/mL in stock NaNO₂ solution,
- B = total mL standard KMnO₄ used,
- C = normality of standard KMnO₄,
- D = total mL standard reductant added,
- E = normality of standard reductant, and
- F = mL stock NaNO₂ solution taken for titration.

Each 1.00 mL 0.01M (0.05N) KMnO₄ consumed by the NaNO₂ solution corresponds to 1750 µg NO₂⁻-N.

f. *Intermediate nitrite solution:* Calculate the volume, G, of stock NO₂⁻ solution required for the intermediate NO₂⁻ solution from $G = 12.5/A$. Dilute the volume G (approximately 50 mL) to 250 mL with water; 1.00 mL = 50.0 µg N. Prepare daily.

g. *Standard nitrite solution:* Dilute 10.00 mL intermediate NO₂⁻ solution to 1000 mL with water; 1.00 mL = 0.500 µg N. Prepare daily.

h. *Standard potassium permanganate titrant, 0.01M (0.05N):* Dissolve 1.6 g KMnO₄ in 1 L distilled water. Keep in a brown glass-stoppered bottle and age for at least 1 week. Carefully decant or pipet supernate without stirring up any sediment. Standardize this solution frequently by the following procedure:

Weigh to the nearest 0.1 mg several 100- to 200-mg samples of anhydrous Na₂C₂O₄ into 400-mL beakers. To each beaker, in turn, add 100 mL distilled water and stir to dissolve. Add 10 mL 1 + 1 H₂SO₄ and heat rapidly to 90 to 95°C. Titrate rapidly with permanganate solution to be standardized, while stirring, to a slight pink end-point color that persists for at least 1 min. Do not let temperature fall below 85°C. If necessary, warm beaker contents during titration; 100 mg will consume about 6 mL solution. Run a blank on distilled water and H₂SO₄.

$$\text{Normality of KMnO}_4 = \frac{\text{g Na}_2\text{C}_2\text{O}_4}{(A - B) \times 0.33505}$$

where:

- A = mL titrant for sample and
- B = mL titrant for blank.

Average the results of several titrations.

4. Procedure

a. *Removal of suspended solids:* If sample contains suspended solids, filter through a 0.45-µm-pore-diam membrane filter.

b. *Color development:* If sample pH is not between 5 and 9, adjust to that range with 1N HCl or NH₄OH as required. To 50.0 mL sample, or to a portion diluted to 50.0 mL, add 2 mL color reagent and mix.

c. *Photometric measurement:* Between 10 min and 2 h after adding color reagent to samples and standards, measure absorbance at 543 nm. As a guide use the following light paths for the indicated NO₂⁻-N concentrations:

Light Path Length cm	NO ₂ ⁻ -N µg/L
1	2–25
5	2–6
10	<2

5. Calculation

Prepare a standard curve by plotting absorbance of standards against NO₂⁻-N concentration. Compute sample concentration directly from curve.

6. Precision and Bias

In a single laboratory using wastewater samples at concentrations of 0.04, 0.24, 0.55, and 1.04 mg NO₃⁻ + NO₂⁻-N/L, the standard deviations were ±0.005, ±0.004, ±0.005, and ±0.01, respectively. In a single laboratory using wastewater samples at concentrations of 0.24, 0.55, and 1.05 mg NO₃⁻ + NO₂⁻-N/L, the recoveries were 100%, 102%, and 100%, respectively.¹

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes. Method 353. 3. U.S. Environmental Protection Agency, Washington, D.C.

8. Bibliography

- BOLTZ, D.F., ed. 1958. Colorimetric Determination of Nonmetals. Interscience Publishers, New York, N.Y.
- NYDAHL, F. 1976. On the optimum conditions for the reduction of nitrate by cadmium. *Talanta* 23:349.

4500-NO₂⁻ C. (Reserved)4500-NO₃⁻ NITROGEN (NITRATE)*4500-NO₃⁻ A. Introduction

1. Selection of Method

Determination of nitrate (NO₃⁻) is difficult because of the relatively complex procedures required, the high probability that interfering constituents will be present, and the limited concentration ranges of the various techniques.

An ultraviolet (UV) technique (Method B) that measures the absorbance of NO₃⁻ at 220 nm is suitable for screening uncontaminated water (low in organic matter).

Screen a sample; if necessary, then select a method suitable for its concentration range and probable interferences. Nitrate may be determined by ion chromatography (Section 4110), capillary ion electrophoresis (Section 4140), or the methods shown here. Applicable ranges are: nitrate electrode method (D), 0.14 to 1400 mg NO₃⁻-N/L; cadmium reduction method (E), 0.01 to 1.0 mg NO₃⁻-N/L; automated hydrazine reduction method (H), 0.01 to

10 mg NO₃⁻-N/L; automated cadmium reduction method (F), 0.1 to 10 mg NO₃⁻-N/L; cadmium reduction flow injection method (I), 0.000 25 to 10 mg NO₃⁻-N/L; second-derivative ultraviolet spectrophotometric method (C), 0.05 to 2 mg NO₃⁻-N/L. For higher NO₃⁻-N concentrations, dilute into the range of the selected method.

The colorimetric and UV second-derivative methods require an optically clear sample. Filter turbid sample through 0.45-μm membrane filter. Test filters for nitrate contamination.

2. Storage of Samples

Start NO₃⁻ determinations promptly after sampling. If storage is necessary, store for up to 2 d at 4°C; disinfected samples are stable much longer (at least 14 d) without acid preservation.

NOTE: If nitrite, NO₂⁻, is present, then acid preservation can cause disproportionation of HNO₂ to NO₃⁻ and NO (nitric oxide). NO can be oxidized and hydrolyzed to nitrate. As a result, nitrate values may be the sum of nitrate and nitrite. Therefore, do not acidify samples for nitrate determination.

* Approved by Standard Methods Committee, 2000.

Joint Task Group: (4500-NO₃⁻-C)—Thomas R. Holm (chair), Martin S. Frant, Karla Alicia Johnson, Scott Stieg; 20th Edition (4500-NO₃⁻-J)—Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

4500-NO₃⁻ B. Ultraviolet Spectrophotometric Screening Method

1. General Discussion

a. Principle: Use this technique only for screening samples that have low organic matter contents, i.e., uncontaminated natural waters and potable water supplies. The NO₃⁻ calibration curve follows Beer's law up to 11 mg N/L.

Measurement of UV absorption at 220 nm enables rapid determination of NO₃⁻. Because dissolved organic matter also may absorb at 220 nm and NO₃⁻ does not absorb at 275 nm, a second measurement made at 275 nm may be used to correct the NO₃⁻ value. The extent of this empirical correction is related to the nature and concentration of organic matter and may vary from one water to another. Consequently, this method is not recommended if a significant correction for organic matter absorbance is required, although it may be useful in monitoring NO₃⁻ levels within a water body with a constant type of organic matter. Correction factors for organic matter absorbance can be established by the method of additions in combination with analysis of the original NO₃⁻ content by another method. Sample filtration is intended to remove possible interference from suspended particles. Acidification with 1N HCl is designed to

prevent interference from hydroxide or carbonate concentrations up to 1000 mg CaCO₃/L. Chloride has no effect on the determination.

b. Interference: Dissolved organic matter, surfactants, NO₂⁻, and Cr⁶⁺ interfere. Various inorganic ions not normally found in natural water, such as chlorite and chlorate, may interfere. Inorganic substances can be compensated for by independent analysis of their concentrations and preparation of individual correction curves. For turbid samples, see ¶ A.1.

2. Apparatus

Spectrophotometer, for use at 220 nm and 275 nm with matched silica cells of 1-cm or longer light path.

3. Reagents

a. Nitrate-free water: Use redistilled or distilled, deionized water of highest purity to prepare all solutions and dilutions.

b. Stock nitrate solution: Dry potassium nitrate (KNO₃) in an oven at 105°C for 24 h. Dissolve 0.7218 g in water and dilute to

1000 mL; 1.00 mL = 100 µg NO₃⁻-N. Preserve with 2 mL CHCl₃/L. This solution is stable for at least 6 months.

c. *Intermediate nitrate solution:* Dilute 100 mL stock nitrate solution to 1000 mL with water; 1.00 mL = 10.0 µg NO₃⁻-N. Preserve with 2 mL CHCl₃/L. This solution is stable for 6 months.

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

4. Procedure

a. *Treatment of sample:* To 50 mL clear sample, filtered if necessary, add 1 mL HCl solution and mix thoroughly.

b. *Preparation of standard curve:* Prepare NO₃⁻ calibration standards in the range 0 to 7 mg NO₃⁻-N/L by diluting to 50 mL the following volumes of intermediate nitrate solution: 0, 1.00, 2.00, 4.00, 7.00 ... 35.0 mL. Treat NO₃⁻ standards in same manner as samples.

c. *Spectrophotometric measurement:* Read absorbance or transmittance against redistilled water set at zero absorbance or 100% transmittance. Use a wavelength of 220 nm to obtain NO₃⁻ reading and a wavelength of 275 nm to determine interference due to dissolved organic matter.

4500-NO₃⁻ C. Second-Derivative Ultraviolet Spectrophotometric Method (PROPOSED)

1. General Discussion

a. *Principle:* Even though nitrate strongly absorbs ultraviolet (UV) light, determining nitrate by measuring the absorbance at one wavelength is not feasible because natural organic matter (NOM) and other solutes also absorb UV light. The spectra of NOM from different water sources usually are different, so the UV nitrate screening procedure (4500-NO₃⁻.B) may not give reliable results. The UV spectrum of nitrate is quite different from that of NOM. In the nitrate spectrum, the absorbance increases rapidly from 230 to 210 nm, whereas in NOM spectra, the absorbance in the same wavelength range increases gradually. Computing the second derivative of a sample spectrum effectively eliminates the background NOM contribution.

b. *Interferences:* The nitrite UV spectrum is similar to that of nitrate. However, nitrite concentrations usually are much lower than nitrate concentrations. Bicarbonate absorbs weakly at wavelengths below 210 nm, but does not affect the second-derivative signal of nitrate. Bromide interferes at seawater concentrations (68 mg Br⁻/L, salinity 35‰) so this method cannot be used to determine nitrate in seawater. Neither Fe nor Cu interferes at 2 mg/L but both metals seriously interfere at 20 mg/L.¹ The method has been tested only for potable water. Its suitability for nitrate determination in wastewater has not been tested.

2. Apparatus

a. *Spectrophotometer,* capable of scanning from 250 to 200 nm. Some spectrophotometers can compute the second derivative of a spectrum. If the spectrophotometer cannot compute second-derivative spectra, then a computer interface is needed.

5. Calculation

For samples and standards, subtract two times the absorbance reading at 275 nm from the reading at 220 nm to obtain absorbance due to NO₃⁻. Construct a standard curve by plotting absorbance due to NO₃⁻ against NO₃⁻-N concentration of standard. Using corrected sample absorbances, obtain sample concentrations directly from standard curve. NOTE: If correction value is more than 10% of the reading at 220 nm, do not use this method.

6. Bibliography

- HOATHER, R.C. & R.F. RACKMAN. 1959. Oxidized nitrogen and sewage effluents observed by ultraviolet spectrophotometry. *Analyst* 84: 549.
- GOLDMAN, E. & R. JACOBS. 1961. Determination of nitrates by ultraviolet absorption. *J. Amer. Water Works Assoc.* 53:187.
- ARMSTRONG, F.A.J. 1963. Determination of nitrate in water by ultraviolet spectrophotometry. *Anal. Chem.* 35:1292.
- NAVONE, R. 1964. Proposed method for nitrate in potable waters. *J. Amer. Water Works Assoc.* 56:781.

b. *Cuvettes,* matched 1-cm quartz.

c. *Pipettor,* adjustable 1 to 5 mL. Use a pipettor that clicks into place for volume adjustment, not one with a sliding scale.*

d. *Cuvette mixer.*†

e. *Computer, cable, and software* for transferring data from the spectrophotometer and computing second-derivative spectra. The cable and software may be available from the spectrophotometer manufacturer. These items are needed only if the spectrophotometer cannot compute second-derivative spectra.

3. Reagents

a. *Nitrate stock solution,* 100 mg NO₃⁻-N/L: Prepare as in Section 4500-NO₃⁻.B.3b or obtain from a commercial source.

b. *Nitrate-free water,* distilled or deionized. Use to prepare standards and dilute samples if necessary.

4. Procedure

a. *Sample treatment:* Filter samples if turbid. Add 1 mL 1M H₂SO₄ or 2 mL 1M HCl to 100 mL sample, mix thoroughly.

b. *Standards:* Dilute 0.5, 1.0, 1.5, 2.0, and 2.5 mL nitrate stock solution to 100 mL to prepare standards containing 0.5, 1.0, 1.5, 2.0, and 2.5 mg N/L.

c. *Measurement:* Pipet 3.0 mL standard or sample into sample cuvette. Discard and pipet another 3.0 mL. Scan from 250 to 200 nm. If absorbance goes off-scale, dilute sample by withdrawing some sample with the macropipet and then pipetting same vol-

* Wheaton pipet sold as Fisher #13-707-61 or VWR #53499-605, or equivalent.

† Bel-Art F37752-0000 or equivalent.

ume of nitrate-free water into the cuvette. For dilutions of 1:3, 1:5, and 1:10, withdraw 2.0, 2.4, and 2.7 mL. Mix with cuvette mixer. Alternatively, perform dilutions in volumetric glassware. Record any dilution. Compute second-derivative spectrum. Find maximum second derivative in the range 230 to 220 nm and record value. If spectrophotometer cannot compute second-derivative spectra, then set it to read the absorbance every 1.0 nm and transfer the spectra to the computer after each scan.

5. Calculation

a. Calibration: Compute second-derivative spectrum and find maximum for each sample, blank, or standard if the spectrophotometer cannot do this. Use the simplified least-squares procedure^{2,3} to simultaneously smooth and differentiate spectra.† Perform least-squares linear regression using the second derivatives of the blank and standard spectra.

$$S = Int + C \times Slp$$

where:

S = maximum second derivative,

C = concentration,

Int = intercept of regression line, and

Slp = slope of regression line.

Thus,

$$C = \frac{S - Int}{Slp}$$

b. Samples: Calculate the nitrate concentration, C , from the equation above.

† A Lotus 1-2-3® worksheet for this calculation can be obtained from Tom Holm, Illinois State Water Survey, 2204 Griffith Drive, Champaign, IL 61820.

6. Precision and Bias

Nitrate concentrations determined by the UV second-derivative method were compared with concentrations determined by ion chromatography for 79 potable-water samples with nitrate concentrations of up to 40 mg NO_3^- -N/L.⁴ The slope of the regression line was 0.94 with a standard error of 0.005. For 65 out of the 79 samples, the difference between nitrate concentrations determined by second-derivative UV spectrophotometry and ion chromatography was 0.5 mg N/L or less. The maximum difference for all samples was 1.6 mg NO_3^- -N/L. The differences between the nitrate concentrations determined by the two methods appeared to be random, but they were not normally distributed.

7. References

- CRUMPTON, W.G., T.M. ISENHARD & P.D. MITCHELL. 1992. Nitrate and organic N analyses with second-derivative spectroscopy. *Limnol. Oceanogr.* 37:907.
- SAVITSKY, A. & M. GOLAY. 1964. Smoothing and differentiation of data by simplified least squares procedures. *Anal. Chem.* 36:1627.
- STEINER, J., Y. TERMONIA & J. DELTOUR. 1972. Comments on smoothing and differentiation of data by simplified least squares. *Anal. Chem.* 44:1906.
- HOLM, T.R., W.R. KELLY, L.F. SIEVERS & D.L. WEBB. 1997. A comparison of ultraviolet spectrophotometry with other methods for the determination of nitrate in water. *Spectroscopy* 12:38.

8. Bibliography

- SIMAL, J., M.S. LAGE & I. IGLESIAS. 1985. Second derivative ultraviolet spectroscopy and sulfamic acid method for determination of nitrates in water. *J. Assoc. Offic. Anal. Chem.* 68:962.
- SUZUKI, N. & R. KURODA. 1987. Direct simultaneous determination of nitrate and nitrite by ultraviolet second-derivative spectrophotometry. *Analyst* 112:1077.

4500- NO_3^- D. Nitrate Electrode Method

1. General Discussion

a. Principle: The NO_3^- ion electrode is a selective sensor that develops a potential across a thin, porous, inert membrane that holds in place a water-immiscible liquid ion exchanger. The electrode responds to NO_3^- ion activity between about 10^{-5} and 10^{-1} M (0.14 to 1400 mg NO_3^- -N/L). The lower limit of detection is determined by the small but finite solubility of the liquid ion exchanger.

b. Interferences: Chloride and bicarbonate ions interfere when their weight ratios to NO_3^- -N are >10 or >5 , respectively. Ions that are potential interferences but do not normally occur at significant levels in potable waters are NO_2^- , CN^- , S^{2-} , Br^- , I^- , ClO_3^- , and ClO_4^- . Although the electrodes function satisfactorily in buffers over the range pH 3 to 9, erratic responses have been noted where pH is not held constant. Because the electrode responds to NO_3^- activity rather than con-

centration, ionic strength must be constant in all samples and standards. Minimize these problems by using a buffer solution containing Ag_2SO_4 to remove Cl^- , Br^- , I^- , S^{2-} , and CN^- , sulfamic acid to remove NO_2^- , a buffer at pH 3 to eliminate HCO_3^- and to maintain a constant pH and ionic strength, and $\text{Al}_2(\text{SO}_4)_3$ to complex organic acids.

2. Apparatus

a. pH meter, expanded-scale or digital, capable of 0.1 mV resolution.

*b. Double-junction reference electrode.** Fill outer chamber with $(\text{NH}_4)_2\text{SO}_4$ solution.

* Orion Model 90-02, or equivalent.

c. *Nitrate ion electrode*:† Carefully follow manufacturer's instructions regarding care and storage.

d. *Magnetic stirrer*: TFE-coated stirring bar.

3. Reagents

a. *Nitrate-free water*: Prepare as described in ¶ B.3a. Use for all solutions and dilutions.

b. *Stock nitrate solution*: Prepare as described in ¶ B.3b.

c. *Standard nitrate solutions*: Dilute 1.0, 10, and 50 mL stock nitrate solution to 100 mL with water to obtain standard solutions of 1.0, 10, and 50 mg NO₃⁻-N/L, respectively.

d. *Buffer solution*: Dissolve 17.32 g Al₂(SO₄)₃ · 18H₂O, 3.43 g Ag₂SO₄, 1.28 g H₃BO₃, and 2.52 g sulfamic acid (H₂NSO₃H), in about 800 mL water. Adjust to pH 3.0 by slowly adding 0.10N NaOH. Dilute to 1000 mL and store in a dark glass bottle.

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

f. *Reference electrode filling solution*: Dissolve 0.53 g (NH₄)₂SO₄ in water and dilute to 100 mL.

4. Procedure

a. *Preparation of calibration curve*: Transfer 10 mL of 1 mg NO₃⁻-N/L standard to a 50-mL beaker, add 10 mL buffer, and stir with a magnetic stirrer. Immerse tips of electrodes and record millivolt reading when stable (after about 1 min). Remove electrodes, rinse, and blot dry. Repeat for 10-mg NO₃⁻-N/L and 50-mg NO₃⁻-N/L standards. Plot potential measurements against NO₃⁻-N concentration on semilogarithmic graph paper, with NO₃⁻-N concentration on the logarithmic axis (abscissa)

† Orion Model 93-07, Corning Model 476134, or equivalent.

4500-NO₃⁻ E. Cadmium Reduction Method

1. General Discussion

a. *Principle*: NO₃⁻ is reduced almost quantitatively to nitrite (NO₂⁻) in the presence of cadmium (Cd). This method uses commercially available Cd granules treated with copper sulfate (CuSO₄) and packed in a glass column.

The NO₂⁻ produced thus is determined by diazotizing with sulfanilamide and coupling with *N*-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye that is measured colorimetrically. A correction may be made for any NO₂⁻ present in the sample by analyzing without the reduction step. The applicable range of this method is 0.01 to 1.0 mg NO₃⁻-N/L. The method is recommended especially for NO₃⁻ levels below 0.1 mg N/L where other methods lack adequate sensitivity.

b. *Interferences*: Suspended matter in the column will restrict sample flow. For turbid samples, see ¶ A.1. Concentrations of iron, copper, or other metals above several milligrams per liter lower reduction efficiency. Add EDTA to samples to eliminate

and potential (in millivolts) on the linear axis (ordinate). A straight line with a slope of +57 ± 3 mV/decade at 25°C should result. Recalibrate electrodes several times daily by checking potential reading of the 10 mg NO₃⁻-N standard and adjusting the calibration control until the reading plotted on the calibration curve is displayed again.

b. *Measurement of sample*: Transfer 10 mL sample to a 50-mL beaker, add 10 mL buffer solution, and stir (for about 1 min) with a magnetic stirrer. Measure standards and samples at about the same temperature. Immerse electrode tips in sample and record potential reading when stable (after about 1 min). Read concentration from calibration curve.

5. Precision

Over the range of the method, precision of ±0.4 mV, corresponding to 2.5% in concentration, is expected.

6. Bibliography

- LANGMUIR, D. & R.I. JACOBSON. 1970. Specific ion electrode determination of nitrate in some fresh waters and sewage effluents. *Environ. Sci. Technol.* 4:835.
- KEENEY, D.R., B.H. BYRNES & J.J. GENSON. 1970. Determination of nitrate in waters with nitrate-selective ion electrode. *Analyst* 95: 383.
- MILHAM, P.J., A.S. AWAD, R.E. PAULL & J.H. BULL. 1970. Analysis of plants, soils and waters for nitrate by using an ion-selective electrode. *Analyst* 95:751.
- SYNNOTT, J.C., S.J. WEST & J.W. ROSS. 1984. Comparison of ion-selective electrode and gas-sensing electrode technique for measurement of nitrate in environmental samples. In Pawlowski et al., eds., *Studies in Environmental Science*, No. 23, Chemistry for Protection of the Environment. Elsevier Press, New York, N.Y.

this interference. Oil and grease will coat the Cd surface. Remove by pre-extraction with an organic solvent (see Section 5520). Residual chlorine can interfere by oxidizing the Cd column, reducing its efficiency. Check samples for residual chlorine (see DPD methods in Section 4500-Cl). Remove residual chlorine by adding sodium thiosulfate (Na₂S₂O₃) solution (Section 4500-NH₃.B.3d). Sample color that absorbs at about 540 nm interferes.

2. Apparatus

a. *Reduction column*: Purchase or construct the column* (Figure 4500-NO₃⁻:1) from a 100-mL volumetric pipet by removing the top portion. The column also can be constructed from two pieces of tubing joined end to end: join a 10-cm length of

* Tudor Scientific Glass Co., 555 Edgefield Road, Belvedere, SC 29841, Cat. TP-1730, or equivalent.

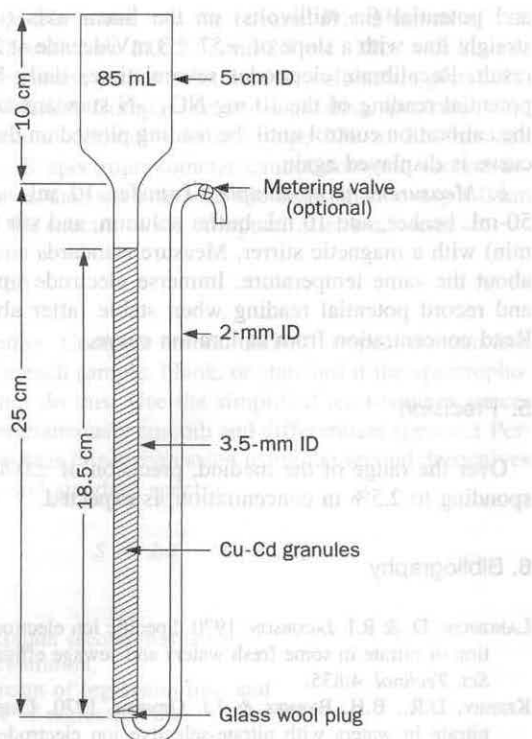


Figure 4500-NO₃⁻:1. Reduction column.

3-cm-ID tubing to a 25-cm length of 3.5-mm-ID tubing. Add a TFE stopcock with metering valve¹ to control flow rate.

b. *Colorimetric equipment:* One of the following is required:

1) *Spectrophotometer*, for use at 543 nm, providing a light path of 1 cm or longer.

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

3. Reagents

a. *Nitrate-free water:* See ¶ B.3a. The absorbance of a reagent blank prepared with this water should not exceed 0.01. Use for all solutions and dilutions.

b. *Copper-cadmium granules:* Wash 25 g new or used 20- to 100-mesh Cd granules[†] with 6N HCl and rinse with water. Swirl Cd with 100 mL 2% CuSO₄ solution for 5 min or until blue color partially fades. Decant and repeat with fresh CuSO₄ until a brown colloidal precipitate begins to develop. Gently flush with water to remove all precipitated Cu.

c. *Color reagent:* Prepare as directed in Section 4500-NO₂⁻-B.3b.

d. *Ammonium chloride-EDTA solution:* Dissolve 13 g NH₄Cl and 1.7 g disodium ethylenediamine tetraacetate in 900 mL water. Adjust to pH 8.5 with conc NH₄OH and dilute to 1 L.

e. *Dilute ammonium chloride-EDTA solution:* Dilute 300 mL NH₄Cl-EDTA solution to 500 mL with water.

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

g. *Copper sulfate solution, 2%:* Dissolve 20 g CuSO₄·5H₂O in 500 mL water and dilute to 1 L.

h. *Stock nitrate solution:* Prepare as directed in ¶ B.3b.

i. *Intermediate nitrate solution:* Prepare as directed in ¶ B.3c.

j. *Stock nitrite solution:* See Section 4500-NO₂⁻-B.3e.

k. *Intermediate nitrite solution:* See Section 4500-NO₂⁻-B.3f.

l. *Working nitrite solution:* Dilute 50.0 mL intermediate nitrite solution to 500 mL with nitrite-free water; 1.00 mL = 5 µg NO₂⁻-N.

4. Procedure

a. *Preparation of reduction column:* Insert a glass wool plug into bottom of reduction column and fill with water. Add sufficient Cu-Cd granules to produce a column 18.5 cm long. Maintain water level above Cu-Cd granules to prevent entrapment of air. Wash column with 200 mL dilute NH₄Cl-EDTA solution. Activate column by passing through it, at 7 to 10 mL/min, at least 100 mL of a solution composed of 25% 1.0 mg NO₃⁻-N/L standard and 75% NH₄Cl-EDTA solution.

b. *Treatment of sample:*

1) *Turbidity removal*—For turbid samples, see ¶ A.1.

2) *pH adjustment*—Adjust pH to between 7 and 9, as necessary, using a pH meter and dilute HCl or NaOH. This insures a pH of 8.5 after adding NH₄Cl-EDTA solution.

3) *Sample reduction*—To 25.0 mL sample or a portion diluted to 25.0 mL, add 75 mL NH₄Cl-EDTA solution and mix. Pour mixed sample into column and collect at a rate of 7 to 10 mL/min. Discard first 25 mL. Collect the rest in original sample flask. There is no need to wash columns between samples, but if columns are not to be reused for several hours or longer, pour 50 mL dilute NH₄Cl-EDTA solution on to the top and let it pass through the system. Store Cu-Cd column in this solution and never let it dry.

4) *Color development and measurement*—As soon as possible, and not more than 15 min after reduction, add 2.0 mL color reagent to 50 mL sample and mix. Between 10 min and 2 h afterward, measure absorbance at 543 nm against a distilled water-reagent blank. NOTE: If NO₃⁻ concentration exceeds the standard curve range (about 1 mg N/L), use remainder of reduced sample to make an appropriate dilution and analyze again.

c. *Standards:* Using the intermediate NO₃⁻-N solution, prepare standards in the range 0.05 to 1.0 mg NO₃⁻-N/L by diluting the following volumes to 100 mL in volumetric flasks: 0.5, 1.0, 2.0, 5.0, and 10.0 mL. Carry out reduction of standards exactly as described for samples. Compare at least one NO₂⁻ standard to a reduced NO₃⁻ standard at the same concentration to verify reduction column efficiency. Reactivate Cu-Cd granules as described in ¶ 3b above when efficiency of reduction falls below about 75%.

5. Calculation

Obtain a standard curve by plotting absorbance of standards against NO₃⁻-N concentration. Compute sample concentrations directly from standard curve. Report as milligrams oxidized N per liter (the sum of NO₃⁻-N plus NO₂⁻-N) unless the concentration of NO₂⁻-N is separately determined and subtracted.

[†] EM Laboratories, Inc., 500 Exec. Blvd., Elmsford, NY, Cat. 2001, or equivalent.

6. Precision and Bias

In a single laboratory using wastewater samples at concentrations of 0.04, 0.24, 0.55, and 1.04 mg NO₃⁻ + NO₂⁻-N/L, the standard deviations were ±0.005, ±0.004, ±0.005, and ±0.01, respectively. In a single laboratory using wastewater with additions of 0.24, 0.55, and 1.05 mg NO₃⁻ + NO₂⁻-N/L, the recoveries were 100%, 102%, and 100%, respectively.²

7. References

1. WOOD, E.D., F.A.J. ARMSTRONG & F.A. RICHARDS. 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. *J. Mar. Biol. Assoc. U.K.* 47:23.

2. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes, Method 353.3. U.S. Environmental Protection Agency, Washington, D.C.

8. Bibliography

- STRICKLAND, J.D.H. & T.R. PARSONS. 1972. A Practical Handbook of Sea Water Analysis, 2nd ed. Bull. No. 167, Fisheries Research Board Canada, Ottawa, Ont.
- NYDAHL, F. 1976. On the optimum conditions for the reduction of nitrate by cadmium. *Talanta* 23:349.
- AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1987. Annual Book of ASTM Standards, Vol. 11.01. American Soc. Testing & Materials, Philadelphia, Pa.

4500-NO₃⁻ F. Automated Cadmium Reduction Method

1. General Discussion

a. *Principle:* See ¶ E.1a.

b. *Interferences:* Sample turbidity may interfere. Remove by filtration before analysis. Sample color that absorbs in the photometric range used for analysis also will interfere.

c. *Application:* Nitrate and nitrite, singly or together in potable, surface, and saline waters and domestic and industrial wastewaters, can be determined over a range of 0.5 to 10 mg N/L.

2. Apparatus

Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the components shown in Figure 4500-NO₃⁻:2.

3. Reagents

a. *Deionized distilled water:* See ¶ B.3a.

b. *Copper sulfate solution:* Dissolve 20 g CuSO₄ · 5H₂O in 500 mL water and dilute to 1 L.

c. *Wash solution:* Use water for unpreserved samples. For samples preserved with H₂SO₄, add 2 mL conc H₂SO₄/L wash water.

d. *Copper-cadmium granules:* See ¶ E.3b.

e. *Hydrochloric acid, HCl, conc.*

f. *Ammonium hydroxide, NH₄OH, conc.*

g. *Color reagent:* To approximately 800 mL water, add, while stirring, 100 mL conc H₃PO₄, 40 g sulfanilamide, and 2 g N-(1-naphthyl)-ethylenediamine dihydrochloride. Stir until dissolved and dilute to 1 L. Store in brown bottle and keep in the dark when not in use. This solution is stable for several months.

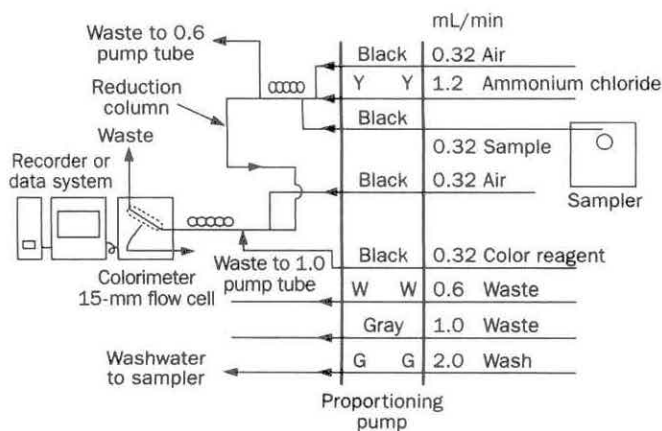


Figure 4500-NO₃⁻:2. Nitrate-nitrite manifold.

h. *Ammonium chloride solution:* Dissolve 85 g NH₄Cl in water and dilute to 1 L. Add 0.5 mL polyoxyethylene 23 lauryl ether.*

i. *Stock nitrate solution:* See ¶ B.3b.

j. *Intermediate nitrate solution:* See ¶ B.3c.

k. *Standard nitrate solutions:* Using intermediate NO₃⁻-N solution and water, prepare standards for calibration curve in appropriate nitrate range. Compare at least one NO₂⁻ standard to a NO₃⁻ standard at the same concentration to verify column reduction efficiency. To examine saline waters prepare standard solutions with the substitute ocean water described in Section 4500-NH₃.G.3g.

* Brij-35, available from ICI Americas, Inc., Wilmington, DE, or equivalent.

1. *Standard nitrite solution:* See 4500-NO₂⁻.B.3g.

4. Procedure

Set up manifold as shown in Figure 4500-NO₃⁻.2 and follow general procedure described by the manufacturer.

If sample pH is below 5 or above 9, adjust to between 5 and 9 with either conc HCl or conc NH₄OH.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against NO₃⁻-N concentration in standards. Compute sample NO₃⁻-N concentration by comparing sample response with standard curve.

6. Precision and Bias

Data obtained in three laboratories with an automated system based on identical chemical principles but having slightly different configurations are given in the table below. Analyses were conducted on four natural water samples containing exact increments of inorganic nitrate:

Increment as NO ₃ ⁻ -N μg/L	Standard Deviation μg N/L	Bias %	Bias μg N/L
290	12	+5.75	+17
350	92	+18.10	+63

4500-NO₃⁻ G. (Reserved)

4500-NO₃⁻ H. Automated Hydrazine Reduction Method

1. General Discussion

a. *Principle:* NO₃⁻ is reduced to NO₂⁻ with hydrazine sulfate. The NO₂⁻ (originally present) plus reduced NO₃⁻ is determined by diazotization with sulfanilamide and coupling with *N*-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye that is measured colorimetrically.

b. *Interferences:* Sample color that absorbs in the photometric range used will interfere. Concentrations of sulfide ion of less than 10 mg/L cause variations of NO₃⁻ and NO₂⁻ concentrations of ±10%.

c. *Application:* NO₃⁻ + NO₂⁻ in potable and surface water and in domestic and industrial wastes can be determined over a range of 0.01 to 10 mg N/L.

2. Apparatus

Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the components shown in Figure 4500-NO₃⁻.3.

Increment as NO ₃ ⁻ -N μg/L	Standard Deviation μg N/L	Bias %	Bias μg N/L
2310	318	+4.47	+103
2480	176	-2.69	-67

In a single laboratory using surface water samples at concentrations of 100, 200, 800, and 2100 μg N/L, the standard deviations were 0, ±40, ±50, and ±50 μg/L, respectively, and at concentrations of 200 and 2200 μg N/L, recoveries were 100 and 96%, respectively.

Precision and bias for the system described herein are believed to be comparable.

7. Bibliography

- FIGURE, J. & J.E. O'BRIEN. 1962. Automation in sanitary chemistry—Parts 1 and 2. Determination of nitrates and nitrites. *Wastes Eng.* 33:128 & 238.
- ARMSTRONG, F.A., C.R. STEARNS & J.D. STRICKLAND. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon AutoAnalyzer and associated equipment. *Deep Sea Res.* 14:381.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes. U.S. Environmental Protection Agency, Washington, D.C.
- AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1987. Annual Book of ASTM Standards, Vol. 11.01. American Soc. Testing & Materials, Philadelphia, Pa.

3. Reagents

a. *Color developing reagent:* To approximately 500 mL water add 200 mL conc phosphoric acid and 10 g sulfanilamide. After sulfanilamide is dissolved completely, add 0.8 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Dilute to 1 L with water, store in a dark bottle, and refrigerate. Solution is stable for approximately 1 month.

b. *Copper sulfate stock solution:* Dissolve 2.5 g CuSO₄ · 5H₂O in water and dilute to 1 L.

c. *Copper sulfate dilute solution:* Dilute 20 mL stock solution to 2 L.

d. *Sodium hydroxide stock solution, 10N:* Dissolve 400 g NaOH in 750 mL water, cool, and dilute to 1 L.

e. *Sodium hydroxide, 1.0N:* Dilute 100 mL stock NaOH solution to 1 L.

f. *Hydrazine sulfate stock solution:* Dissolve 27.5 g N₂H₄ · H₂SO₄ in 900 mL water and dilute to 1 L. This solution

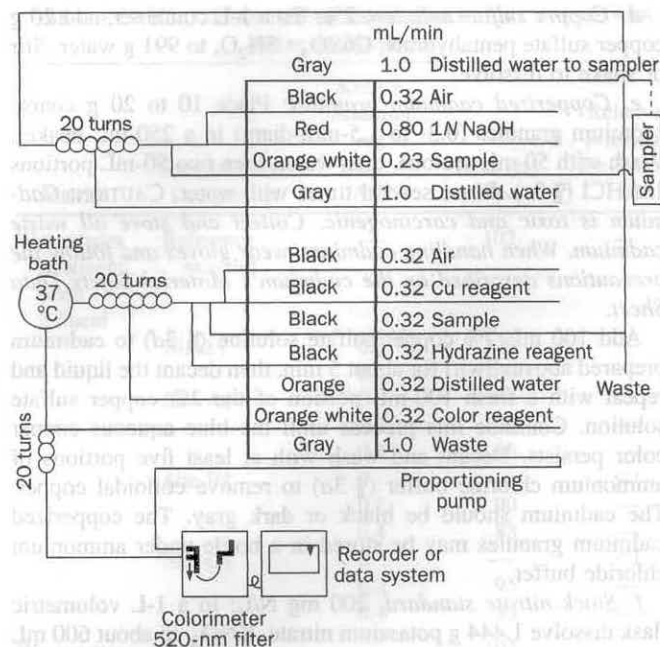


Figure 4500-NO₃⁻:3. Nitrate-nitrite manifold.

is stable for approximately 6 months. CAUTION: Toxic if ingested. Mark container with appropriate warning.

g. Hydrazine sulfate dilute solution: Dilute 22 mL stock solution to 1 L.

h. Stock nitrate solution: See ¶ B.3b.

i. Intermediate nitrate solution: See ¶ B.3c.

j. Standard nitrate solutions: Prepare NO₃⁻ calibration standards in the range 0 to 10 mg/L by diluting to 100 mL the following volumes of stock nitrate solution: 0, 0.5, 1.0, 2.0 . . . 10.0 mL. For standards in the range of 0.01 mg/L use intermediate nitrate solution. Compare at least one nitrite standard to a nitrate standard at the same concentration to verify the efficiency of the reduction.

k. Standard nitrite solution: See ¶s 4500-NO₂⁻.B.3e, f, and g.

4. Procedure

Set up manifold as shown in Figure 4500-NO₃⁻:3 and follow general procedure described by manufacturer. Run a 2.0-mg NO₃⁻-N/L and a 2.0-mg NO₂⁻-N/L standard through the system to check for 100% reduction of nitrate to nitrite. The two peaks should be of equal height; if not, adjust concentration of the hydrazine sulfate solution: If the NO₃⁻ peak is lower than the NO₂⁻ peak, increase concentration of hydrazine sulfate until they are equal; if the NO₃⁻ peak is higher than the NO₂⁻ reduce concentration of hydrazine sulfate. When correct concentration has been determined, no further adjustment should be necessary.

5. Calculation

Prepare a standard curve by plotting response of processed standards against known concentrations. Compute concentrations of samples by comparing response with standard curve.

6. Precision and Bias

In a single laboratory using drinking water, surface water, and industrial waste at concentrations of 0.39, 1.15, 1.76, and 4.75 mg NO₃⁻-N/L, the standard deviations were ±0.02, ±0.01, ±0.02, and ±0.03, respectively. In a single laboratory using drinking water at concentrations of 0.75 and 2.97 mg NO₃⁻-N/L, the recoveries were 99% and 101%.¹

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes. U.S. Environmental Protection Agency, Washington, D.C.

8. Bibliography

KAMPHAKE, L., S. HANNAH & J. COHEN. 1967. Automated analysis for nitrate by hydrazine reduction. *Water Res.* 1:205.

4500-NO₃⁻ I. Cadmium Reduction Flow Injection Method

1. General Discussion

a. Principle: The nitrate in the sample is reduced quantitatively to nitrite by passage of the sample through a copperized cadmium column. The resulting nitrite plus any nitrite originally in the sample is determined as a sum by diazotizing the nitrite with sulfanilamide followed by coupling with *N*-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water-soluble dye has a magenta color; absorbance of the color at 540 nm is proportional to the nitrate + nitrite in the sample. This sum also is known as total oxidized nitrogen (TON).

Nitrite alone can be determined by removing the cadmium column, recalibrating the method, and repeating the sample analyses. A TON and a nitrite FIA method also can be run in

parallel for a set of samples. In this arrangement, the concentrations determined in the nitrite method can be subtracted from the corresponding concentrations determined in the TON method to give the resulting nitrate concentrations of the samples.

Also see Section 4500-NO₂⁻ and Section 4130, Flow Injection Analysis (FIA).

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

Residual chlorine can interfere by oxidizing the cadmium reduction column. Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. Eliminate

this interference by pre-extracting sample with an organic solvent.

Low results would be obtained for samples that contain high concentrations of iron, copper, or other metals. In this method, EDTA is added to the buffer to reduce this interference.

Also see Section 4500-NO₂⁻.B.1b and c and Section 4500-NO₃⁻.A.2 and B.1b.

2. Apparatus

Flow injection analysis equipment consisting of:

- FIA injection valve with sample loop or equivalent.
- Multichannel proportioning pump.
- FIA manifold (Figure 4500-NO₃⁻:4) with flow cell. Relative flow rates only are shown in Figure 4500-NO₃⁻:4. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.
- Absorbance detector, 540 nm, 10-nm bandpass.
- Injection valve control and data acquisition system.

3. Reagents

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

a. **Ammonium chloride buffer:** CAUTION: Fumes. Use a hood. To a tared 1-L container add 800.0 g water, 126 g conc hydrochloric acid, HCl, 55.6 g ammonium hydroxide, NH₄OH, and 1.0 g disodium EDTA. Shake until dissolved. The pH of this buffer should be 8.5.

b. **Sulfanilamide color reagent:** To a tared, dark 1-L container add 876 g water, 170 g 85% phosphoric acid, H₃PO₄, 40.0 g sulfanilamide, and 1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride (NED). Shake until wetted and stir with stir bar for 30 min until dissolved. This solution is stable for 1 month.

c. **Hydrochloric acid, HCl, 1M:** To a 100-mL container, add 92 g water, then add 9.6 g conc HCl. Stir or shake to mix.

d. **Copper sulfate solution, 2%:** To a 1-L container, add 20 g copper sulfate pentahydrate, CuSO₄ · 5H₂O, to 991 g water. Stir or shake to dissolve.

e. **Copperized cadmium granules:** Place 10 to 20 g coarse cadmium granules (0.3- to 1.5-mm-diam) in a 250-mL beaker. Wash with 50 mL acetone, then water, then two 50-mL portions 1M HCl (¶ 3c). Rinse several times with water. CAUTION: Cadmium is toxic and carcinogenic. Collect and store all waste cadmium. When handling cadmium, wear gloves and follow the precautions described on the cadmium's Material Safety Data Sheet.

Add 100 mL 2% copper sulfate solution (¶ 3d) to cadmium prepared above. Swirl for about 5 min, then decant the liquid and repeat with a fresh 100-mL portion of the 2% copper sulfate solution. Continue this process until the blue aqueous copper color persists. Decant and wash with at least five portions of ammonium chloride buffer (¶ 3a) to remove colloidal copper. The cadmium should be black or dark gray. The copperized cadmium granules may be stored in a bottle under ammonium chloride buffer.

f. **Stock nitrate standard, 200 mg N/L:** In a 1-L volumetric flask dissolve 1.444 g potassium nitrate, KNO₃, in about 600 mL water. Add 2 mL chloroform. Dilute to mark and invert to mix. This solution is stable for 6 months.

g. **Stock nitrite standard, 200.0 mg N/L:** In a 1-L volumetric flask dissolve 0.986 g sodium nitrite, NaNO₂, or 1.214 g potassium nitrite, KNO₂, in approximately 800 mL water. Add 2 mL chloroform. Dilute to mark and invert to mix. Refrigerate.

h. **Standard solution:** Prepare nitrate or nitrite standards in the desired concentration range, using the stock standards (¶ 3f or g), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-NO₃⁻:4 and pack column with copperized cadmium granules. Follow methods supplied by column and instrument manufacturer or laboratory's standard operating procedure for this method. Follow quality control procedures outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus TON or nitrite concentration. The calibration curve is linear.

If TON includes measurable nitrite concentrations, it is important that the cadmium column be 100% efficient. If the efficiency is less, the nitrite in the sample will give a positive percent error equal to the difference from 100%, causing an error in TON and nitrate determinations. To measure efficiency of the cadmium column, prepare two calibration curves, one using nitrate standards and one using equimolar nitrite standards. The column efficiency is:

$$\text{Column efficiency} = 100\%$$

$$\times (\text{slope of nitrate curve} / \text{slope of nitrite curve})$$

Determine column efficiency at least weekly.

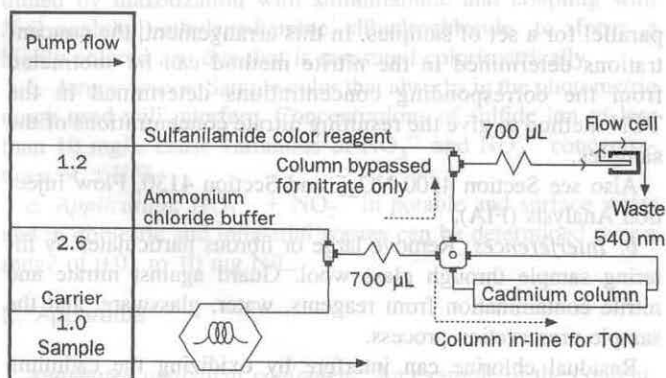


Figure 4500-NO₃⁻:4. FIA nitrate + nitrite manifold.

TABLE 4500-NO₃⁻:I. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg NO ₃ ⁻ - N/L	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	102	—
	Blank†	0.2	100	—
		0.4	100	—
	Site A‡	0	—	<1
		0.2	101	—
		0.4	96	—
		0.4	96	—
	Site B‡	0	—	<1
		0.2	90	—
		0.4	88	—
		0.4	88	—
Wastewater treatment plant effluent	Reference sample*	—	102	—
	Blank†	0.2	100	—
		0.4	95	—
	Site A‡	0	—	0.9
		0.2	95	—
		0.4	102	—
		0.4	102	—
	Site B‡	0	—	0.7
		0.2	91	—
		0.4	101	—
		0.4	101	—
Landfill leachate	Reference sample*	—	98	—
	Blank†	0.2	100	—
		0.4	98	—
	Site A‡	0	—	<1
		0.2	104	—
		0.4	96	—
		0.4	96	—
	Site B‡	0	—	<1
		0.2	95	—
		0.4	94	—
		0.4	94	—
Landfill leachate	Site C‡	0	—	<1
		0.2	91	<1
		0.4	93	—
		0.4	93	—

* U.S. EPA QC sample, 1.98 mg N/L.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate. Typical difference between duplicates: influent, 2%; effluent, 1%; leachate, <1%.

6. Precision and Bias

In the studies described below, nitrate was measured. There was no significant concentration of nitrite in the samples.

a. *Recovery and relative standard deviation:* Table 4500-NO₃⁻:I gives results of single-laboratory studies.

b. *MDL:* A 800-μL sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 2.00-μg N/L standard. These gave a mean of 1.82 μg N/L, a standard deviation of 0.098 μg N/L, and MDL of 0.25 μg N/L. A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagent flow rate.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-N_{org} NITROGEN (ORGANIC)*4500-N_{org} A. Introduction

1. Selection of Method

The kjeldahl methods (B and C) determine nitrogen in the trinegative state. They fail to account for nitrogen in the form of azide, azine, azo, hydrazone, nitrate, nitrite, nitrile, nitro, nitroso, oxime, and semi-carbazone. "Kjeldahl nitrogen" is the sum of organic nitrogen and ammonia nitrogen.

The major factor that influences the selection of a macro- or semi-micro-kjeldahl method to determine organic nitrogen is its concentration. The macro-kjeldahl method is applicable for samples containing either low or high concentrations of organic nitrogen but requires a relatively large sample volume for low concentrations. In the semi-micro-kjeldahl method, which is applicable to samples containing high concentrations of organic nitrogen, the sample volume should be chosen to contain organic plus ammonia nitrogen in the range of 0.2 to 2 mg.

The block digestion method (D) is a micro method with an automated analysis step capable of measuring organic nitrogen as low as 0.1 mg/L when blanks are carefully controlled.

2. Storage of Samples

The most reliable results are obtained on fresh samples. If an immediate analysis is not possible, preserve samples for kjeldahl digestion by acidifying to pH 1.5 to 2.0 with concentrated H₂SO₄ and storing at 4°C. Do not use HgCl₂ because it will interfere with ammonia removal.

3. Interferences

a. Nitrate: During kjeldahl digestion, nitrate in excess of 10 mg/L can oxidize a portion of the ammonia released from the digested organic nitrogen, producing N₂O and resulting in a negative interference. When sufficient organic matter in a low state of oxidation is present, nitrate can be reduced to ammonia, resulting in a positive interference. The conditions under which significant interferences occur are not well defined and there is no proven way to eliminate the interference in conjunction with the kjeldahl methods described herein.

b. Inorganic salts and solids: The acid and salt content of the kjeldahl digestion reagent is intended to produce a digestion

temperature of about 380°C. If the sample contains a very large quantity of salt or inorganic solids that dissolve during digestion, the temperature may rise above 400°C, at which point pyrolytic loss of nitrogen begins to occur. To prevent an excessive digestion temperature, add more H₂SO₄ to maintain the acid-salt balance. Not all salts cause precisely the same temperature rise, but adding 1 mL H₂SO₄/g salt in the sample gives reasonable results. Add the extra acid and the digestion reagent to both sample and reagent blank. Too much acid will lower the digestion temperature below 380°C and result in incomplete digestion and recovery. If necessary, add sodium hydroxide-sodium thiosulfate before the final distillation step to neutralize the excess acid.

Large amounts of salt or solids also may cause bumping during distillation. If this occurs, add more dilution water after digestion.

c. Organic matter: During kjeldahl digestion, H₂SO₄ oxidizes organic matter to CO₂ and H₂O. If a large amount of organic matter is present, a large amount of acid will be consumed, the ratio of salt to acid will increase, and the digestion temperature will increase. If enough organic matter is present, the temperature will rise above 400°C, resulting in pyrolytic loss of nitrogen. To prevent this, add to the digestion flask 10 mL conc H₂SO₄/3 g COD. Alternately, add 50 mL more digestion reagent/g COD. Additional sodium hydroxide-sodium thiosulfate reagent may be necessary to keep the distillation pH high. Because reagents may contain traces of ammonia, treat the reagent blank identically with the samples.

4. Use of a Catalyst

Mercury has been the catalyst of choice for kjeldahl digestion. Because of its toxicity and problems associated with legal disposal of mercury residues, a less toxic catalyst is recommended. Digestion of some samples may be complete or nearly complete without the use of a catalyst. Effective digestion results from the use of a reagent having a salt/acid ratio of 1 g/mL with copper as catalyst (B.3a), and specified temperature (B.2a) and time (B.4c). If a change is made in the reagent formula, report the change and indicate percentage recovery relative to the results for similar samples analyzed using the previous formula.

Before results are considered acceptable, determine nitrogen recovery from samples with known additions of nicotinic acid, to test completeness of digestion; and with ammonium chloride to test for loss of nitrogen.

* Approved by Standard Methods Committee, 1997.

Joint Task Group: 20th Edition (4500-N_{org},D)—Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

4500-N_{org} B. Macro-Kjeldahl Method

1. General Discussion

a. Principle: In the presence of H₂SO₄, potassium sulfate (K₂SO₄), and cupric sulfate (CuSO₄) catalyst, amino nitrogen of many organic materials is converted to ammonium. Free ammonia also is converted to ammonium. After addition of base, the ammonia is distilled from an alkaline medium and absorbed in boric or sulfuric acid. The ammonia may be determined colorimetrically, by ammonia-selective electrode, or by titration with a standard mineral acid.

b. Selection of ammonia measurement method: The sensitivity of colorimetric methods makes them particularly useful for determining organic nitrogen levels below 5 mg/L. The titrimetric and selective electrode methods of measuring ammonia in the distillate are suitable for determining a wide range of organic nitrogen concentrations. Selective electrode methods and automated colorimetric methods may be used for measurement of ammonia in digestate without distillation. Follow equipment manufacturer's instructions.

2. Apparatus

a. Digestion apparatus: Kjeldahl flasks with a total capacity of 800 mL yield the best results. Digest over a heating device adjusted so that 250 mL water at an initial temperature of 25°C can be heated to a rolling boil in approximately 5 min. For testing, preheat heaters for 10 min if gas-operated or 30 min if electric. A heating device meeting this specification should provide the temperature range of 375 to 385°C for effective digestion.

b. Distillation apparatus: See Section 4500-NH₃.B.2a.

c. Apparatus for ammonia determination: See Section 4500-NH₃.C.2, D.2, F.2, or G.2.

3. Reagents

Prepare all reagents and dilutions in ammonia-free water.

All of the reagents listed for the determination of Nitrogen (Ammonia), Section 4500-NH₃.C.3, D.3, F.3, or G.3, are required, plus the following:

a. Digestion reagent: Dissolve 134 g K₂SO₄ and 7.3 g CuSO₄ in about 800 mL water. Carefully add 134 mL conc H₂SO₄. When it has cooled to room temperature, dilute the solution to 1 L with water. Mix well. Keep at a temperature close to 20°C to prevent crystallization.

b. Sodium hydroxide-sodium thiosulfate reagent: Dissolve 500 g NaOH and 25 g Na₂S₂O₃ · 5H₂O in water and dilute to 1 L.

c. Borate buffer solution: See Section 4500-NH₃.B.3b.

d. Sodium hydroxide, NaOH, 6N.

4. Procedure

a. Selection of sample volume and sample preparation: Place a measured volume of sample in an 800-mL kjeldahl flask. Select sample size from the following tabulation:

Organic Nitrogen in Sample mg/L	Sample Size mL
0-1	500
1-10	250
10-20	100
20-50	50.0
50-100	25.0

If necessary, dilute sample to 300 mL, neutralize to pH 7, and dechlorinate as described in Section 4500-NH₃.B.4b.

b. Ammonia removal: Add 25 mL borate buffer and then 6N NaOH until pH 9.5 is reached. Add a few glass beads or boiling chips such as Hengar Granules #12 and boil off 300 mL. If desired, distill this fraction and determine ammonia nitrogen. Alternately, if ammonia has been determined by the distillation method, use residue in distilling flask for organic nitrogen determination.

For sludge and sediment samples, weigh wet sample in a crucible or weighing bottle, transfer contents to a kjeldahl flask, and determine kjeldahl nitrogen. Follow a similar procedure for ammonia nitrogen and organic nitrogen determined by difference. Determinations of organic and kjeldahl nitrogen on dried sludge and sediment samples are not accurate because drying results in loss of ammonium salts. Measure dry weight of sample on a separate portion.

c. Digestion: Cool and add carefully 50 mL digestion reagent (or substitute 6.7 mL conc H₂SO₄, 6.7 g K₂SO₄, and 0.365 g CuSO₄) to distillation flask. Add a few glass beads and, after mixing, heat under a hood or with suitable ejection equipment to remove acid fumes. Boil briskly until the volume is greatly reduced (to about 25 to 50 mL) and copious white fumes are observed (fumes may be dark for samples high in organic matter). Then continue to digest for an additional 30 min. As digestion continues, colored or turbid samples will become transparent and pale green. After digestion, let cool, dilute to 300 mL with water, and mix. Tilt flask away from personnel and carefully add 50 mL sodium hydroxide-thiosulfate reagent to form an alkaline layer at flask bottom. Connect flask to a steamed-out distillation apparatus and swirl flask to insure complete mixing. The pH of the solution should exceed 11.0.

d. Distillation: Distill and collect 200 mL distillate. Use 50 mL indicating boric acid as absorbent solution when ammonia is to be determined by titration. Use 50 mL 0.04N H₂SO₄ solution as absorbent for manual phenate or electrode methods. Extend tip of condenser well below level of absorbent solution and do not let temperature in condenser rise above 29°C. Lower collected distillate free of contact with condenser tip and continue distillation during last 1 or 2 min to cleanse condenser.

e. Final ammonia measurement: Use the titration, ammonia-selective electrode, manual phenate, or automated phenate method, Sections 4500-NH₃.C, D, F, and G, respectively.

f. Standards: Carry a reagent blank and standards through all steps of the procedure.

5. Calculation

See Section 4500-NH₃.C.5, D.5, F.5, or G.5.

6. Precision and Bias

Two analysts in one laboratory prepared reagent water solutions of nicotinic acid and digested them by the macro-kjeldahl method. Ammonia in the distillate was determined by titration. Results are summarized in Table 4500-N_{org}:I.

7. Bibliography

- KJELDAHL, J. 1883. A new method for the determination of nitrogen in organic matter. *Z. Anal. Chem.* 22:366.
- PHELPS, E.B. 1905. The determination of organic nitrogen in sewage by the Kjeldahl process. *J. Infect. Dis.* (Suppl.) 1:225.
- McKENZIE, H.A. & H.S. WALLACE. 1954. The Kjeldahl determination of nitrogen: A critical study of digestion conditions. *Aust. J. Chem.* 7:55.
- MORGAN, G.B., J.B. LACKEY & F.W. GILCREAS. 1957. Quantitative determination of organic nitrogen in water, sewage, and industrial wastes. *Anal. Chem.* 29:833.
- BOLTZ, D.F., ed. 1978. *Colorimetric Determination of Nonmetals*. Interscience Publishers, New York, N.Y.
- JONES, M. & D. BRADSHAW. 1989. Copper: An alternative to mercury; more effective than zirconium in kjeldahl digestion of ecological materials. *Commun. Soil Sci. Plant Anal.* 20:1513.

TABLE 4500-N_{org}:I. PRECISION DATA FOR KJELDAHL NITROGEN METHOD BASED ON MEAN OF TRIPPLICATE ANALYSES OF NICOTINIC ACID

Lab/ Analyst	Nicotinic Acid mg N/L	Recovery of N %	Standard Deviation mg/L	Relative Standard Deviation %
1/1	5	93.3	0.16	3.46
1/2	5	101	0.16	3.17
1/1	10	87.7	0.16	1.84
1/2	10	91.5	0.28	3.06
1/1	20	95.7	0.16	0.84
1/2	20	95.7	0.58	3.03
2/1	0.5	97.4	0.005	1.04
2/2	0.5	95.3	0.027	5.46
3/1	0.5	87.3	0.130	29.9
4/1	0.5	113	0.235	41.7
2/1	1.0	103	0.012	1.15
2/2	1.0	101	0.046	4.63
3/1	1.0	84.3	0.081	9.66
4/1	1.0	99.3	0.396	39.9
2/1	2.0	104	0	0
2/2	2.0	99.2	0.029	1.44
3/1	2.0	89.2	0.071	3.98
4/1	2.0	112	0.139	6.18

4500-N_{org} C. Semi-Micro-Kjeldahl Method

1. General Discussion

See Section 4500-N_{org}.B.1.

2. Apparatus

a. Digestion apparatus: Use kjeldahl flasks with a capacity of 100 mL in a semi-micro-kjeldahl digestion apparatus* equipped with heating elements to accommodate kjeldahl flasks and a suction outlet to vent fumes. The heating elements should provide the temperature range of 375 to 385°C for effective digestion.

b. Distillation apparatus: Use an all-glass unit equipped with a steam-generating vessel containing an immersion heater† (Figure 4500-N_{org}:1).

c. pH meter.

d. Apparatus for ammonia determination: See Section 4500-NH₃.C.2, D.2, F.2, or G.2.

3. Reagents

All of the reagents listed for the determination of Nitrogen (Ammonia) (Section 4500-NH₃.B.3) and Nitrogen (Organic)

macro-kjeldahl (Section 4500-N_{org}.B.3) are required. Prepare all reagents and dilutions with ammonia-free water.

4. Procedure

a. Selection of sample volume: Determine the sample size from the following tabulation:

Organic Nitrogen in Sample mg/L	Sample Size mL
4-40	50
8-80	25
20-200	10
40-400	5

For sludge and sediment samples weigh a portion of wet sample containing between 0.2 and 2 mg organic nitrogen in a crucible or weighing bottle. Transfer sample quantitatively to a 100-mL beaker by diluting it and rinsing the weighing dish several times with small quantities of water. Make the transfer using as small a quantity of water as possible and do not exceed a total volume of 50 mL. Measure dry weight of sample on a separate portion.

b. Ammonia removal: Pipet 50 mL sample or an appropriate volume diluted to 50 mL with water into a 100-mL beaker. Add

* Rotary kjeldahl digestion unit, Kontes, Model 551000-0000, or equivalent.

† ASTM E-147 or equivalent.

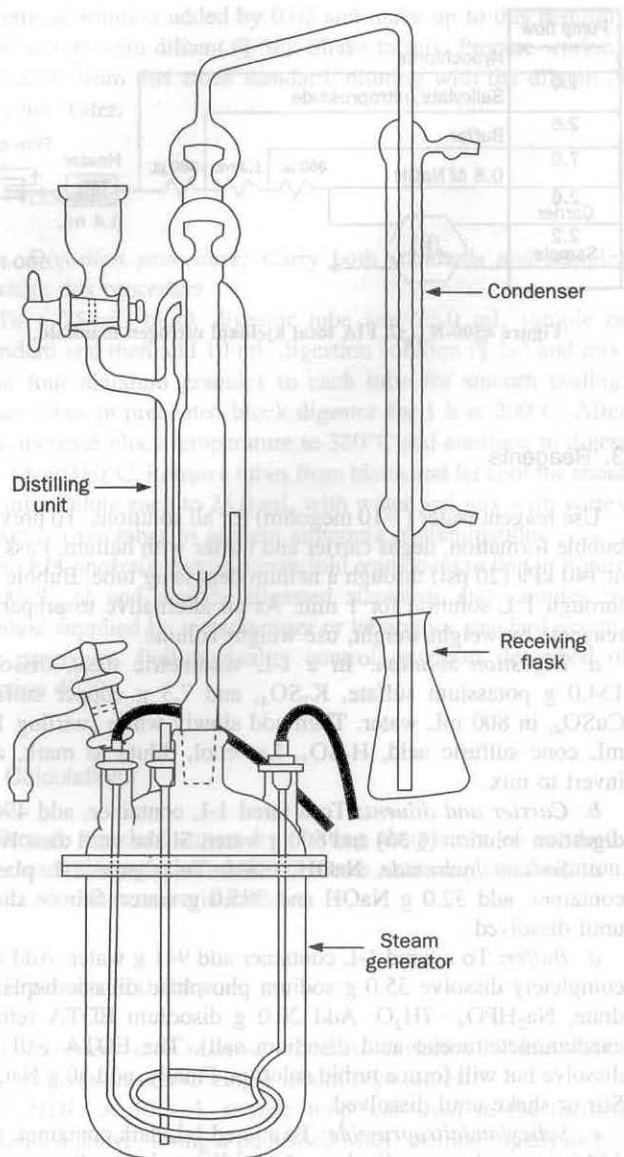


Figure 4500-N_{org}:1. Micro-kjeldahl distillation apparatus.

3 mL borate buffer and adjust to pH 9.5 with 6N NaOH, using a pH meter. Quantitatively transfer sample to a 100-mL kjeldahl

4500-N_{org} D. Block Digestion and Flow Injection Analysis

1. General Discussion

a. Principle: Samples of drinking, ground, and surface waters and of domestic and industrial wastes are digested in a block digester with sulfuric acid and copper sulfate as a catalyst. The digestion recovers nitrogen components of biological origin,

flask and boil off 30 mL. Alternatively, if ammonia removal is not required, digest samples directly as described in ¶ c below. Distillation following this direct digestion yields kjeldahl nitrogen concentration rather than organic nitrogen.

c. Digestion: Carefully add 10 mL digestion reagent to kjeldahl flask containing sample. Add five or six glass beads (3- to 4-mm size) to prevent bumping during digestion. Set each heating unit on the micro-kjeldahl digestion apparatus to its medium setting and heat flasks under a hood or with suitable ejection equipment to remove fumes of SO₃. Continue to boil briskly until solution becomes transparent and pale green and copious fumes are observed. Then turn each heating unit up to its maximum setting and digest for an additional 30 min. Cool. Quantitatively transfer digested sample by diluting and rinsing several times into micro-kjeldahl distillation apparatus so that total volume in distillation apparatus does not exceed 30 mL. Add 10 mL sodium hydroxide-thiosulfate reagent and turn on steam.

d. Distillation: Control rate of steam generation to boil contents in distillation unit so that neither escape of steam from tip of condenser nor bubbling of contents in receiving flask occurs. Distill and collect 30 to 40 mL distillate below surface of 10 mL absorbent solution contained in a 125-mL erlenmeyer flask. Use indicating boric acid for a titrimetric finish. Use 10 mL 0.04N H₂SO₄ solution for collecting distillate for the phenate or electrode methods. Extend tip of condenser well below level of absorbent solution and do not let temperature in condenser rise above 29°C. Lower collected distillate free of contact with condenser tip and continue distillation during last 1 or 2 min to cleanse condenser.

e. Standards: Carry a reagent blank and standards through all steps of procedure and apply necessary correction to results.

f. Final ammonia measurement: Use the titration, ammonia-selective electrode, manual phenate, or automated phenate method, Sections 4500-NH₃-C, D, F, and G, respectively.

5. Calculation

See Section 4500-NH₃-C.5, D.5, F.5, or G.5.

6. Precision and Bias

No data on the precision and bias of the semi-micro-kjeldahl method are available.

7. Bibliography

See Section 4500-N_{org}-B.7.

such as amino acids, proteins, and peptides, as ammonia, but may not recover the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones, and some refractory tertiary amines. Nitrate is not recovered. See Section 4500-N for a discussion of the various forms of nitrogen found in waters and wastewaters, Sections

4500-N_{org}.A and B for a discussion of kjeldahl nitrogen methods, and Section 4130, Flow Injection Analysis (FIA).

The digested sample is injected onto the FIA manifold where its pH is controlled by raising it to a known, basic pH by neutralization with a concentrated buffer. This in-line neutralization converts the ammonium cation to ammonia, and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction that follows. The ammonia thus produced is heated with salicylate and hypochlorite to produce a blue color that is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of EDTA in the buffer prevents precipitation of calcium and magnesium. The resulting peak's absorbance is measured at 660 nm. The peak area is proportional to the concentration of total kjeldahl nitrogen in the original sample.

b. Interferences: Remove large or fibrous particulates by filtering the sample through glass wool.

The main source of interference is ammonia. Ammonia is an airborne contaminant that is removed rapidly from ambient air by the digestion solution. Guard against ammonia contamination in reagents, water, glassware, and the sample preservation process. It is particularly important to prevent ammonia contamination in the sulfuric acid used for the digestion. Open sulfuric acid bottles away from laboratories in which ammonia or ammonium chloride have been used as reagents and store sulfuric acid away from such reagents. Ensure that the open ends of the block digester's tubes can be covered to prevent ammonia from being scrubbed from the fume hood make-up air during the digestion.

If a sample consumes more than 10% of the sulfuric acid during digestion, the pH-dependent color reaction will show a matrix effect. The color reaction buffer will accommodate a range of $5.4\% \pm 0.4\%$ H₂SO₄ (v/v) in the diluted digested sample. Sample matrices with a high concentration of carbohydrates or other organic material may consume more than 10% of the acid during digestion. If this effect is suspected, titrate digested sample with standardized sodium hydroxide to determine whether more than 10% of the sulfuric acid has been consumed during digestion. The block digester also should have a means to prevent loss of sulfuric acid from the digestion tubes during the digestion period.

Also see Sections 4500-N_{org}.A and B.

2. Apparatus

Digestion and flow injection analysis equipment consisting of:

a. Block digester capable of maintaining a temperature of 380°C for 2 h.

b. Digestion tubes capable of being heated to 380°C for 2 h and having a cover to prevent ammonia contamination and loss of sulfuric acid.

c. FIA injection valve with sample loop or equivalent.

d. Multichannel proportioning pump.

e. FIA manifold (Figure 4500-N_{org}.2) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-N_{org}.2. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

f. Absorbance detector, 660 nm, 10-nm bandpass.

g. Injection valve control and data acquisition system.

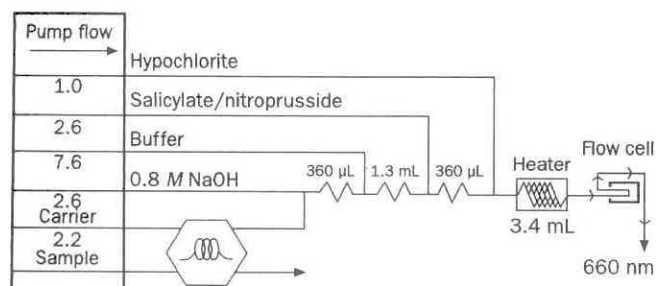


Figure 4500-N_{org}.2. FIA total kjeldahl nitrogen manifold.

3. Reagents

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

a. Digestion solution: In a 1-L volumetric flask, dissolve 134.0 g potassium sulfate, K₂SO₄, and 7.3 g copper sulfate, CuSO₄, in 800 mL water. Then add slowly while swirling 134 mL conc sulfuric acid, H₂SO₄. Let cool, dilute to mark, and invert to mix.

b. Carrier and diluent: To a tared 1-L container, add 496 g digestion solution (§ 3a) and 600 g water. Shake until dissolved.

c. Sodium hydroxide, NaOH, 0.8M: To a tared 1-L plastic container, add 32.0 g NaOH and 985.0 g water. Stir or shake until dissolved.

d. Buffer: To a tared 1-L container add 941 g water. Add and completely dissolve 35.0 g sodium phosphate dibasic heptahydrate, Na₂HPO₄ · 7H₂O. Add 20.0 g disodium EDTA (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add 50 g NaOH. Stir or shake until dissolved.

e. Salicylate/nitroprusside: To a tared 1-L dark container, add 150.0 g sodium salicylate (salicylic acid sodium salt), C₆H₄(OH)(COO)Na, 1.00 g sodium nitroprusside (sodium nitroferrocyanide dihydrate), Na₂Fe(CN)₅NO · 2H₂O, and 908 g water. Stir or shake until dissolved. Prepare fresh monthly.

f. Hypochlorite: To a tared 250-mL container, add 16 g commercial 5.25% sodium hypochlorite bleach solution* and 234 g deionized water. Shake to mix.

g. Stock standard, 250 mg N/L: In a 1-L volumetric flask dissolve 0.9540 g ammonium chloride, NH₄Cl (dried for 2 h at 110°C), in about 800 mL water. Dilute to mark and invert to mix.

h. Standard ammonia solutions: Prepare ammonia standards in desired concentration range, using the stock standard (§ 3g) and diluting with water.

i. Simulated digested standards: To prepare calibration standards without having to digest the standards prepared in § 3h, proceed as follows:

Stock standard, 5.00 mg N/L: To a tared 250-mL container add about 5.0 g stock standard (250 mg N/L). Divide actual

* Regular Clorox, The Clorox Company, Pleasanton, CA, or equivalent.

weight of solution added by 0.02 and make up to this resulting total weight with diluent (§ 3b). Shake to mix. Prepare working standards from this stock standard, diluting with the diluent (§ 3b), not water.

4. Procedure

a. Digestion procedure: Carry both standards and samples through this procedure.

To a 75-mL block digester tube add 25.0 mL sample or standard and then add 10 mL digestion solution (§ 3a) and mix. Add four alundum granules to each tube for smooth boiling. Place tubes in preheated block digester for 1 h at 200°C. After 1 h, increase block temperature to 380°C and continue to digest for 1 h at 380°C. Remove tubes from block and let cool for about 10 min. Dilute each to 25.0 mL with water and mix with vortex mixer. Cover tubes to prevent ammonia contamination.

b. FIA analysis: Set up a manifold equivalent to that in Figure 4500-N_{org}:2 and analyze digested standards and samples by method supplied by manufacturer or laboratory standard operating procedure. Follow quality control protocols described in Section 4020.

5. Calculations

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

6. Precision and Bias

a. Recovery and relative standard deviation: Table 4500-N_{org}:II gives results of single-laboratory studies.

b. MDL: A 130-μL sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 0.1-mg N/L standard. These gave a mean of 0.103 mg N/L, a standard deviation of 0.014 mg N/L, and MDL of 0.034 mg N/L. A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagents flow rate.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

TABLE 4500-N_{org}:II. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg N/L	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	97	—
	Blank†	3.0	97	—
		6.0	99	—
	Site A‡§	0	—	3.3
		3.0	91	—
		6.0	95	—
	Site B‡§	0	—	3.6
		3.0	115	—
		6.0	93	—
	Site C‡§	0	—	5.1
		3.0	97	—
		6.0	107	—
Wastewater treatment plant effluent	Reference sample*	—	92	—
	Blank†	3.0	97	—
		6.0	100	—
	Site A‡	0	—	5.4
		3.0	94	—
		6.0	100	—
	Site B‡	0	—	4.1
		3.0	119	—
		6.0	81	—
	Site C‡	0	—	7.3
		3.0	93	—
		6.0	105	—
Landfill leachate	Reference sample*	—	96	—
	Blank†	3.0	101	—
		6.0	99	—
	Site A‡#	0	—	3.3
		3.0	95	—
		6.0	98	—
	Site B‡#	0	—	4.4
		3.0	134	—
		6.0	85	—
	Site C‡#	0	—	3.8
		3.0	98	—
		6.0	105	—

* U.S. EPA nutrient QC sample, 1.52 mg N/L.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate.

§ Sample dilutions: A - 5-fold; B - 10-fold; C - 5-fold. Typical relative difference between duplicates 3%.

|| Sample dilutions: A - none; B - 2-fold; C - none. Typical relative difference between duplicates 1%.

Sample dilutions: A, B, and C - 25-fold. Typical relative difference between duplicates 4%.

4500-O OXYGEN (DISSOLVED)*

4500-O A. Introduction

1. Significance

Dissolved oxygen (DO) levels in natural and wastewaters depend on the physical, chemical, and biochemical activities in the water body. The analysis for DO is a key test in water pollution and waste treatment process control.

2. Selection of Method

Two methods for DO analysis are described: the Winkler or iodometric method and its modifications and the electrometric

* Approved by Standard Methods Committee, 2001.

method using membrane electrodes. The iodometric method¹ is a titrimetric procedure based on the oxidizing property of DO while the membrane electrode procedure is based on the rate of diffusion of molecular oxygen across a membrane.² The choice of procedure depends on the interferences present, the accuracy desired, and, in some cases, convenience or expedience.

3. References

1. WINKLER, L.W. 1888. The determination of dissolved oxygen in water. *Berlin. Deut. Chem. Ges.* 21:2843.
2. MANCY, K.H. & T. JAFFE. 1966. Analysis of Dissolved Oxygen in Natural and Waste Waters. Publ. No. 999-WP-37, U.S. Public Health Serv., Washington, D.C.

4500-O B. Iodometric Methods

1. Principle

The iodometric test is the most precise and reliable titrimetric procedure for DO analysis. It is based on the addition of divalent manganese solution, followed by strong alkali, to the sample in a glass-stoppered bottle. DO rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxides of higher valency states. In the presence of iodide ions in an acidic solution, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent to the original DO content. The iodine is then titrated with a standard solution of thiosulfate.

The titration end point can be detected visually, with a starch indicator, or electrometrically, with potentiometric or dead-stop techniques.¹ Experienced analysts can maintain a precision of $\pm 50 \mu\text{g/L}$ with visual end-point detection and a precision of $\pm 5 \mu\text{g/L}$ with electrometric end-point detection.^{1,2}

The liberated iodine also can be determined directly by simple absorption spectrophotometers.³ This method can be used on a routine basis to provide very accurate estimates for DO in the microgram-per-liter range provided that interfering particulate matter, color, and chemical interferences are absent.

2. Selection of Method

Before selecting a method consider the effect of interferences, particularly oxidizing or reducing materials that may be present in the sample. Certain oxidizing agents liberate iodine from iodides (positive interference) and some reducing agents reduce iodine to iodide (negative interference). Most organic matter is oxidized partially when the oxidized manganese precipitate is acidified, thus causing negative errors.

Several modifications of the iodometric method are given to minimize the effect of interfering materials.² Among the more commonly used procedures are the azide modification,⁴ the permanganate modification,⁵ the alum flocculation modification,⁶ and the copper sulfate-sulfamic acid flocculation modification.^{7,8} The azide modification (C) effectively removes interference caused by nitrite, which is the most common interference in biologically treated effluents and incubated BOD samples. Use the permanganate modification (D) in the presence of ferrous iron. When the sample contains 5 or more mg ferric iron salts/L, add potassium fluoride (KF) as the first reagent in the azide modification or after the permanganate treatment for ferrous iron. Alternately, eliminate Fe(III) interference by using 85 to 87% phosphoric acid (H_3PO_4) instead of sulfuric acid (H_2SO_4) for acidification. This procedure has not been tested for Fe(III) concentrations above 20 mg/L.

Use the alum flocculation modification (E) in the presence of suspended solids that cause interference and the copper sulfate-sulfamic acid flocculation modification (F) on activated-sludge mixed liquor.

3. Collection of Samples

Collect samples very carefully. Methods of sampling are highly dependent on source to be sampled and, to a certain extent, on method of analysis. Do not let sample remain in contact with air or be agitated, because either condition causes a change in its gaseous content. Samples from any depth in streams, lakes, or reservoirs, and samples of boiler water, need special precautions to eliminate changes in pressure and temperature. Procedures and equipment have been developed for sampling waters under pressure and unconfined waters (e.g., streams,

ivers, and reservoirs). Sampling procedures and equipment needed are described in American Society for Testing and Materials Special Technical Publication No. 148-1 and in U.S. Geological Survey Water Supply Paper No. 1454.

Collect surface water samples in narrow-mouth glass-stoppered BOD bottles of 300-mL capacity with tapered and pointed ground-glass stoppers and flared mouths. Avoid entraining or dissolving atmospheric oxygen. In sampling from a line under pressure, attach a glass or rubber tube to the tap and extend to bottom of bottle. Let bottle overflow two or three times its volume and replace stopper so that no air bubbles are entrained.

Suitable samplers for streams, ponds, or tanks of moderate depth are of the APHA type shown in Figure 4500-O:1. Use a Kemmerer-type sampler for samples collected from depths greater than 2 m. Bleed sample from bottom of sampler through a tube extending to bottom of a 250- to 300-mL BOD bottle. Fill bottle to overflowing (overflow for approximately 10 s), and prevent turbulence and formation of bubbles while filling. Record sample temperature to nearest degree Celsius or more precisely.

4. Preservation of Samples

Determine DO immediately on all samples containing an appreciable oxygen or iodine demand. Samples with no iodine demand may be stored for a few hours without change after adding manganous sulfate (MnSO_4) solution, alkali-iodide solution, and H_2SO_4 , followed by shaking in the usual way. Protect stored samples from strong sunlight and titrate as soon as possible.

For samples with an iodine demand, preserve for 4 to 8 h by adding 0.7 mL conc H_2SO_4 and 1 mL sodium azide solution (2 g NaN_3 /100 mL distilled water) to the BOD bottle. This will arrest biological activity and maintain DO if the bottle is stored at the temperature of collection or water-sealed and kept at 10 to 20°C. As soon as possible, complete the procedure, using 2 mL MnSO_4 solution, 3 mL alkali-iodide solution, and 2 mL conc H_2SO_4 .

5. References

1. POTTER, E.C. & G.E. EVERITT. 1957. Advances in dissolved oxygen microanalysis. *J. Appl. Chem.* 9:642.

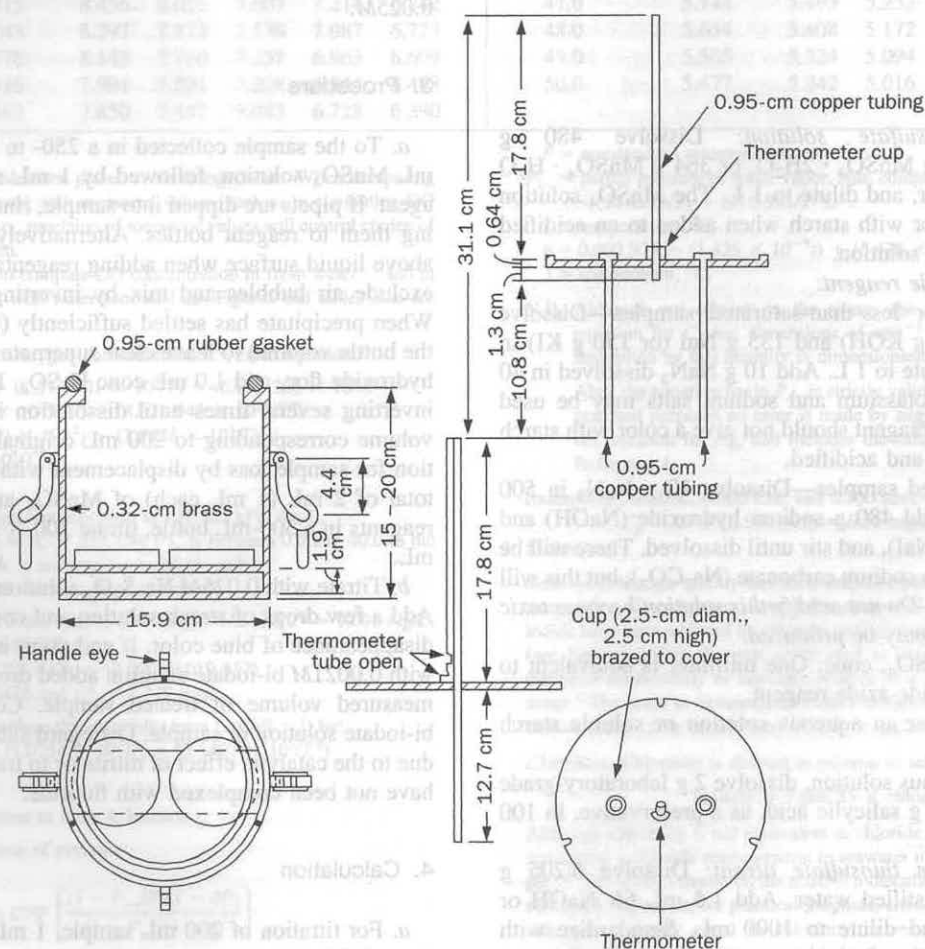


Figure 4500-O:1. DO and BOD sampler assembly.

2. MANCY, K.H. & T. JAFFE. 1966. Analysis of Dissolved Oxygen in Natural and Waste Waters. Publ. No. 99-WP-37, U.S. Public Health Serv., Washington, D.C.
3. OULMAN, C.S. & E.R. BAUMANN. 1956. A colorimetric method for determining dissolved oxygen. *Sewage Ind. Wastes* 28:1461.
4. ALSTERBERG, G. 1925. Methods for the determination of elementary oxygen dissolved in water in the presence of nitrite. *Biochem. Z.* 159:36.
5. RIDEAL, S. & G.G. STEWART. 1901. The determination of dissolved oxygen in waters in the presence of nitrites and of organic matter. *Analyst* 26:141.
6. RUCHHOFT, C.C. & W.A. MOORE. 1940. The determination of biochemical oxygen demand and dissolved oxygen of river mud suspensions. *Ind. Eng. Chem., Anal. Ed.* 12:711.
7. PLACAK, O.R. & C.C. RUCHHOFT. 1941. Comparative study of the azide and Rideal-Stewart modifications of the Winkler method in the determination of biochemical oxygen demand. *Ind. Eng. Chem., Anal. Ed.* 13:12.
8. RUCHHOFT, C.C. & O.R. PLACAK. 1942. Determination of dissolved oxygen in activated-sludge sewage mixtures. *Sewage Works J.* 14: 638.

4500-O C. Azide Modification

1. General Discussion

Use the azide modification for most wastewater, effluent, and stream samples, especially if samples contain more than 50 μg NO_2^- -N/L and not more than 1 mg ferrous iron/L. Other reducing or oxidizing materials should be absent. If 1 mL KF solution is added before the sample is acidified and there is no delay in titration, the method is applicable in the presence of 100 to 200 mg ferric iron/L.

2. Reagents

a. Manganous sulfate solution: Dissolve 480 g $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 400 g $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$, or 364 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ in distilled water, filter, and dilute to 1 L. The MnSO_4 solution should not give a color with starch when added to an acidified potassium iodide (KI) solution.

b. Alkali-iodide-azide reagent:

1) For saturated or less-than-saturated samples—Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in distilled water and dilute to 1 L. Add 10 g NaN_3 dissolved in 40 mL distilled water. Potassium and sodium salts may be used interchangeably. This reagent should not give a color with starch solution when diluted and acidified.

2) For supersaturated samples—Dissolve 10 g NaN_3 in 500 mL distilled water. Add 480 g sodium hydroxide (NaOH) and 750 g sodium iodide (NaI), and stir until dissolved. There will be a white turbidity due to sodium carbonate (Na_2CO_3), but this will do no harm. CAUTION—Do not acidify this solution because toxic hydrazoic acid fumes may be produced.

c. Sulfuric acid, H_2SO_4 , conc: One milliliter is equivalent to about 3 mL alkali-iodide-azide reagent.

d. Starch: Use either an aqueous solution or soluble starch powder mixtures.

To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid, as a preservative, in 100 mL hot distilled water.

e. Standard sodium thiosulfate titrant: Dissolve 6.205 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in distilled water. Add 1.5 mL 6N NaOH or 0.4 g solid NaOH and dilute to 1000 mL. Standardize with bi-iodate solution.

f. Standard potassium bi-iodate solution, 0.0021M: Dissolve 812.4 mg $\text{KH}(\text{IO}_3)_2$ in distilled water and dilute to 1000 mL.

3. Procedure

a. To the sample collected in a 250- to 300-mL bottle, add 1 mL MnSO_4 solution, followed by 1 mL alkali-iodide-azide reagent. If pipets are dipped into sample, rinse them before returning them to reagent bottles. Alternatively, hold pipet tips just above liquid surface when adding reagents. Stopper carefully to exclude air bubbles and mix by inverting bottle a few times. When precipitate has settled sufficiently (to approximately half the bottle volume) to leave clear supernate above the manganese hydroxide floc, add 1.0 mL conc H_2SO_4 . Restopper and mix by inverting several times until dissolution is complete. Titrate a volume corresponding to 200 mL original sample after correction for sample loss by displacement with reagents. Thus, for a total of 2 mL (1 mL each) of MnSO_4 and alkali-iodide-azide reagents in a 300-mL bottle, titrate $200 \times 300 / (300 - 2) = 201$ mL.

b. Titrate with 0.025M $\text{Na}_2\text{S}_2\text{O}_3$ solution to a pale straw color. Add a few drops of starch solution and continue titration to first disappearance of blue color. If end point is overrun, back-titrate with 0.0021M bi-iodate solution added dropwise, or by adding a measured volume of treated sample. Correct for amount of bi-iodate solution or sample. Disregard subsequent recolorations due to the catalytic effect of nitrite or to traces of ferric salts that have not been complexed with fluoride.

4. Calculation

a. For titration of 200 mL sample, 1 mL 0.025M $\text{Na}_2\text{S}_2\text{O}_3 = 1$ mg DO/L.

b. To express results as percent saturation at 101.3 kPa, use the solubility data in Table 4500-O:I. Equations for correcting sol-

TABLE 4500-O:I SOLUBILITY OF OXYGEN IN WATER EXPOSED TO WATER-SATURATED AIR AT ATMOSPHERIC PRESSURE (101.3 kPa)¹

Oxygen Solubility mg/L							Oxygen Solubility mg/L						
Temperature °C	Chlorinity: 0	5.0	10.0	15.0	20.0	25.0	Temperature °C	Chlorinity: 0	5.0	10.0	15.0	20.0	25.0
0.0	14.621	13.728	12.888	12.097	11.355	10.657	26.0	8.113	7.711	7.327	6.962	6.615	6.285
1.0	14.216	13.356	12.545	11.783	11.066	10.392	27.0	7.968	7.575	7.201	6.845	6.506	6.184
2.0	13.829	13.000	12.218	11.483	10.790	10.139	28.0	7.827	7.444	7.079	6.731	6.400	6.085
3.0	13.460	12.660	11.906	11.195	10.526	9.897	29.0	7.691	7.317	6.961	6.621	6.297	5.990
4.0	13.107	12.335	11.607	10.920	10.273	9.664	30.0	7.559	7.194	6.845	6.513	6.197	5.896
5.0	12.770	12.024	11.320	10.656	10.031	9.441	31.0	7.430	7.073	6.733	6.409	6.100	5.806
6.0	12.447	11.727	11.046	10.404	9.799	9.228	32.0	7.305	6.957	6.624	6.307	6.005	5.717
7.0	12.139	11.442	10.783	10.162	9.576	9.023	33.0	7.183	6.843	6.518	6.208	5.912	5.631
8.0	11.843	11.169	10.531	9.930	9.362	8.826	34.0	7.065	6.732	6.415	6.111	5.822	5.546
9.0	11.559	10.907	10.290	9.707	9.156	8.636	35.0	6.950	6.624	6.314	6.017	5.734	5.464
10.0	11.288	10.656	10.058	9.493	8.959	8.454	36.0	6.837	6.519	6.215	5.925	5.648	5.384
11.0	11.027	10.415	9.835	9.287	8.769	8.279	37.0	6.727	6.416	6.119	5.835	5.564	5.305
12.0	10.777	10.183	9.621	9.089	8.586	8.111	38.0	6.620	6.316	6.025	5.747	5.481	5.228
13.0	10.537	9.961	9.416	8.899	8.411	7.949	39.0	6.515	6.217	5.932	5.660	5.400	5.152
14.0	10.306	9.747	9.218	8.716	8.242	7.792	40.0	6.412	6.121	5.842	5.576	5.321	5.078
15.0	10.084	9.541	9.027	8.540	8.079	7.642	41.0	6.312	6.026	5.753	5.493	5.243	5.005
16.0	9.870	9.344	8.844	8.370	7.922	7.496	42.0	6.213	5.934	5.667	5.411	5.167	4.933
17.0	9.665	9.153	8.667	8.207	7.770	7.356	43.0	6.116	5.843	5.581	5.331	5.091	4.862
18.0	9.467	8.969	8.497	8.049	7.624	7.221	44.0	6.021	5.753	5.497	5.252	5.017	4.793
19.0	9.276	8.792	8.333	7.896	7.483	7.090	45.0	5.927	5.665	5.414	5.174	4.944	4.724
20.0	9.092	8.621	8.174	7.749	7.346	6.964	46.0	5.835	5.578	5.333	5.097	4.872	4.656
21.0	8.915	8.456	8.021	7.607	7.214	6.842	47.0	5.744	5.493	5.252	5.021	4.801	4.589
22.0	8.743	8.297	7.873	7.470	7.087	6.723	48.0	5.654	5.408	5.172	4.947	4.730	4.523
23.0	8.578	8.143	7.730	7.337	6.963	6.609	49.0	5.565	5.324	5.094	4.872	4.660	4.457
24.0	8.418	7.994	7.591	7.208	6.844	6.498	50.0	5.477	5.242	5.016	4.799	4.591	4.392
25.0	8.263	7.850	7.457	7.083	6.728	6.390							

NOTE:

- The table provides three decimal places to aid interpolation. When computing saturation values to be used with measured values, such as in computing DO deficit in a receiving water, precision of measured values will control choice of decimal places to be used.
- Equations are available to compute DO concentration in fresh water¹⁻³ and in seawater¹ at equilibrium with water-saturated air. Figures and tables also are available.³

Calculate the equilibrium oxygen concentration, C^* , from equation:

$$\ln C^* = -139.344/11 + (1.575/701 \times 10^5/T) - (6.642/308 \times 10^7/T^2) \\ + (1.243/800 \times 10^{10}/T^3) - (8.621/949 \times 10^{11}/T^4) \\ - \text{Chl} [(3.1929 \times 10^{-2}) - (1.9428 \times 10^1/T) \\ + (3.8673 \times 10^3/T^2)]$$

where:

C^* = equilibrium oxygen concentration at 101.325 kPa, mg/L,

T = temperature (°K) = °C + 273.150, (°C is between 0.0 and 40.0 in the equation; the table is accurate up to 50.0), and

Chl = Chlorinity (see definition in Note 4, below).

Example 1: At 20°C and 0.000 Chl, $\ln C^* = 2.207/442$ and $C^* = 9.092$ mg/L;

Example 2: At 20°C and 15.000 Chl,

$$\ln C^* = (2.207/442) - 15.000/(0.010/657) \\ = 2.0476 \text{ and } C^* = 7.749 \text{ mg/L.}$$

When salinity is used, replace the chlorinity term $(-\text{Chl}[\dots])$ by:
 $-S(1.7674 \times 10^{-2}) - (1.0754 \times 10^1/T) + (2.1407 \times 10^3/T^2)$

where:

S = salinity (see definition in Note 4, below).

- For nonstandard conditions of pressure:

$$C_p = C^*P \left[\frac{(1 - P_{wv}/P)(1 - \theta P)}{(1 - P_{wv})(1 - \theta)} \right]$$

where:

C_p = equilibrium oxygen concentration at nonstandard pressure, mg/L,

C^* = equilibrium oxygen concentration at standard pressure of 1 atm, mg/L.

P = nonstandard pressure, atm,

P_{wv} = partial pressure of water vapor, atm, computed from: $\ln P_{wv} = 11.8571 \\ - (3840.70/T) - (216/961/T^2)$,

T = temperature, °K,

$\theta = 0.000/975 - (1.426 \times 10^{-5}/t) + (6.436 \times 10^{-8}/t^2)$, and

t = temperature, °C.

N.B.: Although not explicit in the above, the quantity in brackets in the equation for C_p has dimensions of atm⁻¹ per Reference 4, so that P multiplied by this quantity is dimensionless.

Also, the equation for $\ln P_{wv}$ is strictly valid for fresh water only, but for practical purposes no error is made by neglecting the effect of salinity. An equation for P_{wv} that includes the salinity factor may be found in Reference 1.

Example 3: At 20°C, 0.000 Chl, and 0.700 atm,

$$C_p = C^*P(0.990/092) = 6.30 \text{ mg/L.}$$

- Definitions:

Salinity: Although salinity has been defined traditionally as the total solids in water after all carbonates have been converted to oxides, all bromide and iodide have been replaced by chloride, and all organic matter has been oxidized (see Section 2520), the new scale used to define salinity is based on the electrical conductivity of seawater relative to a specified solution of KCl in water.⁵ The scale is dimensionless and the traditional dimension of parts per thousand (i.e., g/kg of solution) no longer applies.

Chlorinity: Chlorinity is defined in relation to salinity as follows:

$$\text{Salinity} = 1.80655 \times \text{chlorinity}$$

Although chlorinity is not equivalent to chloride concentration, the factor for converting a chloride concentration in seawater to include bromide, for example, is only 1.0045 (based on the relative molecular weights and amounts of the two ions). Therefore, for practical purposes, chloride concentration (in g/kg of solution) is nearly equal to chlorinity in seawater. For wastewater, it is necessary to know the ions responsible for the solution's electrical conductivity to correct for their effect on oxygen solubility and use of the tabular value. If this is not done, the equation is inappropriate unless the relative composition of the wastewater is similar to that of seawater.

abilities to barometric pressures other than mean sea level and for various chlorinities are given below the table.

5. Precision and Bias

DO can be determined with a precision, expressed as a standard deviation, of about 20 $\mu\text{g/L}$ in distilled water and about 60 $\mu\text{g/L}$ in wastewater and secondary effluents. In the presence of appreciable interference, even with proper modifications, the standard deviation may be as high as 100 $\mu\text{g/L}$. Still greater errors may occur in testing waters having organic suspended solids or heavy pollution. Avoid errors due to carelessness in collecting samples, prolonging the completion of test, or selecting an unsuitable modification.

4500-O D. Permanganate Modification

1. General Discussion

Use the permanganate modification only on samples containing ferrous iron. Interference from high concentrations of ferric iron (up to several hundred milligrams per liter), as in acid mine water, may be overcome by the addition of 1 mL potassium fluoride (KF) and azide, provided that the final titration is made immediately after acidification.

This procedure is ineffective for oxidation of sulfite, thiosulfate, polythionate, or the organic matter in wastewater. The error with samples containing 0.25% by volume of digester waste from the manufacture of sulfite pulp may amount to 7 to 8 mg DO/L. With such samples, use the alkali-hypochlorite modification.¹ At best, however, the latter procedure gives low results, the deviation amounting to 1 mg/L for samples containing 0.25% digester wastes.

2. Reagents

All the reagents required for Method C, and in addition:

a. Potassium permanganate solution: Dissolve 6.3 g KMnO_4 in distilled water and dilute to 1 L.

b. Potassium oxalate solution: Dissolve 2 g $\text{K}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$ in 100 mL distilled water; 1 mL will reduce about 1.1 mL permanganate solution.

c. Potassium fluoride solution: Dissolve 40 g $\text{KF} \cdot 2\text{H}_2\text{O}$ in distilled water and dilute to 100 mL.

3. Procedure

a. To a sample collected in a 250- to 300-mL bottle add, below the surface, 0.70 mL conc H_2SO_4 , 1 mL KMnO_4 solution, and 1 mL KF solution. Stopper and mix by inverting. Never add more than 0.7 mL conc H_2SO_4 as the first step of pretreatment. Add

6. References

1. BENSON, B.B. & D. KRAUSE, JR. 1984. The concentration and isotopic fractionation of oxygen dissolved in freshwater and seawater in equilibrium with the atmosphere. *Limnol. Oceanogr.* 29:620.
2. BENSON, B.B. & D. KRAUSE, JR. 1980. The concentration and isotopic fractionation of gases dissolved in fresh water in equilibrium with the atmosphere: I. Oxygen. *Limnol. Oceanogr.* 25:662.
3. MORTIMER, C.H. 1981. The oxygen content of air-saturated fresh waters over ranges of temperature and atmospheric pressure of limnological interest. *Int. Assoc. Theoret. Appl. Limnol.*, Communication No. 22, Stuttgart, West Germany.
4. SULZER, F. & W.M. WESTGARTH. 1962. Continuous D. O. recording in activated sludge. *Water Sewage Works* 109: 376.
5. UNITED NATIONS EDUCATIONAL, SCIENTIFIC & CULTURAL ORGANIZATION. 1981. Background Papers and Supporting Data on the Practical Salinity Scale 1978. Tech. Paper Mar. Sci. No. 37.

acid with a 1-mL pipet graduated to 0.1 mL. Add sufficient KMnO_4 solution to obtain a violet tinge that persists for 5 min. If the permanganate color is destroyed in a shorter time, add additional KMnO_4 solution, but avoid large excesses.

b. Remove permanganate color completely by adding 0.5 to 1.0 mL $\text{K}_2\text{C}_2\text{O}_4$ solution. Mix well and let stand in the dark to facilitate the reaction. Excess oxalate causes low results; add only enough $\text{K}_2\text{C}_2\text{O}_4$ to decolorize the KMnO_4 completely without an excess of more than 0.5 mL. Complete decolorization in 2 to 10 min. If it is impossible to decolorize the sample without adding a large excess of oxalate, the DO result will be inaccurate.

c. From this point the procedure closely parallels that in Section 4500-O.C.3. Add 1 mL MnSO_4 solution and 3 mL alkali-iodide-azide reagent. Stopper, mix, and let precipitate settle a short time; acidify with 2 mL conc H_2SO_4 . When 0.7 mL acid, 1 mL KF solution, 1 mL KMnO_4 solution, 1 mL $\text{K}_2\text{C}_2\text{O}_4$ solution, 1 mL MnSO_4 solution, and 3 mL alkali-iodide-azide (or a total of 7.7 mL reagents) are used in a 300-mL bottle, take $200 \times 300 / (300 - 7.7) = 205$ mL for titration.

This correction is slightly in error because the KMnO_4 solution is nearly saturated with DO and 1 mL would add about 0.008 mg oxygen to the DO bottle. However, because precision of the method (standard deviation, 0.06 mL thiosulfate titration, or 0.012 mg DO) is 50% greater than this error, a correction is unnecessary. When substantially more KMnO_4 solution is used routinely, use a solution several times more concentrated so that 1 mL will satisfy the permanganate demand.

4. Reference

1. THERIAULT, E.J. & P.D. MCNAMEE. 1932. Dissolved oxygen in the presence of organic matter, hypochlorites, and sulfite wastes. *Ind. Eng. Chem., Anal. Ed.* 4:59.

4500-O E. Alum Flocculation Modification

1. General Discussion

Samples high in suspended solids may consume appreciable quantities of iodine in acid solution. The interference due to solids may be removed by alum flocculation.

2. Reagents

All the reagents required for the azide modification (Section 4500-O.C.2) and in addition:

a. *Alum solution*: Dissolve 10 g aluminum potassium sulfate, $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, in distilled water and dilute to 100 mL.

b. *Ammonium hydroxide*, NH_4OH , conc.

3. Procedure

Collect sample in a glass-stoppered bottle of 500 to 1000 mL capacity, using the same precautions as for regular DO samples. Add 10 mL alum solution and 1 to 2 mL conc NH_4OH . Stopper and invert gently for about 1 min. Let sample settle for about 10 min and siphon clear supernate into a 250- to 300-mL DO bottle until it overflows. Avoid sample aeration and keep siphon submerged at all times. Continue sample treatment as in Section 4500-O.C.3 or an appropriate modification.

4500-O F. Copper Sulfate-Sulfamic Acid Flocculation Modification

1. General Discussion

This modification is used for biological flocs such as activated sludge mixtures, which have high oxygen utilization rates.

2. Reagents

All the reagents required for the azide modification (Section 4500-O.C.2) and, in addition:

Copper sulfate-sulfamic acid inhibitor solution: Dissolve 32 g technical-grade $\text{NH}_2\text{SO}_2\text{OH}$ without heat in 475 mL distilled water. Dissolve 50 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 500 mL distilled water. Mix the two solutions and add 25 mL conc acetic acid.

3. Procedure

Add 10 mL $\text{CuSO}_4\text{-NH}_2\text{SO}_2\text{OH}$ inhibitor to a 1-L glass-stoppered bottle. Insert bottle in a special sampler designed so that bottle fills from a tube near bottom and overflows only 25 to 50% of bottle capacity. Collect sample, stopper, and mix by inverting. Let suspended solids settle and siphon relatively clear supernatant liquor into a 250- to 300-mL DO bottle. Continue sample treatment as rapidly as possible by the azide (Section 4500-O.C.3) or other appropriate modification.

4500-O G. Membrane Electrode Method

1. General Discussion

Various modifications of the iodometric method have been developed to eliminate or minimize effects of interferences; nevertheless, the method still is inapplicable to a variety of industrial and domestic wastewaters.¹ Moreover, the iodometric method is not suited for field testing and cannot be adapted easily for continuous monitoring or for DO determinations in situ.

Polarographic methods using the dropping mercury electrode or the rotating platinum electrode have not been reliable always for the DO analysis in domestic and industrial wastewaters because impurities in the test solution can cause electrode poisoning or other interferences.^{2,3} With membrane-covered electrode systems these problems are minimized, because the sensing element is protected by an oxygen-permeable plastic membrane that serves as a diffusion barrier against impurities.⁴⁻⁶ Under

steady-state conditions the current is directly proportional to the DO concentration.*

Membrane electrodes of the polarographic⁴ as well as the galvanic⁵ type have been used for DO measurements in lakes and reservoirs,⁸ for stream survey and control of industrial effluents,^{9,10} for continuous monitoring of DO in activated sludge units,¹¹ and for estuarine and oceanographic studies.¹² Being completely submersible, membrane electrodes are suited for analysis in situ. Their portability and ease of operation and maintenance make them particularly convenient for field applications. In laboratory investigations, membrane electrodes have been used for continuous DO analysis in bacterial cultures, including the BOD test.^{5,13}

Membrane electrodes provide an excellent method for DO analysis in polluted waters, highly colored waters, and strong waste effluents. They are recommended for use especially under

* Fundamentally, the current is directly proportional to the activity of molecular oxygen.⁷

conditions that are unfavorable for use of the iodometric method, or when that test and its modifications are subject to serious errors caused by interferences.

a. Principle: Oxygen-sensitive membrane electrodes of the polarographic or galvanic type are composed of two solid metal electrodes in contact with supporting electrolyte separated from the test solution by a selective membrane. The basic difference between the galvanic and the polarographic systems is that in the former the electrode reaction is spontaneous (similar to that in a fuel cell), while in the latter an external source of applied voltage is needed to polarize the indicator electrode. Polyethylene and fluorocarbon membranes are used commonly because they are permeable to molecular oxygen and are relatively rugged.

Membrane electrodes are commercially available in some variety. In all these instruments the "diffusion current" is linearly proportional to the concentration of molecular oxygen. The current can be converted easily to concentration units (e.g., milligrams per liter) by a number of calibration procedures.

Membrane electrodes exhibit a relatively high temperature coefficient largely due to changes in the membrane permeability.⁶ The effect of temperature on the electrode sensitivity, ϕ (microamperes per milligram per liter), can be expressed by the following simplified relationship:⁶

$$\log \phi = 0.43 m t + b$$

where:

t = temperature, °C.

m = constant that depends on the membrane material, and

b = constant that largely depends on membrane thickness.

If values of ϕ and m are determined for one temperature (ϕ_0 and t_0), it is possible to calculate the sensitivity at any desired temperature (ϕ and t) as follows:

$$\log \phi = \log \phi_0 + 0.43 m (t - t_0)$$

Nomographic charts for temperature correction can be constructed easily⁷ and are available from some manufacturers. An example is shown in Figure 4500-O:2, in which, for simplicity, sensitivity is plotted versus temperature on semilogarithmic coordinates. Check one or two points frequently to confirm original calibration. If calibration changes, the new calibration should be parallel to the original, provided that the same membrane material is used.

Temperature compensation also can be made automatically by using thermistors in the electrode circuit.⁴ However, thermistors may not compensate fully over a wide temperature range. For certain applications where high accuracy is required, use calibrated nomographic charts to correct for temperature effect.

To use the DO membrane electrode in estuarine waters or in wastewaters with varying ionic strength, correct for effect of salting-out on electrode sensitivity.^{6,7} This effect is particularly significant for large changes in salt content. Electrode sensitivity varies with salt concentration according to the following relationship:

$$\log \phi_s = 0.43 m_s C_s + \log \phi_0$$

where:

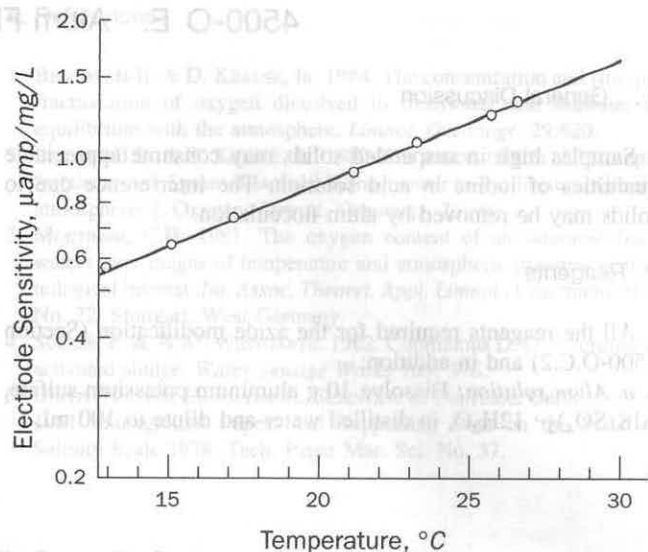


Figure 4500-O:2. Effect of temperature on electrode sensitivity.

ϕ_s, ϕ_0 = sensitivities in salt solution and distilled water, respectively,
 C_s = salt concentration (preferably ionic strength), and
 m_s = constant (salting-out coefficient).

If ϕ_0 and m_s are determined, it is possible to calculate sensitivity for any value of C_s . Conductivity measurements can be used to approximate salt concentration (C_s). This is particularly applicable to estuarine waters. Figure 4500-O:3 shows calibration curves for sensitivity of varying salt solutions at different temperatures.

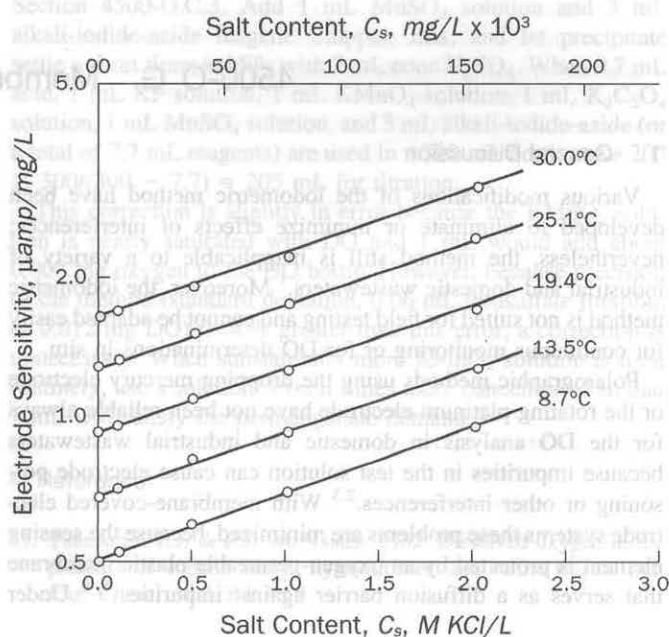


Figure 4500-O:3. The salting-out effect at different temperatures.

b. Interference: Plastic films used with membrane electrode systems are permeable to a variety of gases besides oxygen, although none is depolarized easily at the indicator electrode. Prolonged use of membrane electrodes in waters containing such gases as hydrogen sulfide (H_2S) tends to lower cell sensitivity. Eliminate this interference by frequently changing and calibrating the membrane electrode.

c. Sampling: Because membrane electrodes offer the advantage of analysis *in situ* they eliminate errors caused by sample handling and storage. If sampling is required, use the same precautions suggested for the iodometric method.

2. Apparatus

Oxygen-sensitive membrane electrode, polarographic or galvanic, with appropriate meter.

3. Procedure

a. Calibration: Follow manufacturer's calibration procedure exactly to obtain guaranteed precision and accuracy. Generally, calibrate membrane electrodes by reading against air or a sample of known DO concentration (determined by iodometric method) as well as in a sample with zero DO. (Add excess sodium sulfite, Na_2SO_3 , and a trace of cobalt chloride, $CoCl_2$, to bring DO to zero.) Preferably calibrate with samples of water under test. Avoid an iodometric calibration where interfering substances are suspected. The following illustrate the recommended procedures:

1) Fresh water—For unpolluted samples where interfering substances are absent, calibrate in the test solution or distilled water, whichever is more convenient.

2) Salt water—Calibrate directly with samples of seawater or waters having a constant salt concentration in excess of 1000 mg/L.

3) Fresh water containing pollutants or interfering substances—Calibrate with distilled water because erroneous results occur with the sample.

4) Salt water containing pollutants or interfering substances—Calibrate with a sample of clean water containing the same salt content as the sample. Add a concentrated potassium chloride (KCl) solution (see Conductivity, Section 2510 and Table 2510:I) to distilled water to produce the same specific conductance as that in the sample. For polluted ocean waters, calibrate with a sample of unpolluted seawater.

5) Estuary water containing varying quantities of salt—Calibrate with a sample of uncontaminated seawater or distilled or tap water. Determine sample chloride or salt concentration and revise calibration to account for change of oxygen solubility in the estuary water.⁷

b. Sample measurement: Follow all precautions recommended by manufacturer to insure acceptable results. Take care in changing membrane to avoid contamination of sensing element and also trapping of minute air bubbles under the membrane, which can lead to lowered response and high residual current. Provide sufficient sample flow across membrane surface to overcome erratic response (see Figure 4500-O:4 for a typical example of the effect of stirring).

c. Validation of temperature effect: Check frequently one or two points to verify temperature correction data.

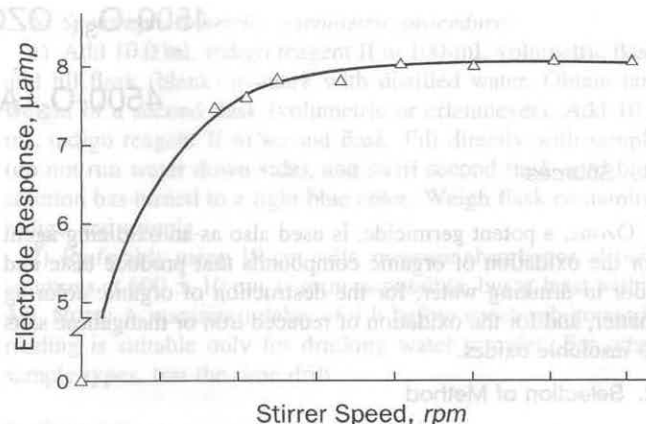


Figure 4500-O:4. Typical trend of effect of stirring on electrode response.

4. Precision and Bias

With most commercially available membrane electrode systems an accuracy of ± 0.1 mg DO/L and a precision of ± 0.05 mg DO/L can be obtained.

5. References

1. McKEOWN, J.J., L.C. BROWN & G.W. GOVE. 1967. Comparative studies of dissolved oxygen analysis methods. *J. Water Pollut. Control Fed.* 39:1323.
2. LYNN, W.R. & D.A. OKUN. 1955. Experience with solid platinum electrodes in the determination of dissolved oxygen. *Sewage Ind. Wastes* 27:4.
3. MANCY, K.H. & D.A. OKUN. 1960. Automatic recording of dissolved oxygen in aqueous systems containing surface active agents. *Anal. Chem.* 32:108.
4. CARRITT, D.E. & J.W. KANWISHER. 1959. An electrode system for measuring dissolved oxygen. *Anal. Chem.* 31:5.
5. MANCY, K.H. & W.C. WESTGARTH. 1962. A galvanic cell oxygen analyzer. *J. Water Pollut. Control Fed.* 34:1037.
6. MANCY, K.H., D.A. OKUN & C.N. REILLEY. 1962. A galvanic cell oxygen analyzer. *J. Electroanal. Chem.* 4:65.
7. MANCY, K.H. & T. JAFFE. 1966. Analysis of Dissolved Oxygen in Natural and Waste Waters. Publ. No. 999-WP-37, U.S. Public Health Serv., Washington, D.C.
8. WEISS, C.M. & R.T. OGLESBY. 1963. Instrumentation for monitoring water quality in reservoirs. American Water Works Assoc. 83rd Annual Conf., New York, N.Y.
9. CLEARY, E.J. 1962. Introducing the ORSANCO robot monitor. *Proc. Water Quality Meas. Instrum.* Publ. No. 108, U.S. Public Health Serv., Washington, D.C.
10. MACKERETH, F.J.H. 1964. An improved galvanic cell for determination of oxygen concentrations in fluids. *J. Sci. Instrum.* 41:38.
11. SULZER, F. & W.M. WESTGARTH. 1962. Continuous D.O. recording in activated sludge. *Water Sewage Works* 109:376.
12. DUXBURY, A.C. 1963. Calibration and use of a galvanic type oxygen electrode in field work. *Limnol. Oceanogr.* 8:483.
13. LIPNER, H.J., L.R. WITHERSPOON & V.C. CHAMPEAUS. 1964. Adaptation of a galvanic cell for microanalysis of oxygen. *Anal. Chem.* 36:204.

4500-O₃ OZONE (RESIDUAL)*4500-O₃ A. Introduction

1. Sources

Ozone, a potent germicide, is used also as an oxidizing agent for the oxidation of organic compounds that produce taste and odor in drinking water, for the destruction of organic coloring matter, and for the oxidation of reduced iron or manganese salts to insoluble oxides.

2. Selection of Method

Ozone residual in water is determined by the indigo method. Residual ozone decays rapidly. Depending on water quality, the

ozone residual half-life may be several seconds to a few minutes. Methods also are available for determining ozone in process gases.^{1,2}

3. References

1. RAKNESS, K.L., G. GORDON, B. LANGLAIS, W. MASSCHELEIN, N. MATSUMOTO, Y. RICHARD, C.M. ROBSON & I. SOMIYA. 1996. Guideline for measurement of ozone concentration in the process gas from an ozone generator. *Ozone: Sci. Eng.* 18:209.
2. RAKNESS, K.L., L.D. DEMERS, B.D. BLANK & D.J. HENRY. 1996. Gas phase ozone concentration comparisons from a commercial UV meter and KI wet-chemistry tests. *Ozone: Sci. Eng.* 18:231.

* Approved by Standard Methods Committee, 1997.

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4500-O₃ B. Indigo Colorimetric Method

1. General Discussion

The indigo colorimetric method is quantitative, selective, and simple; it replaces methods based on the measurement of total oxidant. The method is applicable to lake water, river infiltrate, manganese-containing groundwaters, extremely hard groundwaters, and even biologically treated domestic wastewaters.

a. Principle: In acidic solution, ozone rapidly decolorizes indigo. The decrease in absorbance is linear with increasing concentration. The proportionality constant at 600 nm is $0.42 \pm 0.01/\text{cm}/\text{mg/L}$ ($\Delta E = 20\,000/M \cdot \text{cm}$) compared to the ultraviolet absorption of pure ozone of $E = 2950/M \cdot \text{cm}$ at 258 nm.¹

b. Interferences: Hydrogen peroxide (H₂O₂) and organic peroxides decolorize the indigo reagent very slowly. H₂O₂ does not interfere if ozone is measured in less than 6 h after adding reagents. Organic peroxides may react more rapidly. Fe(III) does not interfere. Mn(II) does not interfere but it is oxidized by ozone to forms that decolorize the reagent. Correct for this decolorization by making the measurement relative to a blank in which the ozone has been destroyed selectively. Without the corrective procedure, 0.1 mg/L ozonated manganese gives a response of about 0.08 mg/L apparent ozone. Chlorine also interferes. Low concentrations of chlorine (<0.1 mg/L) can be masked by malonic acid. Bromine, which can be formed by oxidation of Br⁻, interferes (1 mole HOBr corresponds to 0.4 mole ozone). In the presence of HOBr or chlorine in excess of 0.1 mg/L, an accurate measurement of ozone cannot be made with this method.

c. Minimum detectable concentration: For the spectrophotometric procedure using thermostated cells and a high-quality photometer, the low-range procedure will measure down to 2 µg O₃/L. The practical lower limit for residual measurement is 10 to 20 µg/L.

d. Sampling: React sample with indigo as quickly as possible, because the residual may decay rapidly. Avoid loss of ozone residual due to off-gassing during sample collection. Do not run sample down side of flask. Add sample so that completely decolorized zones are eliminated quickly by swirling or stirring.

2. Apparatus

Photometer: Spectrophotometer or filter colorimeter for use at 600 ± 10 nm.

3. Reagents

a. Indigo stock solution: Add about 500 mL distilled water and 1 mL conc phosphoric acid to a 1-L volumetric flask. With stirring, add 770 mg potassium indigo trisulfonate, C₁₆H₇N₂O₁₁S₃K₃ (use only high-grade reagent, commercially available at about 80 to 85% purity). Fill to mark with distilled water. A 1:100 dilution exhibits an absorbance of 0.20 ± 0.010 cm at 600 nm. The stock solution is stable for about 4 months when stored in the dark. Discard when absorbance of a 1:100 dilution falls below 0.16/cm. Do not change concentration of dye for higher ranges of ozone residual. Volume of dye used may be adjusted.

b. Indigo reagent I: To a 1-L volumetric flask add 20 mL indigo stock solution, 10 g sodium dihydrogen phosphate (NaH₂PO₄), and 7 mL conc phosphoric acid. Dilute to mark. Prepare solution fresh when its absorbance decreases to less than 80% of its initial value, typically within a week.

c. Indigo reagent II: Proceed as with indigo reagent I, but add 100 mL indigo stock solution instead of 20 mL.

d. *Malonic acid reagent*: Dissolve 5 g malonic acid in water and dilute to 100 mL.

e. *Glycine reagent*: Dissolve 7 g glycine in water and dilute to 100 mL.

4. Procedure

a. Spectrophotometric, volumetric procedure:

1) Concentration range 0.01 to 0.1 mg O₃/L—Add 10.0 mL indigo reagent I to each of two 100-mL volumetric flasks. Fill one flask (blank) to mark with distilled water. Fill other flask to mark with sample. Measure absorbance of both solutions at 600 ± 10 nm as soon as possible but at least within 4 h. Preferably use 10-cm cells. Calculate the ozone concentration from the difference between the absorbances found in sample and blank (§ 5a below). (NOTE: A maximum delay of 4 h before spectrophotometric reading can be tolerated only for drinking water samples. For other sample types that cannot be read immediately, determine the relationship between time and absorbance.)

2) Range 0.05 to 0.5 mg O₃/L—Proceed as above using 10.0 mL indigo reagent II instead of reagent I. Preferably measure absorbance in 4- or 5-cm cells.

3) Concentrations greater than 0.3 mg O₃/L—Proceed using indigo reagent II, but for these higher ozone concentrations use a correspondingly smaller sample volume. Dilute resulting mixture to 100 mL with distilled water.

4) Control of interferences—In presence of low chlorine concentration (<0.1 mg/L), place 1 mL malonic acid reagent in both flasks before adding sample and/or filling to mark. Measure absorbance as soon as possible, within 60 min (Br⁻, Br₂, and HOBr are only partially masked by malonic acid).

In presence of manganese prepare a blank solution using sample, in which ozone is selectively destroyed by addition of glycine. Place 0.1 mL glycine reagent in 100-mL volumetric flask (blank) and 10.0 mL indigo reagent II in second flask (sample). Pipet exactly the same volume of sample into each flask. Adjust dose so that decolorization in second flask is easily visible but complete bleaching does not result (maximum 80 mL).

Insure that pH of glycine/sample mixture in blank flask (before adding indigo) is not below 6 because reaction between ozone and glycine becomes very slow at low pH. Stopper flasks and mix by carefully inverting. Add 10.0 mL indigo reagent II to blank flask only 30 to 60 s after sample addition. Fill both flasks to the mark with ozone-free water and mix thoroughly. Measure absorbance of both solutions at comparable contact times of approximately 30 to 60 min (after this time, residual manganese oxides further discolor indigo only slowly and the drift of absorbance in blank and sample become comparable). Reduced absorbance in blank flask results from manganese oxides while that in sample flask is due to ozone plus manganese oxide.

5) Calibration—Because ozone is unstable, base measurements on known and constant loss of absorbance of the indigo reagent ($f = 0.42 + 0.01/\text{cm}/\text{mg O}_3/\text{L}$). For maximum accuracy analyze the lot of potassium indigo trisulfonate (no commercial lot has been found to deviate from $f = 0.42$) using the iodometric procedure.

When using a filter photometer, readjust the conversion factor, f , by comparing photometer sensitivity with absorbance at 600 nm by an accurate spectrophotometer.

b. Spectrophotometric, gravimetric procedure:

1) Add 10.0 mL indigo reagent II to 100-mL volumetric flask and fill flask (blank) to mark with distilled water. Obtain tare weight of a second flask (volumetric or erlenmeyer). Add 10.0 mL indigo reagent II to second flask. Fill directly with sample (do not run water down side), and swirl second flask until blue solution has turned to a light blue color. Weigh flask containing indigo and sample.

2) Preferably using 10-cm cells, measure absorbance of both solutions at 600 ± 10 nm as soon as possible, but at least within 4 h. NOTE: A maximum delay of 4 h before spectrophotometric reading is suitable only for drinking water samples. For other sample types, test the time drift.

5. Calculations

a. Spectrophotometric, volumetric method:

$$\text{mg O}_3/\text{L} = \frac{100 \times \Delta A}{f \times b \times V}$$

where:

ΔA = difference in absorbance between sample and blank,

b = path length of cell, cm,

V = volume of sample, mL (normally 90 mL), and

$f = 0.42$.

The factor f is based on a sensitivity factor of 20 000/cm for the change of absorbance (600 nm) per mole of added ozone per liter. It was calibrated by iodometric titration. The UV absorbance of ozone in pure water may serve as a secondary standard: the factor $f = 0.42$ corresponds to an absorption coefficient for aqueous ozone, $E = 2950/M \cdot \text{cm}$ at 258 nm.

b. Spectrophotometric, gravimetric method:

$$\text{mg O}_3/\text{L} = \frac{(A_B \times 100) - (A_S \times V_T)}{f \times V_S \times b}$$

where:

A_B, A_S = absorbance of blank and sample, respectively,

V_S = volume of sample, mL = [(final weight - tare weight) g × 1.0 mL/g] - 10 mL,

V_T = total volume of sample plus indigo, mL = (final weight - tare weight) g × 1.0 mL/g,

b = path length of cell, cm, and

$f = 0.42$ (see § a above).

6. Precision and Bias

For the spectrophotometric volumetric procedure in the absence of interferences, the relative error is less than 5% without special sampling setups. In laboratory testing this may be reduced to 1%. No data are available for the spectrophotometric gravimetric procedure.

Because this method is based on the differences in absorbance between the sample and blank (ΔA) the method is not applicable in the presence of chlorine. If the manganese content exceeds the ozone, precision is reduced. If the ratio of manganese to ozone is

less than 10:1, ozone concentrations above 0.02 mg/L may be determined with a relative error of less than 20%.

7. Reference

1. HOIGNÉ, J. & H. BADER. 1980. Bestimmung von Ozon und Chlordinoxid im Wasser mit der Indigo-Methode. *Vom Wasser* 55:261.

8. Bibliography

- BADER, H. & J. HOIGNÉ. 1981. Determination of ozone in water by the indigo method. *Water Res.* 15:449.
- BADER, H. & J. HOIGNÉ. 1982. Determination of ozone in water by the indigo method. A submitted standard method. *Ozone: Sci. Eng.* 4:169.
- GILBERT, E. & J. HOIGNÉ. 1983. Messung von Ozon in Wasserwerken; Vergleich der DPD- und Indigo-Methode. *GWF-Wasser/Abwasser* 124:527.

- HAAG, W.R. & J. HOIGNÉ. 1983. Ozonation of bromide-containing waters: kinetics of formation of hypobromous acid and bromate. *Environ. Sci. Technol.* 17:261.
- STRAKA, M.R., G. GORDON & G.E. PACEY. 1985. Residual aqueous ozone determination by gas diffusion flow injection analysis. *Anal. Chem.* 57:1799.
- GORDON, G. & G.E. PACEY. 1986. An introduction to the chemical reactions of ozone pertinent to its analysis. In R.G. Rice, L.J. Bollyky & W.J. Lacy, eds. *Analytical Aspects of Ozone Treatment of Water and Wastewater—A Monograph*. Lewis Publishers, Inc., Chelsea, Mich.
- COFFEY, B.C., K.G. GRAFF, A.A. MOFIDI & J.T. GRAMITH. 1995. On-line monitoring of ozone disinfection effectiveness within an over-under baffled contactor. Proc. Annu. Conf., American Water Works Assoc., Anaheim, Calif. American Water Works Assoc., Denver, Colo.
- METROPOLITAN WATER DISTRICT OF SOUTHERN CALIFORNIA. 1996. Demonstration-Scale Evaluation of Ozone/PEROXONE." American Water Works Assoc. Research Foundation.

4500-P PHOSPHORUS*

4500-P A. Introduction

1. Occurrence

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates (pyro-, meta-, and other polyphosphates), and organically bound phosphates. They occur in solution, in particles or detritus, or in the bodies of aquatic organisms.

These forms of phosphate arise from a variety of sources. Small amounts of orthophosphate or certain condensed phosphates are added to some water supplies during treatment. Larger quantities of the same compounds may be added during laundering or other cleaning, because these materials are major constituents of many commercial cleaning preparations. Phosphates are used extensively in the treatment of boiler waters. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm runoff and to a lesser extent with melting snow. Organic phosphates are formed primarily by biological processes. They are contributed to sewage by body wastes and food residues, and also may be formed from orthophosphates in biological treatment processes or by receiving-water biota.

Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water. In instances where phosphate is a growth-limiting nutrient, the discharge of raw or treated wastewater, agricultural drainage, or certain industrial wastes to that water may stimulate

the growth of photosynthetic aquatic micro- and macroorganisms in nuisance quantities.

Phosphates also occur in bottom sediments and in biological sludges, both as precipitated inorganic forms and incorporated into organic compounds.

2. Definition of Terms

Phosphorus analyses embody two general procedural steps: (a) conversion of the phosphorus form of interest to dissolved orthophosphate, and (b) colorimetric determination of dissolved orthophosphate. The separation of phosphorus into its various forms is defined analytically but the analytical differentiations have been selected so that they may be used for interpretive purposes.

Filtration through a 0.45- μ m-pore-diam membrane filter separates dissolved from suspended forms of phosphorus. No claim is made that filtration through 0.45- μ m filters is a true separation of suspended and dissolved forms of phosphorus; it is merely a convenient and replicable analytical technique designed to make a gross separation. Prefiltration through a glass fiber filter may be used to increase the filtration rate.

Phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the sample are termed "reactive phosphorus." While reactive phosphorus is largely a measure of orthophosphate, a small fraction of any condensed phosphate present usually is hydrolyzed unavoidably in the procedure. Reactive phosphorus occurs in both dissolved and suspended forms.

Acid hydrolysis at boiling-water temperature converts dissolved and particulate condensed phosphates to dissolved or-

* Approved by Standard Methods Committee, 1999.
Joint Task Group: (4500-P.J)—William Nivens (chair), Prem H. Arora, Lori J. Emery, James G. Poff, Steven C. Schindler; 20th Edition (4500-P.G, H, I)—Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

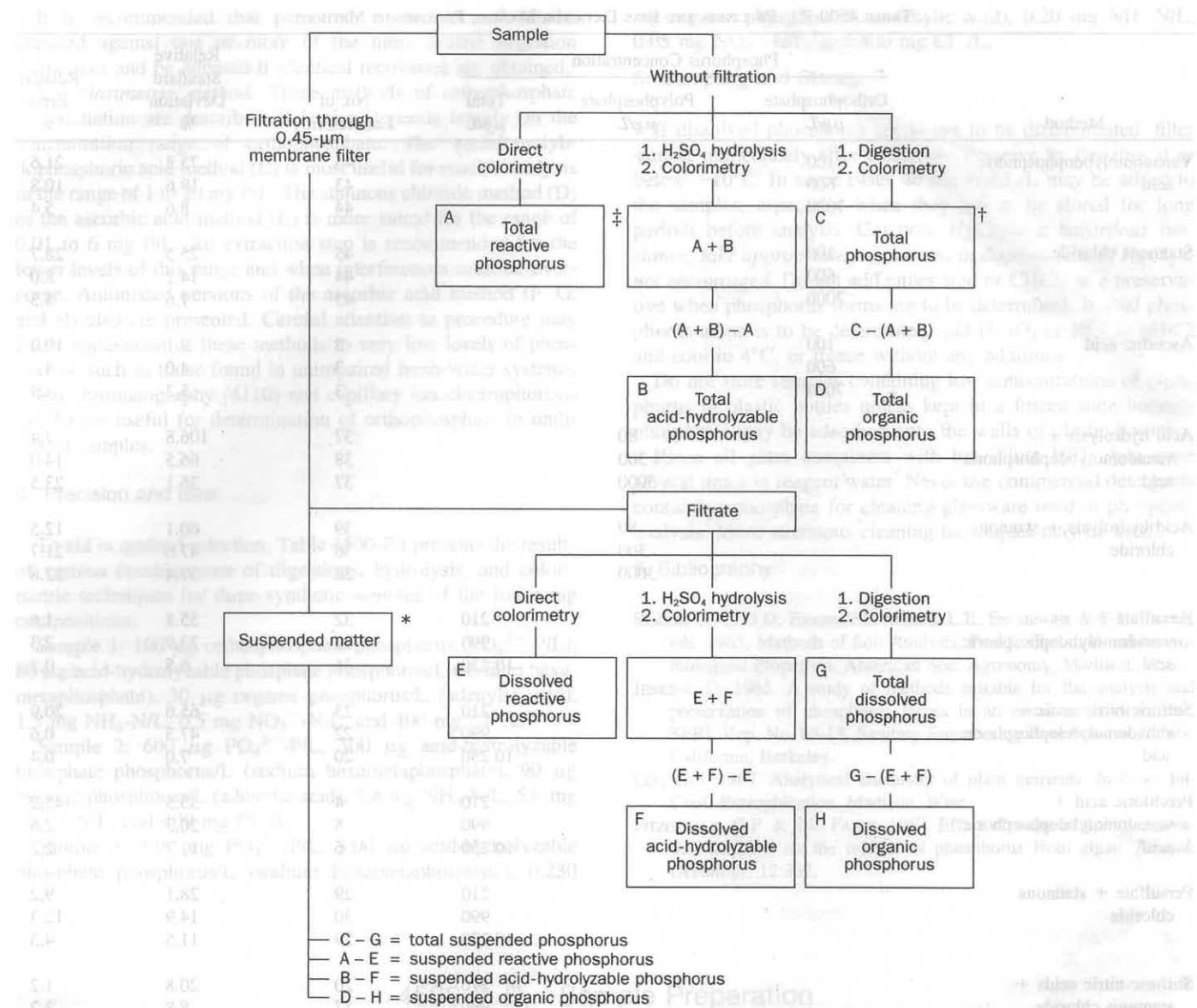


Figure 4500-P:1. Steps for analysis of phosphate fractions.

* Direct determination of phosphorus on the membrane filter containing suspended matter will be required where greater precision than that obtained by difference is desired. Digest filter with HNO_3 and follow by perchloric acid. Then perform colorimetry.

† Total phosphorus measurements on highly saline samples may be difficult because of precipitation of large quantities of salt as a result of digestion techniques that drastically reduce sample volume. For total phosphorus analyses on such samples, directly determine total dissolved phosphorus and total suspended phosphorus and add the results.

‡ In determination of total dissolved or total suspended reactive phosphorus, anomalous results may be obtained on samples containing large amounts of suspended sediments. Very often results depend largely on the degree of agitation and mixing to which samples are subjected during analysis because of a time-dependent desorption of orthophosphate from the suspended particles.

thophosphate. The hydrolysis unavoidably releases some phosphate from organic compounds, but this may be reduced to a

minimum by judicious selection of acid strength and hydrolysis time and temperature. The term “acid-hydrolyzable phosphorus” is preferred over “condensed phosphate” for this fraction.

The phosphate fractions that are converted to orthophosphate only by oxidation destruction of the organic matter present are considered “organic” or “organically bound” phosphorus. The severity of the oxidation required for this conversion depends on the form—and to some extent on the amount—of the organic phosphorus present. Like reactive phosphorus and acid-hydrolyzable phosphorus, organic phosphorus occurs both in the dissolved and suspended fractions.

The total phosphorus as well as the dissolved and suspended phosphorus fractions each may be divided analytically into the three chemical types that have been described: reactive, acid-hydrolyzable, and organic phosphorus. Figure 4500-P:1 shows

TABLE 4500-P.I. PRECISION AND BIAS DATA FOR MANUAL PHOSPHORUS METHODS

Method	Phosphorus Concentration			No. of Laboratories	Relative Standard Deviation %	Relative Error %
	Orthophosphate $\mu\text{g/L}$	Polyphosphate $\mu\text{g/L}$	Total $\mu\text{g/L}$			
Vanadomolybdophosphoric acid	100			45	75.2	21.6
	600			43	19.6	10.8
	7000			44	8.6	5.4
Stannous chloride	100			45	25.5	28.7
	600			44	14.2	8.0
	7000			45	7.6	4.3
Ascorbic acid	100			3	9.1	10.0
	600			3	4.0	4.4
	7000			3	5.2	4.9
Acid hydrolysis + vanadomolybdophosphoric acid		80		37	106.8	7.4
		300		38	66.5	14.0
		3000		37	36.1	23.5
Acid hydrolysis + stannous chloride		80		39	60.1	12.5
		300		36	47.6	21.7
		3000		38	37.4	22.8
Persulfate + vanadomolybdophosphoric acid			210	32	55.8	1.6
			990	32	23.9	2.3
			10 230	31	6.5	0.3
Sulfuric-nitric acids + vanadomolybdophosphoric acid			210	23	65.6	20.9
			990	22	47.3	0.6
			10 230	20	7.0	0.4
Perchloric acid + vanadomolybdophosphoric acid			210	4	33.5	45.2
			990	5	20.3	2.6
			10 230	6	11.7	2.2
Persulfate + stannous chloride			210	29	28.1	9.2
			990	30	14.9	12.3
			10 230	29	11.5	4.3
Sulfuric-nitric acids + stannous chloride			210	20	20.8	1.2
			990	17	8.8	3.2
			10 230	19	7.5	0.4

the steps for analysis of individual phosphorus fractions. As indicated, determinations usually are conducted only on the unfiltered and filtered samples. Suspended fractions generally are determined by difference; however, they may be determined directly by digestion of the material retained on a glass-fiber filter.

3. Selection of Method

a. Digestion methods: Because phosphorus may occur in combination with organic matter, a digestion method to determine total phosphorus must be able to oxidize organic matter effectively to release phosphorus as orthophosphate. Three digestion methods are given in Section 4500-P.B.3, 4, and 5. The perchloric acid method, the most drastic and time-consuming method, is

recommended only for particularly difficult samples such as sediments. The nitric acid-sulfuric acid method is recommended for most samples. By far the simplest method is the persulfate oxidation technique. Persulfate oxidation is coupled with ultraviolet light for a more efficient digestion in an automated in-line digestion/determination by flow injection analysis (4500-P.I).

The persulfate oxidation method in Section 4500-P.J renders a digestate that can be analyzed for both total nitrogen and total phosphorus. This procedure can be used for both parameters because it occurs over a broad pH range. During the initial stage of the digestion, sample pH is alkaline ($\text{pH} > 12$); during the final stage, sample pH becomes acidic. As a result, nitrogenous compounds are oxidized to nitrate and phosphorus compounds to orthophosphate.

It is recommended that persulfate oxidation methods be checked against one or more of the more drastic digestion techniques and be adopted if identical recoveries are obtained.

b. Colorimetric method: Three methods of orthophosphate determination are described. Selection depends largely on the concentration range of orthophosphate. The vanadomolybdophosphoric acid method (C) is most useful for routine analysis in the range of 1 to 20 mg P/L. The stannous chloride method (D) or the ascorbic acid method (E) is more suited for the range of 0.01 to 6 mg P/L. An extraction step is recommended for the lower levels of this range and when interferences must be overcome. Automated versions of the ascorbic acid method (F, G, and H) also are presented. Careful attention to procedure may allow application of these methods to very low levels of phosphorus, such as those found in unimpaired fresh-water systems.

Ion chromatography (4110) and capillary ion electrophoresis (4140) are useful for determination of orthophosphate in undigested samples.

4. Precision and Bias

To aid in method selection, Table 4500-P:I presents the results of various combinations of digestions, hydrolysis, and colorimetric techniques for three synthetic samples of the following compositions:

Sample 1: 100 μg orthophosphate phosphorus ($\text{PO}_4^{3-}\text{-P/L}$), 80 μg acid-hydrolyzable phosphate phosphorus/L (sodium hexametaphosphate), 30 μg organic phosphorus/L (adenylic acid), 1.5 mg $\text{NH}_3\text{-N/L}$, 0.5 mg $\text{NO}_3^-\text{-N/L}$, and 400 mg $\text{Cl}^-\text{/L}$.

Sample 2: 600 μg $\text{PO}_4^{3-}\text{-P/L}$, 300 μg acid-hydrolyzable phosphate phosphorus/L (sodium hexametaphosphate), 90 μg organic phosphorus/L (adenylic acid), 0.8 mg $\text{NH}_3\text{-N/L}$, 5.0 mg $\text{NO}_3^-\text{-N/L}$, and 400 mg $\text{Cl}^-\text{/L}$.

Sample 3: 7.00 mg $\text{PO}_4^{3-}\text{-P/L}$, 3.00 μg acid-hydrolyzable phosphate phosphorus/L (sodium hexametaphosphate), 0.230

mg organic phosphorus/L (adenylic acid), 0.20 mg $\text{NH}_3\text{-N/L}$, 0.05 mg $\text{NO}_3^-\text{-N/L}$, and 400 mg $\text{Cl}^-\text{/L}$.

5. Sampling and Storage

If dissolved phosphorus forms are to be differentiated, filter sample immediately after collection. Preserve by freezing at or below -10°C . In some cases 40 mg $\text{HgCl}_2\text{/L}$ may be added to the samples, especially when they are to be stored for long periods before analysis. CAUTION: *HgCl₂ is a hazardous substance; take appropriate precautions in disposal; use of HgCl₂ is not encouraged.* Do not add either acid or CHCl_3 as a preservative when phosphorus forms are to be determined. If total phosphorus alone is to be determined, add H_2SO_4 or HCl to $\text{pH}<2$ and cool to 4°C , or freeze without any additions.

Do not store samples containing low concentrations of phosphorus in plastic bottles unless kept in a frozen state because phosphates may be adsorbed onto the walls of plastic bottles.

Rinse all glass containers with hot dilute HCl , then rinse several times in reagent water. Never use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis. More strenuous cleaning techniques may be used.

6. Bibliography

- BLACK, C.A., D.D. EVANS, J.L. WHITE, L.E. ENSMINGER & F.E. CLARK, eds. 1965. *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*. American Soc. Agronomy, Madison, Wisc.
- JENKINS, D. 1965. A study of methods suitable for the analysis and preservation of phosphorus forms in an estuarine environment. SERL Rep. No. 65-18, Sanitary Engineering Research Lab., Univ. California, Berkeley.
- LEE, G.F. 1967. Analytical chemistry of plant nutrients. In *Proc. Int. Conf. Eutrophication*, Madison, Wisc.
- FITZGERALD, G.P. & S.L. FAUST. 1967. Effect of water sample preservation methods on the release of phosphorus from algae. *Limnol. Oceanogr.* 12:332.

4500-P B. Sample Preparation

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

1. Preliminary Filtration

Filter samples for determination of dissolved reactive phosphorus, dissolved acid-hydrolyzable phosphorus, and total dissolved phosphorus through $0.45\text{-}\mu\text{m}$ membrane filters. A glass fiber filter may be used to prefilter hard-to-filter samples.

Wash membrane filters by soaking in distilled water before use because they may contribute significant amounts of phosphorus to samples containing low concentrations of phosphate. Use one of two washing techniques: (a) soak 50 filters in 2 L distilled water for 24 h; (b) soak 50 filters in 2 L distilled water for 1 h, change distilled water, and soak filters an additional 3 h. Membrane filters also may be washed by running several 100-mL portions of distilled water through them. This procedure

requires more frequent determination of blank values to ensure consistency in washing and to evaluate different lots of filters.

2. Preliminary Acid Hydrolysis

The acid-hydrolyzable phosphorus content of the sample is defined operationally as the difference between reactive phosphorus as measured in the untreated sample and phosphate found after mild acid hydrolysis. Generally, it includes condensed phosphates such as pyro-, tripoly-, and higher-molecular-weight species such as hexametaphosphate. In addition, some natural waters contain organic phosphate compounds that are hydrolyzed to orthophosphate under the test conditions. Polyphosphates generally do not respond to reactive phosphorus tests but can be hydrolyzed to orthophosphate by boiling with acid.

After hydrolysis, determine reactive phosphorus by a colorimetric method (C, D, or E). Interferences, precision, bias, and sensitivity will depend on the colorimetric method used.

a. Apparatus:

Autoclave or pressure cooker, capable of operating at 98 to 137 kPa.

b. Reagents:

1) Phenolphthalein indicator aqueous solution.

2) Strong acid solution: Slowly add 300 mL conc H_2SO_4 to about 600 mL distilled water. When cool, add 4.0 mL conc HNO_3 and dilute to 1 L.

3) Sodium hydroxide, NaOH, 6N.

c. Procedure: To 100-mL sample or a portion diluted to 100 mL, add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add strong acid solution dropwise, to just discharge the color. Then add 1 mL more.

Boil gently for at least 90 min, adding distilled water to keep the volume between 25 and 50 mL. Alternatively, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa. Cool, neutralize to a faint pink color with NaOH solution, and restore to the original 100-mL volume with distilled water.

Prepare a calibration curve by carrying a series of standards containing orthophosphate (see colorimetric method C, D, or E) through the hydrolysis step. Do not use orthophosphate standards without hydrolysis, because the salts added in hydrolysis cause an increase in the color intensity in some methods.

Determine reactive phosphorus content of treated portions, using Method C, D, or E. This gives the sum of polyphosphate and orthophosphate in the sample. To calculate its content of acid-hydrolyzable phosphorus, determine reactive phosphorus in a sample portion that has not been hydrolyzed, using the same colorimetric method as for treated sample, and subtract.

3. Perchloric Acid Digestion

a. Apparatus:

- 1) Hot plate: A 30- × 50-cm heating surface is adequate.
- 2) Safety shield.
- 3) Safety goggles.
- 4) Erlenmeyer flasks, 125-mL, acid-washed and rinsed with distilled water.

b. Reagents:

- 1) Nitric acid, HNO_3 , conc.
- 2) Perchloric acid, $\text{HClO}_4 \cdot 2\text{H}_2\text{O}$, purchased as 70 to 72% HClO_4 , reagent-grade.
- 3) Sodium hydroxide, NaOH, 6N.
- 4) Methyl orange indicator solution.
- 5) Phenolphthalein indicator aqueous solution.

c. Procedure: CAUTION—Heated mixtures of HClO_4 and organic matter may explode violently. Avoid this hazard by taking the following precautions: (a) Do not add HClO_4 to a hot solution that may contain organic matter. (b) Always initiate digestion of samples containing organic matter with HNO_3 . Complete digestion using the mixture of HNO_3 and HClO_4 . (c) Do not fume with HClO_4 in ordinary hoods. Use hoods especially constructed for HClO_4 fuming or a glass fume eradicator* connected to a water pump. (d) Never let samples being digested with HClO_4 evaporate to dryness.

Measure sample containing the desired amount of phosphorus (this will be determined by whether Method C, D, or E is to be

used) into a 125-mL erlenmeyer flask. Acidify to methyl orange with conc HNO_3 , add another 5 mL conc HNO_3 , and evaporate on a steam bath or hot plate to 15 to 20 mL.

Add 10 mL each of conc HNO_3 and HClO_4 to the 125-mL conical flask, cooling the flask between additions. Add a few boiling chips, heat on a hot plate, and evaporate gently until dense white fumes of HClO_4 just appear. If solution is not clear, cover neck of flask with a watch glass and keep solution barely boiling until it clears. If necessary, add 10 mL more HNO_3 to aid oxidation.

Cool digested solution and add 1 drop aqueous phenolphthalein solution. Add 6N NaOH solution until the solution just turns pink. If necessary, filter neutralized solution and wash filter liberally with distilled water. Make up to 100 mL with distilled water.

Determine the $\text{PO}_4^{3-}\text{-P}$ content of the treated sample by Method C, D, or E.

Prepare a calibration curve by carrying a series of standards containing orthophosphate (see Method C, D, or E) through digestion step. Do not use orthophosphate standards without treatment.

4. Sulfuric Acid-Nitric Acid Digestion

a. Apparatus:

1) Digestion rack: An electrically or gas-heated digestion rack with provision for withdrawal of fumes is recommended. Digestion racks typical of those used for micro-kjeldahl digestions are suitable.

2) Micro-kjeldahl flasks.

b. Reagents:

- 1) Sulfuric acid, H_2SO_4 , conc.
- 2) Nitric acid, HNO_3 , conc.
- 3) Phenolphthalein indicator aqueous solution.
- 4) Sodium hydroxide, NaOH, 1N.

c. Procedure: Into a micro-kjeldahl flask, measure a sample containing the desired amount of phosphorus (this is determined by the colorimetric method used). Add 1 mL conc H_2SO_4 and 5 mL conc HNO_3 .

Digest to a volume of 1 mL and then continue until solution becomes colorless to remove HNO_3 .

Cool and add approximately 20 mL distilled water, 0.05 mL (1 drop) phenolphthalein indicator, and as much 1N NaOH solution as required to produce a faint pink tinge. Transfer neutralized solution, filtering if necessary to remove particulate material or turbidity, into a 100-mL volumetric flask. Add filter washings to flask and adjust sample volume to 100 mL with distilled water.

Determine phosphorus by Method C, D, or E, for which a separate calibration curve has been constructed by carrying standards through the acid digestion procedure.

5. Persulfate Digestion Method

a. Apparatus:

- 1) Hot plate: A 30- × 50-cm heating surface is adequate.
- 2) Autoclave: An autoclave or pressure cooker capable of developing 98 to 137 kPa may be used in place of a hot plate.
- 3) Glass scoop, to hold required amounts of persulfate crystals.

b. Reagents:

* GFS Chemical Co., Columbus, OH, or equivalent.

1) *Phenolphthalein indicator aqueous solution.*
 2) *Sulfuric acid solution:* Carefully add 300 mL conc H_2SO_4 to approximately 600 mL distilled water and dilute to 1 L with distilled water.

3) *Ammonium persulfate*, $(\text{NH}_4)_2\text{S}_2\text{O}_8$, solid, or potassium persulfate, $\text{K}_2\text{S}_2\text{O}_8$, solid.

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

c. *Procedure:* Use 50 mL or a suitable portion of thoroughly mixed sample. Add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add H_2SO_4 solution dropwise to just discharge the color. Then add 1 mL H_2SO_4 solution and either 0.4 g solid $(\text{NH}_4)_2\text{S}_2\text{O}_8$ or 0.5 g solid $\text{K}_2\text{S}_2\text{O}_8$.

Boil gently on a preheated hot plate for 30 to 40 min or until a final volume of 10 mL is reached. Organophosphorus compounds such as AMP may require as much as 1.5 to 2 h for complete digestion. Cool, dilute to 30 mL with distilled water, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH . Alternatively, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa.

4500-P C. Vanadomolybdophosphoric Acid Colorimetric Method

1. General Discussion

a. *Principle:* In a dilute orthophosphate solution, ammonium molybdate reacts under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow color is proportional to phosphate concentration.

b. *Interference:* Positive interference is caused by silica and arsenate only if the sample is heated. Negative interferences are caused by arsenate, fluoride, thorium, bismuth, sulfide, thiosulfate, thiocyanate, or excess molybdate. Blue color is caused by ferrous iron but this does not affect results if ferrous iron concentration is less than 100 mg/L. Sulfide interference may be removed by oxidation with bromine water. Ions that do not interfere in concentrations up to 1000 mg/L are Al^{3+} , Fe^{3+} , Mg^{2+} , Ca^{2+} , Ba^{2+} , Sr^{2+} , Li^+ , Na^+ , K^+ , NH_4^+ , Cd^{2+} , Mn^{2+} , Pb^{2+} , Hg^{2+} , Sn^{2+} , Cu^{2+} , Ni^{2+} , Ag^+ , U^{4+} , Zr^{4+} , AsO_3^{3-} , Br^- , CO_3^{2-} , ClO_4^- , CN^- , IO_3^- , SiO_4^{4-} , NO_3^- , NO_2^- , SO_4^{2-} , SO_3^{2-} , pyrophosphate, molybdate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate, and salicylate. If HNO_3 is used in the test, Cl^- interferes at 75 mg/L.

c. *Minimum detectable concentration:* The minimum detectable concentration is 200 $\mu\text{g P/L}$ in 1-cm spectrophotometer cells.

2. Apparatus

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

- 1) *Spectrophotometer*, for use at 400 to 490 nm.
- 2) *Filter photometer*, provided with a blue or violet filter exhibiting maximum transmittance between 400 and 470 nm.

The wavelength at which color intensity is measured depends on sensitivity desired, because sensitivity varies tenfold with wavelengths 400 to 490 nm. Ferric iron causes interference at

Cool, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH . Make up to 100 mL with distilled water. In some samples a precipitate may form at this stage, but do not filter. For any subsequent subdividing of the sample, shake well. The precipitate (which is possibly a calcium phosphate) redissolves under the acid conditions of the colorimetric reactive phosphorus test. Determine phosphorus by Method C, D, or E, for which a separate calibration curve has been constructed by carrying standards through the persulfate digestion procedure.

6. Bibliography

- LEE, G.F., N.L. CLESCERI & G.P. FITZGERALD. 1965. Studies on the analysis of phosphates in algal cultures. *J. Air Water Pollut.* 9:715.
 SHANNON, J.E. & G.F. LEE. 1966. Hydrolysis of condensed phosphates in natural waters. *J. Air Water Pollut.* 10:735.
 GALES, M.E., JR., E.C. JULIAN & R.C. KRONER. 1966. Method for quantitative determination of total phosphorus in water. *J. Amer. Water Works Assoc.* 58:1363.

low wavelengths, particularly at 400 nm. A wavelength of 470 nm usually is used. Concentration ranges for different wavelengths are:

P Range mg/L	Wavelength nm
1.0–5.0	400
2.0–10	420
4.0–18	470

b. *Acid-washed glassware:* Use acid-washed glassware for determining low concentrations of phosphorus. Phosphate contamination is common because of its absorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with hot dilute HCl and rinse well with distilled water. Preferably, reserve the glassware only for phosphate determination, and after use, wash and keep filled with water until needed. If this is done, acid treatment is required only occasionally.

c. *Filtration apparatus and filter paper.**

3. Reagents

a. *Phenolphthalein indicator aqueous solution.*

b. *Hydrochloric acid*, HCl , 1 + 1. H_2SO_4 , HClO_4 , or HNO_3 may be substituted for HCl . The acid concentration in the determination is not critical but a final sample concentration of 0.5N is recommended.

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

* Whatman No. 42 or equivalent.

† Darco G60 or equivalent.

d. Vanadate-molybdate reagent:

1) *Solution A:* Dissolve 25 g ammonium molybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, in 300 mL distilled water.

2) *Solution B:* Dissolve 1.25 g ammonium metavanadate, NH_4VO_3 , by heating to boiling in 300 mL distilled water. Cool and add 330 mL conc HCl. Cool Solution B to room temperature, pour Solution A into Solution B, mix, and dilute to 1 L.

e. Standard phosphate solution: Dissolve in distilled water 219.5 mg anhydrous KH_2PO_4 and dilute to 1000 mL; 1.00 mL = 50.0 $\mu\text{g PO}_4^{3-}\text{-P}$.

4. Procedure

a. Sample pH adjustment: If sample pH is greater than 10, add 0.05 mL (1 drop) phenolphthalein indicator to 50.0 mL sample and discharge the red color with 1 + 1 HCl before diluting to 100 mL.

b. Color removal from sample: Remove excessive color in sample by shaking about 50 mL with 200 mg activated carbon in an erlenmeyer flask for 5 min and filter to remove carbon. Check each batch of carbon for phosphate because some batches produce high reagent blanks.

c. Color development in sample: Place 35 mL or less of sample, containing 0.05 to 1.0 mg P, in a 50-mL volumetric flask. Add 10 mL vanadate-molybdate reagent and dilute to the mark with distilled water. Prepare a blank in which 35 mL distilled water is substituted for the sample. After 10 min or more, measure absorbance of sample versus a blank at a wavelength of 400 to 490 nm, depending on sensitivity desired (see ¶ 2a above). The color is stable for days and its intensity is unaffected by variation in room temperature.

d. Preparation of calibration curve: Prepare a calibration curve by using suitable volumes of standard phosphate solution and proceeding as in ¶ 4c. When ferric ion is low enough not to interfere, plot a family of calibration curves of one series of standard solutions for various wavelengths. This permits a wide

latitude of concentrations in one series of determinations. Analyze at least one standard with each set of samples.

5. Calculation

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

6. Precision and Bias

See Table 4500-P:I.

7. Bibliography

- KITSON, R.E. & M.G. MELLON. 1944. Colorimetric determination of phosphorus as molybdovanadophosphoric acid. *Ind. Eng. Chem., Anal. Ed.* 16:379.
- BOLTZ, D.F. & M.G. MELLON. 1947. Determination of phosphorus, germanium, silicon, and arsenic by the heteropoly blue method. *Ind. Eng. Chem., Anal. Ed.* 19:873.
- GREENBERG, A.E., L.W. WEINBERGER & C.N. SAWYER. 1950. Control of nitrite interference in colorimetric determination of phosphorus. *Anal. Chem.* 22:499.
- YOUNG, R.S. & A. GOLLEDGE. 1950. Determination of hexametaphosphate in water after threshold treatment. *Ind. Chem.* 26:13.
- GRISWOLD, B.L., F.L. HUMOLLER & A.R. MCINTYRE. 1951. Inorganic phosphates and phosphate esters in tissue extracts. *Anal. Chem.* 23:192.
- BOLTZ, D.F., ed. 1958. *Colorimetric Determination of Nonmetals*. Interscience Publishers, New York, N.Y.
- AMERICAN WATER WORKS ASSOCIATION. 1958. Committee report. Determination of orthophosphate, hydrolyzable phosphate, and total phosphate in surface waters. *J. Amer. Water Works Assoc.* 50:1563.
- JACKSON, M.L. 1958. *Soil Chemical Analysis*. Prentice-Hall, Englewood Cliffs, N.J.
- ABBOT, D.C., G.E. EMSDEN & J.R. HARRIS. 1963. A method for determining orthophosphate in water. *Analyst* 88:814.
- PROFT, G. 1964. Determination of total phosphorus in water and wastewater as molybdovanadophosphoric acid. *Limnologia* 2:407.

4500-P D. Stannous Chloride Method

1. General Discussion

a. Principle: Molybdophosphoric acid is formed and reduced by stannous chloride to intensely colored molybdenum blue. This method is more sensitive than Method C and makes feasible measurements down to 7 $\mu\text{g P/L}$ by use of increased light path length. Below 100 $\mu\text{g P/L}$ an extraction step may increase reliability and lessen interference.

b. Interference: See Section 4500-P.C.1b.

c. Minimum detectable concentration: The minimum detectable concentration is about 3 $\mu\text{g P/L}$. The sensitivity at 0.3010 absorbance is about 10 $\mu\text{g P/L}$ for an absorbance change of 0.009.

2. Apparatus

The same apparatus is required as for Method C, except that a pipetting bulb is required for the extraction step. Set spectrophotometer at 625 nm in the measurement of benzene-isobutanol ex-

tracts and at 690 nm for aqueous solutions. If the instrument is not equipped to read at 690 nm, use a wavelength of 650 nm for aqueous solutions, with somewhat reduced sensitivity and precision.

3. Reagents

a. Phenolphthalein indicator aqueous solution.

b. Strong-acid solution: Prepare as directed in Section 4500-P.B.2b2).

c. Ammonium molybdate reagent I: Dissolve 25 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 175 mL distilled water. Cautiously add 280 mL conc H_2SO_4 to 400 mL distilled water. Cool, add molybdate solution, and dilute to 1 L.

d. Stannous chloride reagent I: Dissolve 2.5 g fresh $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 100 mL glycerol. Heat in a water bath and stir with a glass rod to hasten dissolution. This reagent is stable and requires neither preservatives nor special storage.

e. Standard phosphate solution: Prepare as directed in Section 4500-P.C.3e.

f. Reagents for extraction:

1) *Benzene-isobutanol solvent*: Mix equal volumes of benzene and isobutyl alcohol. (CAUTION—This solvent is highly flammable.)

2) *Ammonium molybdate reagent II*: Dissolve 40.1 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in approximately 500 mL distilled water. Slowly add 396 mL ammonium molybdate reagent I. Cool and dilute to 1 L.

3) *Alcoholic sulfuric acid solution*: Cautiously add 20 mL conc H_2SO_4 to 980 mL methyl alcohol with continuous mixing.

4) *Dilute stannous chloride reagent II*: Mix 8 mL stannous chloride reagent I with 50 mL glycerol. This reagent is stable for at least 6 months.

4. Procedure

a. *Preliminary sample treatment*: To 100 mL sample containing not more than 200 μg P and free from color and turbidity, add 0.05 mL (1 drop) phenolphthalein indicator. If sample turns pink, add strong acid solution dropwise to discharge the color. If more than 0.25 mL (5 drops) is required, take a smaller sample and dilute to 100 mL with distilled water after first discharging the pink color with acid.

b. *Color development*: Add, with thorough mixing after each addition, 4.0 mL molybdate reagent I and 0.5 mL (10 drops) stannous chloride reagent I. Rate of color development and intensity of color depend on temperature of the final solution, each 1°C increase producing about 1% increase in color. Hence, hold samples, standards, and reagents within 2°C of one another and in the temperature range between 20 and 30°C .

c. *Color measurement*: After 10 min, but before 12 min, using the same specific interval for all determinations, measure color photometrically at 690 nm and compare with a calibration curve, using a distilled water blank. Light path lengths suitable for various concentration ranges are as follows:

Approximate P Range mg/L	Light Path cm
0.3–2	0.5
0.1–1	2
0.007–0.2	10

Always run a blank on reagents and distilled water. Because the color at first develops progressively and later fades, maintain

equal timing conditions for samples and standards. Prepare at least one standard with each set of samples or once each day that tests are made. The calibration curve may deviate from a straight line at the upper concentrations of the 0.3 to 2.0-mg/L range.

d. *Extraction*: When increased sensitivity is desired or interferences must be overcome, extract phosphate as follows: Pipet a 40-mL sample, or one diluted to that volume, into a 125-mL separatory funnel. Add 50.0 mL benzene-isobutanol solvent and 15.0 mL molybdate reagent II. Close funnel at once and shake vigorously for exactly 15 s. If condensed phosphate is present, any delay will increase its conversion to orthophosphate. Remove stopper and withdraw 25.0 mL of separated organic layer, using a pipet with safety bulb. Transfer to a 50-mL volumetric flask, add 15 to 16 mL alcoholic H_2SO_4 solution, swirl, add 0.50 mL (10 drops) dilute stannous chloride reagent II, swirl, and dilute to the mark with alcoholic H_2SO_4 . Mix thoroughly. After 10 min, but before 30 min, read against the blank at 625 nm. Prepare blank by carrying 40 mL distilled water through the same procedure used for the sample. Read phosphate concentration from a calibration curve prepared by taking known phosphate standards through the same procedure used for samples.

5. Calculation

Calculate as follows:

a. Direct procedure:

$$\text{mg P/L} = \frac{\text{mg P (in approximately 104.5 mL final volume)} \times 1000}{\text{mL sample}}$$

b. Extraction procedure:

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

6. Precision and Bias

See Table 4500-P:I.

4500-P E. Ascorbic Acid Method

1. General Discussion

a. *Principle*: Ammonium molybdate and antimony potassium tartrate react in acid medium with orthophosphate to form a heteropoly acid—phosphomolybdic acid—that is reduced to intensely colored molybdenum blue by ascorbic acid.

b. *Interference*: Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate.

Concentrations as low as 0.1 mg As/L interfere with the phosphate determination. Hexavalent chromium and NO_2^- interfere to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L. Sulfide (Na_2S) and silicate do not interfere at concentrations of 1.0 and 10 mg/L.

c. *Minimum detectable concentration*: Approximately 10 μg P/L. P ranges are as follows:

Approximate P Range mg/L	Light Path cm
0.30–2.0	0.5
0.15–1.30	1.0
0.01–0.25	5.0

2. Apparatus

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

1) *Spectrophotometer*, with infrared phototube for use at 880 nm, providing a light path of 2.5 cm or longer.

2) *Filter photometer*, equipped with a red color filter and a light path of 0.5 cm or longer.

b. *Acid-washed glassware:* See Section 4500-P.C.2b.

3. Reagents

a. *Sulfuric acid, H₂SO₄, 5N:* Dilute 70 mL conc H₂SO₄ to 500 mL with distilled water.

b. *Antimony potassium tartrate solution:* Dissolve 1.3715 g K(SbO)C₄H₄O₆ · ½H₂O in 400 mL distilled water in a 500-mL volumetric flask and dilute to volume. Store in a glass-stoppered bottle.

c. *Ammonium molybdate solution:* Dissolve 20 g (NH₄)₆Mo₇O₂₄ · 4H₂O in 500 mL distilled water. Store in a glass-stoppered bottle.

d. *Ascorbic acid, 0.1M:* Dissolve 1.76 g ascorbic acid in 100 mL distilled water. The solution is stable for about 1 week at 4°C.

e. *Combined reagent:* Mix the above reagents in the following proportions for 100 mL of the combined reagent: 50 mL 5N H₂SO₄, 5 mL antimony potassium tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. *Mix after addition of each reagent.* Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 h.

f. *Stock phosphate solution:* See Section 4500-P.C.3e.

g. *Standard phosphate solution:* Dilute 50.0 mL stock phosphate solution to 1000 mL with distilled water; 1.00 mL = 2.50 µg P.

4. Procedure

a. *Treatment of sample:* Pipet 50.0 mL sample into a clean, dry test tube or 125-mL erlenmeyer flask. Add 0.05 mL (1 drop) phenolphthalein indicator. If a red color develops add 5N H₂SO₄ solution dropwise to just discharge the color. Add 8.0 mL combined reagent and mix thoroughly. After at least 10 min but no more than 30 min, measure absorbance of each sample at 880 nm, using reagent blank as the reference solution.

b. *Correction for turbidity or interfering color:* Natural color of water generally does not interfere at the high wavelength used. For highly colored or turbid waters, prepare a blank by adding all reagents except ascorbic acid and antimony potassium tartrate to the sample. Subtract blank absorbance from absorbance of each sample.

c. *Preparation of calibration curve:* Prepare individual calibration curves from a series of six standards within the phosphate ranges indicated in ¶ 1c above. Use a distilled water blank with the combined reagent to make photometric readings for the calibration curve. Plot absorbance vs. phosphate concentration to give a straight line passing through the origin. Test at least one phosphate standard with each set of samples.

5. Calculation

$$\text{mg P/L} = \frac{\text{mg P (in approximately 58 mL)} \times 1000}{\text{final volume} \times 1000} \times \text{mL sample}$$

6. Precision and Bias

The precision and bias values given in Table 4500-P:I are for a single-solution procedure given in the 13th edition. The present procedure differs in reagent-to-sample ratios, no addition of solvent, and acidity conditions. It is superior in precision and bias to the previous technique in the analysis of both distilled water and river water at the 228-µg P/L level (Table 4500-P:II).

7. References

- EDWARDS, G.P., A.H. MOLOF & R.W. SCHNEEMAN. 1965. Determination of orthophosphate in fresh and saline waters. *J. Amer. Water Works Assoc.* 57:917.

TABLE 4500-P:II. COMPARISON OF PRECISION AND BIAS OF ASCORBIC ACID METHODS

Ascorbic Acid Method	Phosphorus Concentration, Dissolved Orthophosphate µg/L	No. of Laboratories	Relative Standard Deviation %		Relative Error %	
			Distilled Water	River Water	Distilled Water	River Water
13th Edition ¹	228	8	3.87	2.17	4.01	2.08
Current method ²	228	8	3.03	1.75	2.38	1.39

2. MURPHY, J. & J. RILEY. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27:31.

8. Bibliography

- SLETTEN, O. & C.M. BACH. 1961. Modified stannous chloride reagent for orthophosphate determination. *J. Amer. Water Works Assoc.* 53: 1031.

4500-P F. Automated Ascorbic Acid Reduction Method

1. General Discussion

a. Principle: Ammonium molybdate and antimony potassium tartrate react with orthophosphate in an acid medium to form an antimony-phosphomolybdate complex, which, on reduction with ascorbic acid, yields an intense blue color suitable for photometric measurement.

b. Interferences: As much as 50 mg Fe³⁺/L, 10 mg Cu/L, and 10 mg SiO₂/L can be tolerated. High silica concentrations cause positive interference.

In terms of phosphorus, the results are high by 0.005, 0.015, and 0.025 mg/L for silica concentrations of 20, 50, and 100 mg/L, respectively. Salt concentrations up to 20% (w/v) cause an error of less than 1%. Arsenate (AsO₄³⁻) is a positive interference.

Eliminate interference from NO₂⁻ and S²⁻ by adding an excess of bromine water or a saturated potassium permanganate (KMnO₄) solution. Remove interfering turbidity by filtration before analysis. Filter samples for total or total hydrolyzable phosphorus only after digestion. Sample color that absorbs in the photometric range used for analysis also will interfere. See also Section 4500-P.E.1b.

c. Application: Orthophosphate can be determined in potable, surface, and saline waters as well as domestic and industrial

- STRICKLAND, J.D.H. & T.R. PARSONS. 1965. *A Manual of Sea Water Analysis*, 2nd ed. Fisheries Research Board of Canada, Ottawa.

wastewaters over a range of 0.001 to 10.0 mg P/L when photometric measurements are made at 650 to 660 or 880 nm in a 15-mm or 50-mm tubular flow cell. Determine higher concentrations by diluting sample. Although the automated test is designed for orthophosphate only, other phosphorus compounds can be converted to this reactive form by various sample pretreatments described in Section 4500-P.B.1, 2, and 5.

2. Apparatus

a. Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-P:2. A flow cell of 15 or 50 mm and a filter of 650 to 660 or 880 nm may be used.

b. Hot plate or autoclave.

c. Acid-washed glassware: See Section 4500-P.C.2b.

3. Reagents

a. Antimony potassium tartrate solution: Dissolve 0.3 g K(SbO)C₄H₄O₆ · ½H₂O in approximately 50 mL distilled water and dilute to 100 mL. Store at 4°C in a dark, glass-stoppered bottle.

b. Ammonium molybdate solution: Dissolve 4 g (NH₄)₆Mo₇O₂₄ · 4H₂O in 100 mL distilled water. Store in a plastic bottle at 4°C.

c. Ascorbic acid solution: See Section 4500-P.E.3d.

d. Combined reagent: See Section 4500-P.E.3e.

e. Dilute sulfuric acid solution: Slowly add 140 mL conc H₂SO₄ to 600 mL distilled water. When cool, dilute to 1 L.

f. Ammonium persulfate, (NH₄)₂S₂O₈, crystalline.

g. Phenolphthalein indicator aqueous solution.

h. Stock phosphate solution: Dissolve 439.3 mg anhydrous KH₂PO₄, dried for 1 h at 105°C, in distilled water and dilute to 1000 mL; 1.00 mL = 100 µg P.

i. Intermediate phosphate solution: Dilute 100.0 mL stock phosphate solution to 1000 mL with distilled water; 1.00 mL = 10.0 µg P.

j. Standard phosphate solutions: Prepare a suitable series of standards by diluting appropriate volumes of intermediate phosphate solution.

4. Procedure

Set up manifold as shown in Figure 4500-P:2 and follow the general procedure described by the manufacturer.

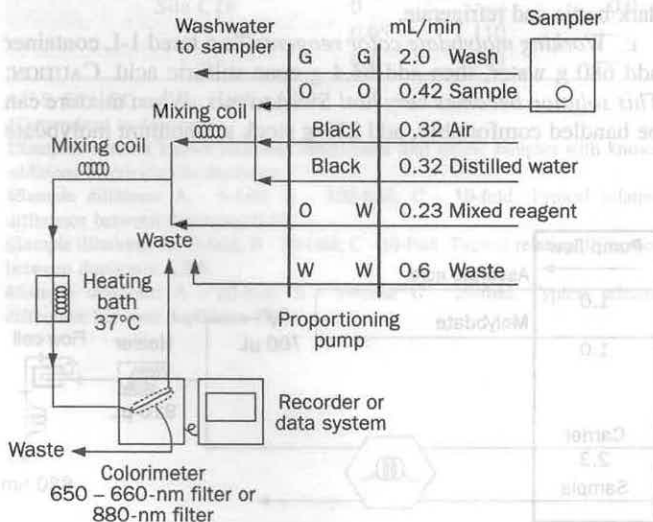


Figure 4500-P:2. Phosphate manifold for automated analytical system.

Add 0.05 mL (1 drop) phenolphthalein indicator solution to approximately 50 mL sample. If a red color develops, add H_2SO_4 (¶ 3e) dropwise to just discharge the color.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against P concentration in standards. Compute sample P concentration by comparing sample response with standard curve.

6. Precision and Bias

Six samples were analyzed in a single laboratory in septuplicate. At an average PO_4^{3-} concentration of 0.340 mg/L, the average deviation was 0.015 mg/L. The coefficient of variation

was 6.2%. In two samples with added PO_4^{3-} , recoveries were 89 and 96%.

7. Bibliography

- HENRIKSEN, A. 1966. An automatic method for determining orthophosphate in sewage and highly polluted waters. *Analyst* 91:652.
- LOBRING, L.B. & R.L. BOOTH. 1973. Evaluation of the AutoAnalyzer II; A progress report. In *Advances in Automated Analysis: 1972 Technicon International Congress*. Vol. 8, p. 7. Mediad, Inc., Tarrytown, N.Y.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020, National Environmental Research Center, Cincinnati, Ohio.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. MDQARL Method Study 4, Automated Methods. National Environmental Research Center, Cincinnati, Ohio.

4500-P G. Flow Injection Analysis for Orthophosphate

1. General Discussion

a. Principle: The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex that absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

Also see Sections 4500-P.A, B, and F, and Section 4130, Flow Injection Analysis (FIA).

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

Silica forms a pale blue complex that also absorbs at 880 nm. This interference is generally insignificant because a silica concentration of approximately 30 mg/L would be required to produce a 0.005 mg P/L positive error in orthophosphate.

Concentrations of ferric iron greater than 50 mg/L cause a negative error due to competition with the complex for the reducing agent ascorbic acid. Treat samples high in iron with sodium bisulfite to eliminate this interference, as well as the interference due to arsenates.

Glassware contamination is a problem in low-level phosphorus determinations. Wash glassware with hot dilute HCl and rinse with reagent water. Commercial detergents are rarely needed but, if they are used, use special phosphate-free preparations.

Also see Section 4500-P.F.

2. Apparatus

Flow injection analysis equipment consisting of:

- FIA injection valve* with sample loop or equivalent.
- Multichannel proportioning pump.*
- FIA manifold* (Figure 4500-P:3) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-P:3. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

d. Absorbance detector, 880 nm, 10-nm bandpass.

e. Injection valve control and data acquisition system.

3. Reagents

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

a. Stock ammonium molybdate solution: To a tared 1-L container add 40.0 g ammonium molybdate tetrahydrate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$ and 983 g water. Mix with a magnetic stirrer for at least 4 h. Store in plastic and refrigerate.

b. Stock antimony potassium tartrate solution: To a 1-L dark, tared container add 3.0 g antimony potassium tartrate (potassium antimonyl tartrate hemihydrate), $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O}$, and 995 g water. Mix with a magnetic stirrer until dissolved. Store in a dark bottle and refrigerate.

c. Working molybdate color reagent: To a tared 1-L container add 680 g water, then add 64.4 g conc sulfuric acid. CAUTION: This solution becomes very hot! Swirl to mix. When mixture can be handled comfortably, add 213 g stock ammonium molybdate

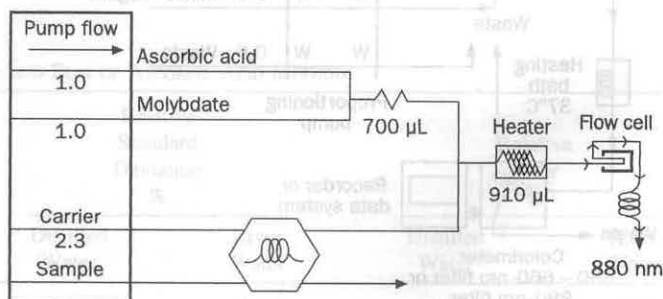


Figure 4500-P:3. FIA orthophosphate manifold.

TABLE 4500-P:III. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg P/L	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	101	—
	Blank†	0.05	96	—
		0.1	95	—
	Site A‡§	0	—	0.7
		0.05	98	—
		0.1	101	—
	Site B‡§	0	—	5
		0.05	75	—
		0.1	91	—
	Site C‡§	0	—	0.6
		0.05	88	—
		0.1	97	—
Wastewater treatment plant effluent	Reference sample*	—	100	—
	Blank†	0.05	96	—
		0.1	96	—
	Site A‡	0	—	0.7
		0.05	94	—
		0.1	96	—
	Site B‡	0	—	0.3
		0.05	94	—
		0.1	99	—
	Site C‡	0	—	0.5
		0.05	109	—
		0.1	107	—
Landfill leachate	Reference sample*	—	98	—
	Blank†	0.05	94	—
		0.1	95	—
	Site A‡#	0	—	0.9
		0.05	105	—
		0.1	106	—
	Site B‡#	0	—	6.7
		0.05	89	—
		0.1	94	—
	Site C‡#	0	—	0.9
		0.05	110	—
		0.1	109	—

* U.S. EPA QC sample, 0.109 mg P/L.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate.

§ Sample dilutions: A - 5-fold; B - 100-fold; C - 10-fold. Typical relative difference between duplicates 0.5%.

|| Sample dilutions: A - 5-fold; B - 20-fold; C - 10-fold. Typical relative difference between duplicates 0.3%.

Sample dilutions: A - 20-fold; B - 10-fold; C - 20-fold. Typical relative difference between duplicates 1%.

solution (¶ 3a) and 72.0 g stock antimony potassium tartrate solution (¶ 3b). Shake and degas with helium.

d. *Ascorbic acid solution*: To a tared 1-L container, add 60.0 g granular ascorbic acid and 975 g water. Stir or shake until dissolved. Degas this reagent with helium, then add 1.0 g dodecyl sulfate, $\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$, stirring gently to mix. Prepare fresh weekly.

e. *Stock orthophosphate standard*, 25.00 mg P/L: In a 1-L volumetric flask dissolve 0.1099 g primary standard grade anhydrous potassium phosphate monobasic (KH_2PO_4) that has been dried for 1 h at 105°C in about 800 mL water. Dilute to mark with water and invert to mix.

f. *Standard orthophosphate solutions*: Prepare orthophosphate standards in desired concentration range, using stock standard (¶ 3e) and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-P:3 and follow method supplied by manufacturer or laboratory standard operating procedure. Use quality control protocols outlined in Section 4020.

5. Calculations

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

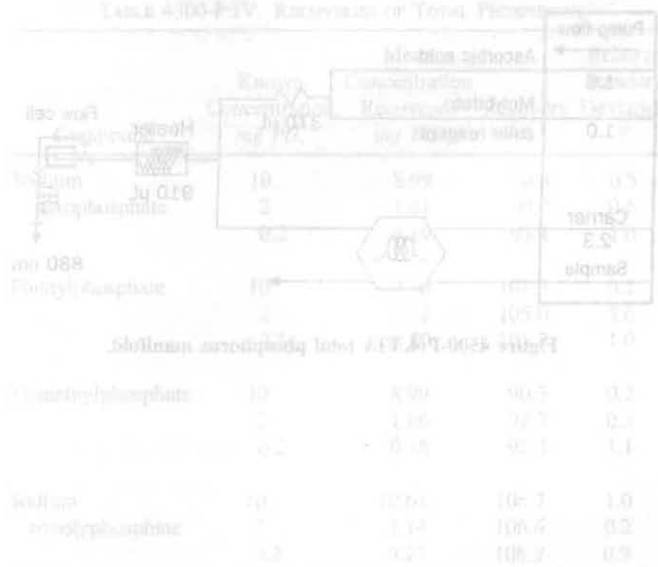
6. Precision and Bias

a. *Recovery and relative standard deviation*: Table 4500-P:III gives results of single-laboratory studies.

b. *MDL*: A 700- μL sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 5.0- μg P/L standard. These gave a mean of 5.26 μg P/L, a standard deviation of 0.264 μg P/L, and MDL of 0.67 μg P/L.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430.



4500-P H. Manual Digestion and Flow Injection Analysis for Total Phosphorus

1. General Discussion

a. Principle: Polyphosphates are converted to the orthophosphate form by a sulfuric acid digestion and organic phosphorus is converted to orthophosphate by a persulfate digestion. When the resulting solution is injected onto the manifold, the orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex that absorbs light at 880 nm. The absorbance is proportional to the concentration of total phosphorus in the sample.

See Section 4500-P.A for a discussion of the various forms of phosphorus found in waters and wastewaters, Section 4500-P.B for a discussion of sample preparation and digestion, and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: See 4500-P.G.1b.

2. Apparatus

Digestion and flow injection analysis equipment consisting of:

a. Hotplate or autoclave.

b. FIA injection valve with sample loop or equivalent.

c. Multichannel proportioning pump.

d. FIA manifold (Figure 4500-P:4) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-P:4. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He

through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

Prepare reagents listed in 4500-P.G.3a, b, d, e, and f, and in addition:

a. Sulfuric acid carrier, H_2SO_4 , 0.13M: To a tared 1-L container add 993 g water, then add 13.3 g conc H_2SO_4 . Shake carefully to mix. Degas daily. Prepare fresh weekly.

b. Molybdate color reagent: To a tared 1-L container add 694 g water, then add 38.4 g conc H_2SO_4 . CAUTION: *The solution becomes very hot!* Swirl to mix. When mixture can be handled comfortably, add 72.0 g stock antimony potassium tartrate (§ G.3b) and 213 g stock ammonium molybdate (§ G.3a). Shake to mix, and degas.

4. Procedure

See Section 4500-P.B.4 or 5 for digestion procedures. Carry both standards and samples through the digestion. The resulting solutions should be about 0.13M in sulfuric acid to match the concentration of the carrier. If the solutions differ more than 10% from this concentration, adjust concentration of carrier's sulfuric acid to match that of digested samples.

Set up a manifold equivalent to that in Figure 4500-P:4 and analyze digested samples and standards by following method supplied by manufacturer or laboratory's standard operating procedure. Use quality control protocols outlined in Section 4020.

5. Calculations

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

6. Precision and Bias

a. MDL: A 780- μL sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 3.5- μg P/L standard. These gave a mean of 3.53 μg P/L, a standard deviation of 0.82 μg P/L, and MDL of 2.0 μg P/L. The MDL is limited mainly by the precision of the digestion.

b. Precision study: Ten injections of a 100.0- μg P/L standard gave a percent relative standard deviation of 0.3%.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430.

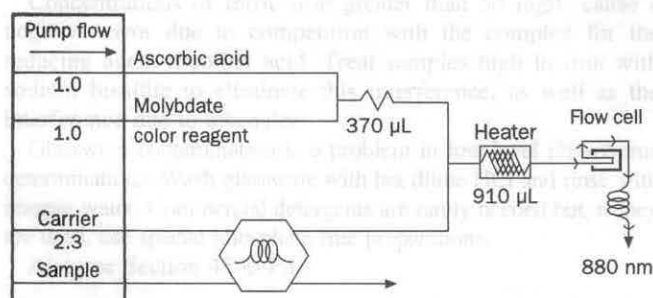


Figure 4500-P:4. FIA total phosphorus manifold.

4500-P I. In-line UV/Persulfate Digestion and Flow Injection Analysis for Total Phosphorus

1. General Discussion

a. Principle: Organic phosphorus is converted in-line to orthophosphate by heat, ultraviolet radiation, and persulfate digestion. At the same time, inorganic polyphosphates are converted to orthophosphate by in-line sulfuric acid digestion. The digestion processes occur before sample injection. A portion of the digested sample is then injected and its orthophosphate concentration determined by the flow injection method described in Section 4500-P.H.1.

See Section 4500-P.A for a discussion of the various forms of phosphorus found in waters and wastewaters, Section 4500-P.B for a discussion of sample preparation and digestion, and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: See 4500-P.G.1b.

2. Apparatus

Flow injection analysis equipment consisting of:

- FIA injection valve** with sample loop or equivalent.
- Multichannel proportioning pump.**
- FIA manifold** (Figure 4500-P:5) with tubing heater, in-line ultraviolet digestion fluidics including a debubbler consisting of a gas-permeable TFE membrane and its holder, and flow cell. Relative flow rates only are shown in Figure 4500-P:5. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE. The block marked "UV" should consist of TFE tubing irradiated by a mercury discharge ultraviolet lamp emitting radiation at 254 nm.
- Absorbance detector**, 880 nm, 10-nm bandpass.
- Injection valve control and data acquisition system.**

3. Reagents

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

a. Digestion reagent 1: To a tared 1-L container, add 893.5 g water, then slowly add 196.0 g sulfuric acid, H_2SO_4 . CAUTION: This solution becomes very hot! Prepare weekly. Degas before using.

b. Digestion reagent 2: To a tared 1-L container, add 1000 g water, then add 26 g potassium persulfate, $\text{K}_2\text{S}_2\text{O}_8$. Mix with a magnetic stirrer until dissolved. Prepare weekly. Degas before using.

c. Sulfuric acid carrier, 0.71M: To a tared 1-L container, slowly add 70 g H_2SO_4 to 962 g water. Add 5 g sodium chloride, NaCl. Let cool, then degas with helium. Add 1.0 g sodium dodecyl sulfate. Invert to mix. Prepare weekly.

d. Stock ammonium molybdate: To a tared 1-L container add 40.0 g ammonium molybdate tetrahydrate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, and 983 g water. Mix with a magnetic stirrer for at least 4 h. The solution can be stored in plastic for up to 2 months if refrigerated.

e. Stock antimony potassium tartrate: To a 1-L dark, plastic, tared container add 3.0 g antimony potassium tartrate (potassium antimonyl tartrate trihydrate), $\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2 \cdot 3\text{H}_2\text{O}$, and 995 g water. Mix with a magnetic stirrer until dissolved. The solution can be stored in a dark plastic container for up to 2 months if refrigerated.

f. Molybdate color reagent: To a tared 1-L container add 715 g water, then 213 g stock ammonium molybdate (§ 3e) and 72.0 g stock antimony potassium tartrate (§ 3f). Add and dissolve 22.8 g sodium hydroxide, NaOH. Shake and degas with helium. Prepare weekly.

g. Ascorbic acid: To a tared 1-L container add 70.0 g ascorbic acid and 975 g water. Mix with a magnetic stirrer until dissolved. Degas with helium. Add 1.0 g sodium dodecyl sulfate. Mix with a magnetic stirrer. Prepare fresh every 2 d.

h. Stock orthophosphate standard, 1000 mg P/L: In a 1-L volumetric flask dissolve 4.396 g primary standard grade anhydrous potassium phosphate monobasic, KH_2PO_4 (dried for 1 h at

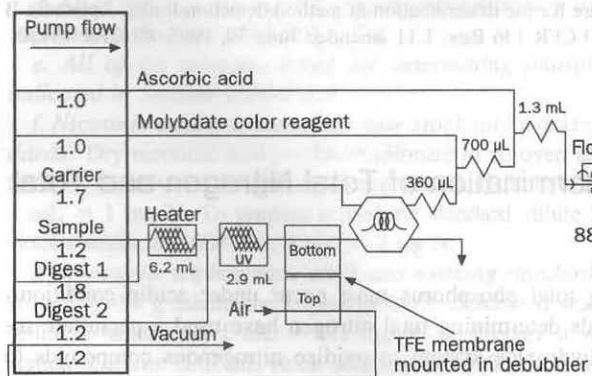


Figure 4500-P:5. FIA in-line total phosphorus manifold.

TABLE 4500-P:IV. RECOVERIES OF TOTAL PHOSPHORUS

Compound	Known Concentration mg P/L	Mean Concentration Recovered mg P/L	Relative Recovery %	Standard Deviation %
Sodium pyrophosphate	10	8.99	90.3	0.5
	2	1.81	90.2	0.6
	0.2	0.19	93.4	1.0
Phenylphosphate	10	10.10	101.5	0.2
	2	2.12	105.0	5.6
	0.2	0.20	101.3	1.0
Trimethylphosphate	10	8.99	90.3	0.2
	2	1.86	92.7	0.3
	0.2	0.18	95.3	1.1
Sodium tripolyphosphate	10	10.61	106.7	1.0
	2	2.14	106.6	0.2
	0.2	0.22	108.9	0.9

TABLE 4500-P:V. COMPARISON OF MANUAL AND IN-LINE TOTAL PHOSPHORUS METHODS

Samples	Concentration by Manual Persulfate Digestion Method mg P/L	Concentration by In-Line Digestion Method mg P/L	Relative Difference %
Influent (I2)	5.93	5.52	-6.9
Influent (I3)	5.03	4.50	-10.5
Influent (I5)	2.14	2.11	-1.4
Influent (I6)	1.88	1.71	-9.0
Effluent (E1)	3.42	2.87	-16.1
Effluent (E2)	3.62	3.55	-1.9
Effluent (E3)	3.26	3.34	+2.4
Effluent (E4)	8.36	8.16	-2.4
Effluent (E5)	0.65	0.71	+9.2
Effluent (E6)	0.74	0.81	+9.5
Phenylphosphate	1.95	1.91	-2.1
Trimethylphosphate	1.87	1.80	-3.7
Sodium pyrophosphate	1.90	1.73	-8.9
Sodium tripolyphosphate	1.84	1.73	-6.0

105°C), in about 800 mL water. Dilute to mark with water and invert to mix. Prepare monthly.

i. *Standard solutions:* Prepare orthophosphate standards in desired concentration range, using stock orthophosphate standards (¶ 3i), and diluting with water. If the samples are preserved with sulfuric acid, ensure that stock standard and diluted standards solutions are of the same concentration.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-P:5 and follow method supplied by manufacturer or laboratory's standard operating procedure. Use quality control procedures described in Section 4020.

5. Calculations

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

Verify digestion efficiency by determining tripolyphosphate and trimethylphosphate standards at regular intervals. In the concentration range of the method, the recovery of either of these compounds should be >95%.

4500-P J. Persulfate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorus

1. General Discussion

a. *Principle:* The oxidation of nitrogenous compounds for determining total nitrogen must occur in an alkaline medium. Conversely, the oxidation of phosphorus compounds for deter-

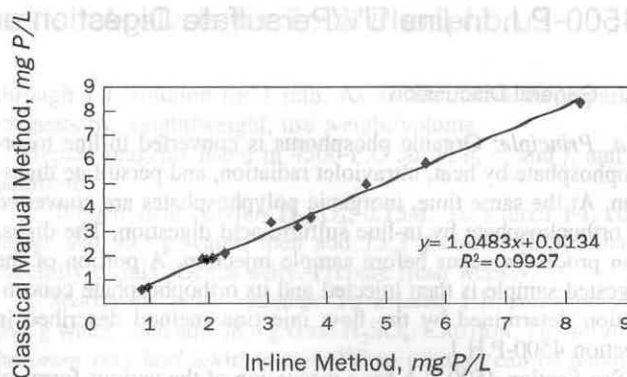


Figure 4500-P:6. Correlation between manual and in-line total phosphorus methods.

6. Precision and Bias

a. *MDL:* A 390-μL sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 0.10-mg P/L orthophosphate standard. These gave a mean of 0.10 mg P/L, a standard deviation of 0.003 mg P/L, and MDL of 0.007 mg P/L.

b. *Precision of recovery study:* Ten injections of a 10.0-mg P/L trimethylphosphate standard gave a mean percent recovery of 98% and a percent relative standard deviation of 0.8%.

c. *Recovery of total phosphorus:* Two organic and two inorganic complex phosphorus compounds were determined in triplicate at three concentrations. The results are shown in Table 4500-P:IV.

d. *Comparison of in-line digestion with manual digestion method:* Samples from a wastewater treatment plant influent and effluent and total phosphorus samples at 2.0 mg P/L were determined in duplicate with both manual persulfate digestion followed by the method in Section 4500-P:H and in-line digestion method. Table 4500-P:V gives the results of this comparison, and Figure 4500-P:6 shows the correlation between manual and in-line total phosphorus methods.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits, Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430.

mining total phosphorus must occur under acidic conditions. Methods determining total nitrogen have used a persulfate-sodium hydroxide system to oxidize nitrogenous compounds to nitrate. Accordingly, methods determining total phosphorus have used persulfate in an acidic medium.

During the initial stage of the digestion, sample pH is alkaline ($\text{pH} > 12$). In the final stage of the digestion, the sodium hydroxide is consumed, causing sample pH to become acidic ($\text{pH} < 2$). By means of this broad pH range, the method allows for the oxidation of both nitrogen and phosphorus compounds. The digested sample is analyzed for nitrate and orthophosphate, yielding total nitrogen and total phosphorus results.

b. Selection of nitrate/orthophosphate measurement methods: Using a dual-channel autoanalyzer that performs nitrate-nitrite by the cadmium reduction method and orthophosphate by the ascorbic acid reduction method, total nitrogen and total phosphorus can be measured simultaneously. Alternatively, other methods for orthophosphate and nitrate can be used.

2. Apparatus

Clean all glassware with HCl before use.

a. Autoclave, capable of achieving a temperature of 120°C for a minimum of 120 min.

b. Glass culture tubes, 13-mm-OD $\times$ 100-mm-long with autoclavable caps.

c. Autopipettor, capable of pipetting a 6.0-mL portion.

d. Repeating pipettor, capable of pipetting 1.25-mL portion.

e. Erlenmeyer flask, 3000-mL.

f. Aluminum foil.

g. Automated continuous-flow instrument system for nitrate and phosphate determination: The suggested analytical instruments are described in Sections 4500- NO_3^- .F and 4500-P.F.

3. Reagents

a. Deionized water, high-quality, free of phosphorus and nitrogen compounds. Prepare by ion-exchange or distillation methods as directed in 4500- NH_3 .B.3a and 4500- NO_3^- .B.3a.

b. Sodium hydroxide, 3N: Dissolve 120 g low-nitrogen NaOH in 800 mL deionized water in a 1000-mL volumetric flask. Cool and dilute to volume.

c. Oxidizing reagent: Dissolve 64 g low-nitrogen ($<0.001\%$ N) potassium persulfate, $\text{K}_2\text{S}_2\text{O}_8$, in 500 mL deionized water. Use low heat if necessary. Add 80 mL 3N NaOH, prepared from low-nitrogen sodium hydroxide, and dilute to 1000 mL. Store in a brown bottle at room temperature.

d. All of the reagents listed for determining nitrate + nitrite as indicated in Section 4500- NO_3^- .F.3.

e. All of the reagents listed for determining phosphate as indicated in Section 4500-P.F.3.

f. Nicotinic acid p-toluenesulfonate stock and working standards: Dry nicotinic acid p-toluenesulfonate in an oven at 105°C for 24 h. Dissolve 2.1084 g in deionized water and dilute to 100 mL; 1 mL = 1 mg N. To prepare a working standard, dilute 2.0 mL stock solution to 1000 mL; 1 mL = 2 μg N.

g. Adenosine triphosphate stock and working standards: Dissolve 0.6514 g adenosine triphosphate in deionized water and dilute to 1000 mL; 1 mL = 0.1 mg P. To prepare a working standard, dilute 20.0 mL stock solution to 1000 mL; 1 mL = 2 μg P. To prepare a low-range working standard, dilute 1.0 mL stock solution to 1000 mL; 1 mL = 0.1 μg P.

4. Procedure

a. Calibration curve: Prepare a minimum of five standards over the desired calibration ranges using a stock calibration standard containing both nitrate and orthophosphate. Treat standards in the same manner as samples. Include blanks in calibration curves.

b. Sample preparation: If necessary, dilute sample with deionized water so that expected nitrogen and phosphorus concentrations fall within the range of the calibration standards. Samples preserved with acid cannot be analyzed by this digestion method.

c. Digestion check standards: Analyze quality-control standards containing organic nitrogen and phosphorus on each analytical run (see ¶s 3f and g for suggested standards and preparation procedures). These standards provide reference checks on the calibration and test the efficiency of the digestion.

d. Digestion: Pipet 6.0 mL of sample or standard into the culture tubes. Add 1.25 mL oxidizing reagent to each tube using a repeating pipettor. Cover the tubes with loose-fitting plastic caps. Prepare an autoanalyzer wash water in an erlenmeyer flask by adding oxidation reagent to deionized water in the same proportion as was added to the samples. Cover flask with foil. Autoclave samples and wash water for 55 min at 120°C . Cool to room temperature. Add 0.05 mL of 3N NaOH to each tube before proceeding to nitrate + nitrite and phosphate analyses. Shake to mix. Add same proportion of 3N NaOH to digested wash water.

e. Final nitrate + nitrite measurement: Use the automated cadmium reduction method for the determination of nitrate-nitrite after digestion. See Section 4500- NO_3^- .F. Other nitrate analysis methods may be applicable; however, precision and bias data do not exist for these methods on this matrix at this time.

f. Final phosphate measurement: Use the automated ascorbic acid reduction method for the determination of phosphate after digestion. See Section 4500-P.F. Other phosphate analysis methods may be applicable; however, precision and bias data do not exist for these methods at this time.

5. Calculation

Prepare nitrogen and phosphorus standard curves by plotting the instrument response of standards against standard concentrations. Compute the nitrogen and phosphorus concentrations by comparing the sample response with the standard curve. Where necessary, multiply sample concentration by the appropriate dilution factor to determine final concentration.

6. Quality Control

Use protocols specified in Section 4020 to verify performance. These include daily use of reagent blanks, laboratory-fortified blanks, and known additions. Regulatory analysis may require additional quality control procedures.

7. Precision and Bias

a. Total nitrogen:

1) Nitrogen digestion check standards — Four different organic nitrogen standards (2 mg N/L) were analyzed by a single

laboratory on three separate analytical runs yielding the following results:

Nitrogen Compound	Mean		Recovery		Standard		Standard	
	mg	N/L	of N	%	Deviation	mg N/L	Deviation	%
Urea	2.03		102		0.021		1.0	
Ammonium <i>p</i> -toluenesulfonate	1.93		97		0.037		1.9	
Glycine <i>p</i> -toluenesulfonate	1.94		97		0.034		1.8	
Nicotinic acid <i>p</i> -toluenesulfonate	1.86		93		0.044		2.4	

2) Mixed ammonia-nitrate solution — A mixed ammonia-nitrate sample (0.55 mg N/L) was analyzed nine times, yielding the following results:

Nitrogen Compounds	Mean		Recovery		Standard		Standard	
	mg/L		of N	%	Deviation	mg/L	Deviation	%
Ammonium sulfate & potassium nitrate	0.557		101		0.012		2.1	

3) Samples — A single laboratory analyzed five samples in quadruplicate on three separate occasions. Samples analyzed for total nitrogen included surface water and diluted wastewater samples. At an average concentration of 15.0 mg N/L, the average standard deviation was 0.143 mg/L.

b. Phosphorus:

1) Adenosine triphosphate solutions — Two concentrations (2 mg P/L and 100 µg P/L) adenosine triphosphate were analyzed by a single laboratory on two separate analytical runs yielding the following results:

Phosphorus Compound	Mean		Recovery	
	mg	P/L	of P	%
Adenosine triphosphate, 2 mg P/L	2.05		103	
Adenosine triphosphate, 0.100 µg/L	0.103		103	

4500-KMnO₄ POTASSIUM PERMANGANATE*

4500-KMnO₄ A. Introduction

1. Occurrence and Significance

Potassium permanganate, KMnO₄, has been widely used in both potable and nonpotable water sources. It has been applied to water supplies to remove taste, odor, color, iron, manganese, and sulfides and to control trihalomethanes (THMs) and zebra mus-

2) Samples — A single laboratory analyzed five samples in quadruplicate on three separate occasions. Samples were analyzed for total phosphorus over two different calibration ranges. Surface water and diluted wastewater samples were analyzed utilizing a low calibration range (0 to 250 µg P/L, method detection level (MDL) = 2 µg P/L). At an average concentration of 1670 µg P/L, the average standard deviation was 29.4 µg/L. Surface water and undiluted wastewater samples were analyzed using a high calibration range (0 to 6 mg P/L, MDL = 0.05 mg P/L). At an average concentration of 1.97 mg P/L, the average standard deviation was 0.028 mg/L.

8. Bibliography

- KOROLEFF, F. 1972. Determination of total nitrogen in natural waters by means of persulfate oxidation. In S.R. Carlberg, ed. New Baltic Montreal. ICES Cooperative Res. Rep., Ser. A, No. 29, p. 73.
- KOROLEFF, F. 1977. Simultaneous persulfate oxidation of phosphorus and nitrogen compounds in water. In K. Grasshoff, Report of the Baltic Intercalibration Workshop. Annex. Interim Commission for the Protection of the Environment of the Baltic Sea, p. 52.
- NYDAHL, F. 1978. On the peroxodisulphate oxidation of total nitrogen in waters to nitrate. *Water Res.* 12: 1123.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Method 365.1 in Methods for Chemical Analysis of Water and Wastes. EPA 600/4-79/020, U.S. Environmental Protection Agency, Cincinnati, Ohio.
- VALDERRAMA, J.C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Marine Chemistry* 10:109.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. 1987. Handbook of Methods for Acid Deposition Studies: Laboratory Analyses for Surface Water Chemistry. EPA 600/4-87/026, U.S. Environmental Protection Agency, Washington, D.C., p. 1811.
- AMEEL, J.J., R.P. AXLER & C.J. OWEN. 1993. Persulfate Digestion for Determination of Total Nitrogen and Phosphorus in Low Nutrient Waters. Center for Water and the Environmental, Natural Resources Research Inst. *Amer. Environ. Lab.* Oct 1993:2.

sels. Municipal and industrial waste treatment facilities use potassium permanganate for odor control, toxic pollutant destruction, bio-augmentation, and grease removal.

Potassium permanganate is produced as a dark black-purple crystalline material. It has a solubility in water of 60 g/L at 20°C. The color of potassium permanganate solutions ranges from faint pink (dilute) to deep purple (concentrated). Under normal conditions the solid material is stable. However, as with all oxidizing agents, avoid contact with acids, peroxides, and all combustible organic or readily oxidizable materials.

* Approved by Standard Methods Committee, 1997.

Joint Task Group: 20th Edition—Philip A. Vella (chair), Bernard Bubnis, Richard E. DeBlois, Gilbert Gordon.

2. Sampling and Storage

If kept dry, solid potassium permanganate may be stored indefinitely. Potassium permanganate solutions, made in oxidant-demand-free water, are stable for long periods of time if kept in an amber bottle out of direct sunlight.¹ For samples obtained from other water sources (those having an oxidant

demand), analyze potassium permanganate on site, as soon as possible after sample collection.

3. Reference

1. DAY, R.A. & A.L. UNDERWOOD. 1986. Quantitative Analysis, 5th ed. Prentice-Hall, Englewood Cliffs, N.J.

4500-KMnO₄ B. Spectrophotometric Method

1. General Discussion

a. Principle: This method is a direct determination of aqueous potassium permanganate solutions. The concentration (pink to violet color) is directly proportional to the absorbance as measured at 525 nm. The concentrations are best determined from the light-absorbing characteristics of a filtered sample by means of a spectrophotometer.

b. Application: This method is applicable to ground and surface waters.

c. Interference: Turbidity and manganese dioxide interfere. Remove by the filtration methods described below. Other color-producing compounds also interfere. Compensate for color by using an untreated sample to zero the spectrophotometer.

d. Minimum detectable concentration: As a guide, use the following light paths for the indicated KMnO₄ concentrations measured at 525 nm. Sample dilution may be required, depending on the initial concentration.

Cell Path Length cm	Range mg KMnO ₄ /L	Expected Absorbance for 1-mg/L KMnO ₄ Solution
1	0.5–100	0.016
2.5	0.2–25	0.039
5	0.1–20	0.078

2. Apparatus

a. Photometric equipment: Use one of the following:

1) *Spectrophotometer*, for use at a wavelength of 525 nm and providing a light path of 1 cm or longer.

2) *Filter photometer*, equipped with a filter having a maximum transmittance at or near 525 nm and providing a light path of 1 cm or longer.

*b. Filtration apparatus.**

c. Filters: Use 0.22-μm filters that do not react with KMnO₄ (or smallest glass fiber filters available).

3. Reagents

Use potassium-permanganate-demand-free water (¶ e below) for all reagent preparation and dilutions.

a. Calcium chloride solution, CaCl₂, 1M: Dissolve 111 g CaCl₂ in water and dilute to 1 L.

* Millipore or equivalent.

b. Sulfuric acid, H₂SO₄, 20%: Add 20 g conc H₂SO₄ slowly, with stirring, to 80 mL water. After cooling, adjust final volume to 100 mL.

c. Sodium oxalate, Na₂C₂O₄, primary standard.

d. Sodium thiosulfate solution, Na₂S₂O₃ · 5H₂O, 0.019M: Dissolve 0.471 g Na₂S₂O₃ · 5H₂O in water and dilute to 100 mL.

e. Potassium-permanganate-demand-free water: Add one small crystal KMnO₄ to 1 L distilled or deionized water; let stand. After 1 to 2 d, a residual pink color should be present; if not, discard and obtain better-quality water or increase permanganate added. Redistill in an all-borosilicate-glass apparatus and discard initial 50 mL distillate. Collect distillate fraction that is free of permanganate: a red color with DPD reagent (see Section 4500-Cl.F.2b) indicates presence of permanganate.

f. Potassium permanganate standard, KMnO₄, 0.006M: Dissolve 1.000 g KMnO₄ in water and dilute to 1000 mL. Standardize as follows: Accurately weigh about 0.1 g primary standard grade sodium oxalate and dissolve it in 150 mL water in a 250-mL erlenmeyer flask. Add 20 mL 20% H₂SO₄ and heat to 70 to 80°C. Titrate the warm oxalate solution with the potassium permanganate standard until a pink coloration persists (60 s). Calculate the potassium permanganate concentration:

$$\text{mg KMnO}_4/\text{L} = \frac{W \times 1000}{2.1197 \times V}$$

where:

W = weight of sodium oxalate, mg, and
V = mL KMnO₄ titrant.

4. Procedure

a. Calibration of photometric equipment: Prepare calibration curve by diluting standardized potassium permanganate solution. Make dilutions appropriate for the cell path length and range desired. Plot absorbance (y axis) versus KMnO₄ concentration (x axis). Calculate a best-fit line through the points. Preferably perform a calibration check with a known KMnO₄ standard before any analysis to ensure that equipment is in proper working order.

b. Potassium permanganate analysis: Check zero on spectrophotometer at 525 nm with deionized water. If the water is soft (i.e., <40 mg/L hardness as CaCO₃), add 1 mL CaCl₂ solution/L sample (111 mg/L as CaCl₂) to aid in removal of any colloidal manganese dioxide and suspended solids. Pass 50 mL sample through a 0.22-μm filter. Rinse spectrophotometer cell with two or three portions of filtrate. Fill cell and check that no air bubbles

are present in the solution or on the sides of the cell. Measure absorbance at 525 nm (Reading A). For best results, minimize time between filtration and reading absorbance. To 100 mL sample, add 0.1 mL CaCl_2 solution. Add 0.1 mL sodium thio-sulfate solution per 1 mg/L KMnO_4 (based on Reading A). Pass through a 0.22- μm filter and measure absorbance (Reading B).

5. Calculation

$$\text{Correct absorbance} = A - B$$

where:

A = absorbance of sample, and

B = absorbance of blank.

Compare corrected absorbance value obtained with the calibration curve and report the corresponding value as milligrams potassium permanganate per liter.

6. Precision and Bias

Based on the results obtained by eight analysts in a single laboratory, the overall precision (pooled standard deviation) was determined to be 0.035 mg/L for a 1-cm cell. The mean recovery for 12 measurements at 4 initial concentrations was 98%. The method detection level (MDL), as determined using Method 1030, was 0.083 mg KMnO_4/L .

4500-SiO₂ SILICA*

4500-SiO₂ A. Introduction

1. Occurrence and Significance

Silicon does not occur free in nature, but rather as free silica (SiO_2) in coarsely crystalline (quartz, rock crystal, amethyst, etc.) and microcrystalline (flint, chert, jasper, etc.) varieties of quartz, the major component of sand and sandstone. Silicon is found in combination with other elements in silicates, represented by feldspar, hornblende, mica, asbestos, and other clay minerals. Silicates also occur in rocks such as granite, basalt, and shale. Silicon therefore is usually reported as silica (SiO_2) when rocks, sediments, soils, and water are analyzed. The average abundance of silica in different rock types is 7 to 80%, in typical soils 50 to 80%, and in surface and groundwater 14 mg/L.

The common aqueous forms of silica are H_4SiO_4 and H_3SiO_4^- . In the presence of magnesium, it can form scale deposits in boilers and in steam turbines. It is considered a nonessential trace element for most plants, but essential for most animals. Chronic exposure to silica dust can be toxic. There is no U.S. EPA drinking water standard MCL for silica.

A more complete discussion of the occurrence and chemistry of silica in natural waters is available.¹

2. Selection of Method

Perform analyses by the electrothermal atomic absorption method (3113B) or one of the colorimetric methods (C, D, E, or F), depending on the fraction to be measured. The inductively coupled plasma mass spectrometric method (3125) or the inductively coupled plasma method (3120) also may be applied successfully in most cases (with lower detection limits), even though silica is not specifically listed as an analyte in the method.

Methods 3120 and 3125 determine total silica. Methods C, D, E, and F determine molybdate-reactive silica. As noted in Sec-

tion 4500-SiO₂.C.4, it is possible to convert other forms of silica to the molybdate-reactive form for determination by these methods. Method 3111D determines more than one form of silica. It will determine all dissolved silica and some colloiddally dispersed silica. The determination of silica present in micrometer and submicrometer particles will depend on the size distribution, composition, and structure of the particles; thus Method 3111D cannot be said to determine total silica.

Method C is recommended for relatively pure waters containing from 0.4 to 25 mg SiO_2/L . As with most colorimetric methods, the range can be extended, if necessary, by diluting, by concentrating, or by varying the light path. Interferences due to tannin, color, and turbidity are more severe with this method than with Method D. Moreover, the yellow color produced by Method C has a limited stability and attention to timing is necessary. When applicable, however, it offers greater speed and simplicity than Method D because one reagent fewer is used; one timing step is eliminated; and many natural waters can be analyzed without dilution, which is not often the case with Method D. Method D is recommended for the low range, from 0.04 to 2 mg SiO_2/L . This range also can be extended if necessary. Such extension may be desirable if interference is expected from tannin, color, or turbidity. A combination of factors renders Methods D, E, and F less susceptible than Method C to those interferences; also, the blue color in Methods D, E, and F is more stable than the yellow color in Method C. However, many samples will require dilution because of the high sensitivity of the method. Permanent artificial color standards are not available for the blue color developed in Method D.

The yellow color produced by Method C and the blue color produced by Methods D, E, and F are affected by high concentrations of salts. With seawater the yellow color intensity is decreased by 20 to 35% and the blue color intensity is increased by 10 to 15%. When waters of high ionic strength are analyzed by these methods, use silica standards of approximately the same ionic strengths.²

Method E or F may be used where large numbers of samples are analyzed regularly. Method 3111D is recommended for broad-range use. Although Method 3111D is usable from 1 to

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300 mg SiO₂/L, optimal results are obtained from about 20 to 300 mg/L. The range can be extended upward by dilution if necessary. This method is rapid and does not require any timing step.

The inductively coupled plasma method (3120) also may be used in analyses for silica.

3. Sampling and Storage

Collect samples in bottles of polyethylene, other plastic, or hard rubber, especially if there will be a delay between collection and analysis. Borosilicate glass is less desirable, particularly with waters of pH above 8 or with seawater, in which cases a significant amount of silica in the glass can dissolve. Freezing to preserve samples for analysis of other constituents can lower soluble silica values by as much as 20 to 40% in waters that have

a pH below 6. Do not acidify samples for preservation because silica precipitates in acidic solutions.

4. References

1. HEM, J.D. 1985. Study and interpretation of the Chemical Characteristics of Natural Water, 3rd ed. U.S. Geol. Surv. Water Supply Pap. No. 2254.
2. FANNING, K.A. & M.E.Q. PILSON. 1973. On the spectrophotometric determination of dissolved silica in natural waters. *Anal. Chem.* 45:136.

5. Bibliography

- ROY, C.J. 1945. Silica in natural waters. *Amer. J. Sci.* 243:393.
- VAIL, J.G. 1952. The Soluble Silicates, Their Properties and Uses. Reinhold Publishing Corp., New York, N.Y. Vol. 1, pp. 95-97, 100-161.

4500-SiO₂ B. (Reserved)*

4500-SiO₂ C. Molybdosilicate Method

1. General Discussion

a. Principle: Ammonium molybdate at pH approximately 1.2 reacts with silica and any phosphate present to produce heteropoly acids. Oxalic acid is added to destroy the molybdophosphoric acid but not the molybdosilicic acid. Even if phosphate is known to be absent, the addition of oxalic acid is highly desirable and is a mandatory step in both this method and Method D. The intensity of the yellow color is proportional to the concentration of "molybdate-reactive" silica. In at least one of its forms, silica does not react with molybdate even though it is capable of passing through filter paper and is not noticeably turbid. It is not known to what extent such "unreactive" silica occurs in waters. Terms such as "colloidal," "crystalloidal," and "ionic" have been used to distinguish among various forms of silica but such terminology cannot be substantiated. "Molybdate-unreactive" silica can be converted to the "molybdate-reactive" form by heating or fusing with alkali. Molybdate-reactive or unreactive does not imply reactivity, or lack of it, toward other reagents or processes.

b. Interference: Because both apparatus and reagents may contribute silica, avoid using glassware as much as possible and use reagents low in silica. Also, make a blank determination to correct for silica so introduced. In both this method and Method D, tannin, large amounts of iron, color, turbidity, sulfide, and phosphate interfere. Treatment with oxalic acid eliminates interference from phosphate and decreases interference from tannin. If necessary, use photometric compensation to cancel interference from color or turbidity.

c. Minimum detectable concentration: Approximately 1 mg SiO₂/L can be detected in 50-mL nessler tubes.

2. Apparatus

- a. Platinum dishes,** 100-mL.
- b. Colorimetric equipment:** One of the following is required:
- 1) *Spectrophotometer*, for use at 410 nm, providing a light path of 1 cm or longer. See Table 4500-SiO₂:I for light path selection.
 - 2) *Filter photometer*, providing a light path of 1 cm or longer and equipped with a violet filter having maximum transmittance near 410 nm.
 - 3) *Nessler tubes*, matched, 50-mL, tall form.

3. Reagents

For best results, set aside and use batches of chemicals low in silica. Use distilled reagent water in making reagents and dilutions. Store all reagents in plastic containers to guard against high blanks.

TABLE 4500-SiO₂:I. SELECTION OF LIGHT PATH LENGTH FOR VARIOUS SILICA CONCENTRATIONS

Light Path cm	Method C		Method D	
	Silica in 55 mL Final Volume μg		Silica in 55 mL Final Volume μg	
	650 nm Wavelength	815 nm Wavelength	650 nm Wavelength	815 nm Wavelength
1	200-1300	40-300	20-100	
2	100-700	20-150	10-50	
5	40-250	7-50	4-20	
10	20-130	4-30	2-10	

a. Sodium bicarbonate, NaHCO_3 , powder.
 b. Sulfuric acid, H_2SO_4 , 1N.
 c. Hydrochloric acid, HCl , 1 + 1.
 d. Ammonium molybdate reagent: Dissolve 10 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in water, with stirring and gentle warming, and dilute to 100 mL. Filter if necessary. Adjust to pH 7 to 8 with silica-free NH_4OH or NaOH and store in a polyethylene bottle to stabilize. (If the pH is not adjusted, a precipitate gradually forms. If the solution is stored in glass, silica may leach out and cause high blanks.) If necessary, prepare silica-free NH_4OH by passing gaseous NH_3 into distilled water contained in a plastic bottle.

e. Oxalic acid solution: Dissolve 7.5 g $\text{H}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$ in water and dilute to 100 mL.

f. Stock silica solution: Dissolve 4.73 g sodium metasilicate nonahydrate, $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$, in water and dilute to 1000 mL. For work of highest accuracy, analyze 100.0-mL portions by the gravimetric method.¹ Store in a tightly stoppered plastic bottle.

g. Standard silica solution: Dilute 10.00 mL stock solution to 1000 mL with water; 1.00 mL = 10.0 μg SiO_2 . Calculate exact concentration from concentration of stock silica solution. Store in a tightly stoppered plastic bottle.

h. Permanent color solutions:

1) Potassium chromate solution: Dissolve 630 mg K_2CrO_4 in water and dilute to 1 L.

2) Borax solution: Dissolve 10 g sodium borate decahydrate, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, in water and dilute to 1 L.

4. Procedure

a. Color development: To 50.0 mL sample add in rapid succession 1.0 mL 1 + 1 HCl and 2.0 mL ammonium molybdate reagent. Mix by inverting at least six times and let stand for 5 to 10 min. Add 2.0 mL oxalic acid solution and mix thoroughly. Read color after 2 min but before 15 min, measuring time from addition of oxalic acid. Because the yellow color obeys Beer's law, measure photometrically or visually.

b. To detect the presence of molybdate-unreactive silica, digest sample with NaHCO_3 before color development. This digestion is not necessarily sufficient to convert all molybdate-unreactive silica to the molybdate-reactive form. Complex silicates and higher silica polymers may require extended fusion with alkali at high temperatures or digestion under pressure for complete conversion. Omit digestion if all the silica is known to react with molybdate.

Prepare a clear sample by filtration if necessary. Place 50.0 mL, or a smaller portion diluted to 50 mL, in a 100-mL platinum dish. Add 200 mg silica-free NaHCO_3 and digest on a steam bath for 1 h. Cool and add slowly, with stirring, 2.4 mL 1N H_2SO_4 . Do not interrupt analysis but proceed at once with remaining steps. Transfer quantitatively to a 50-mL nessler tube and make up to mark with water. (Tall-form 50-mL nessler tubes are convenient for mixing even if the solution subsequently is transferred to an absorption cell for photometric measurement.)

c. Preparation of standards: If NaHCO_3 pretreatment is used, add to the standards (approximately 45 mL total volume) 200 mg NaHCO_3 and 2.4 mL 1N H_2SO_4 , to compensate both for the slight amount of silica introduced by the reagents and for the effect of the salt on color intensity. Dilute to 50.0 mL.

d. Correction for color or turbidity: Prepare a special blank for every sample that needs such correction. Carry two identical portions of each such sample through the procedure, including NaHCO_3 treatment if this is used. To one portion add all reagents as directed in ¶ 4a preceding. To the other portion add HCl and oxalic acid but no molybdate. Adjust photometer to zero absorbance with the blank containing no molybdate before reading absorbance of molybdate-treated sample.

e. Photometric measurement: Prepare a calibration curve from a series of approximately six standards to cover the optimum ranges cited in Table 4500-SiO₂:I. Follow direction of ¶ 4a above on suitable portions of standard silica solution diluted to 50.0 mL in nessler tubes. Set photometer at zero absorbance with water and read all standards, including a reagent blank, against water. Plot micrograms silica in the final (55 mL) developed solution against photometer readings. Run a reagent blank and at least one standard with each group of samples to confirm that the calibration curve previously established has not shifted.

f. Visual comparison: Make a set of permanent artificial color standards, using K_2CrO_4 and borax solutions. Mix liquid volumes specified in Table 4500-SiO₂:II and place them in well-stoppered, appropriately labeled 50-mL nessler tubes. Verify correctness of these permanent artificial standards by comparing them visually against standards prepared by analyzing portions of the standard silica solution. Use permanent artificial color standards only for visual comparison.

5. Calculation

$$\text{mg SiO}_2/\text{L} = \frac{\mu\text{g SiO}_2 \text{ (in 55 mL final volume)}}{\text{mL sample}}$$

Report whether NaHCO_3 digestion was used.

6. Precision and Bias

A synthetic sample containing 5.0 mg SiO_2/L , 10 mg Cl^-/L , 0.20 mg $\text{NH}_3\text{-N}/\text{L}$, 1.0 mg NO_3^-/L , 1.5 mg organic N/L , and 10.0 mg $\text{PO}_4^{3-}/\text{L}$ in distilled water was analyzed in 19 laboratories by the molybdosilicate method with a relative standard deviation of 14.3% and a relative error of 7.8%.

Another synthetic sample containing 15.0 mg SiO_2/L , 200 mg Cl^-/L , 0.800 mg $\text{NH}_3\text{-N}/\text{L}$, 1.0 mg NO_3^-/L , 0.800 mg organic N/L , and 5.0 mg $\text{PO}_4^{3-}/\text{L}$ in distilled water was analyzed in 19

TABLE 4500-SiO₂:II. PREPARATION OF PERMANENT COLOR STANDARDS FOR VISUAL DETERMINATION OF SILICA

Values in Silica μg	Potassium Chromate Solution mL	Borax Solution mL	Water mL
0	0.0	25	30
100	1.0	25	29
200	2.0	25	28
400	4.0	25	26
500	5.0	25	25
750	7.5	25	22
1000	10.0	25	20

laboratories by the molybdosilicate method, with a relative standard deviation of 8.4% and a relative error of 4.2%.

A third synthetic sample containing 30.0 mg SiO₂/L, 400 mg Cl⁻/L, 1.50 mg NH₃-N/L, 1.0 mg NO₃⁻-N/L, 0.200 mg organic N/L, and 0.500 mg PO₄³⁻/L, in distilled water was analyzed in 20 laboratories by the molybdosilicate method, with a relative standard deviation of 7.7% and a relative error of 9.8%.

All results were obtained after sample digestion with NaHCO₃.

7. Reference

1. EATON, A.D., L.S. CLESCERI & A.E. GREENBERG, eds. 1995. Standard Methods for the Examination of Water and Wastewater., 19th ed. American Public Health Assoc., American Water Works Assoc., & Water Environment Fed., Washington, D.C.

8. Bibliography

- DIENERT, F. & F. WANDENBULCKE. 1923. On the determination of silica in waters. *Bull. Soc. Chim. France* 33:1131, *Compt. Rend.* 176:1478.
- DIENERT, F. & F. WANDENBULCKE. 1924. A study of colloidal silica. *Compt. Rend.* 178:564.

SWANK, H.W. & M.G. MELLON. 1934. Colorimetric standards for silica. *Ind. Eng. Chem., Anal. Ed.* 6:348.

TOURKY, A.R. & D.H. BANGHAM. 1936. Colloidal silica in natural waters and the "silicomolybdate" colour test. *Nature* 138:587.

BIRNBAUM, N. & G.H. WALDEN. 1938. Co-precipitation of ammonium silicomolybdate and ammonium phosphomolybdate. *J. Amer. Chem. Soc.* 60:66.

KAHLER, H.L. 1941. Determination of soluble silica in water: A photometric method. *Ind. Eng. Chem., Anal. Ed.* 13:536.

NOLL, C.A. & J.J. MAGUIRE. 1942. Effect of container on soluble silica content of water samples. *Ind. Eng. Chem., Anal. Ed.* 14:569.

SCHWARTZ, M.C. 1942. Photometric determination of silica in the presence of phosphates. *Ind. Eng. Chem., Anal. Ed.* 14:893.

GUTTER, H. 1945. Influence of pH on the composition and physical aspects of the ammonium molybdates. *Compt. Rend.* 220:146.

MILTON, R.F. 1951. Formation of silicomolybdate. *Analyst* 76:431.

KILLEFFER, D.H. & A. LINZ. 1952. Molybdenum Compounds, Their Chemistry and Technology. Interscience Publishers, New York, N.Y. pp. 1-2, 42-45, 67-82, 87-92.

STRICKLAND, J.D.H. 1952. The preparation and properties of silicomolybdic acid. *J. Amer. Chem. Soc.* 74:862, 868, 872.

CHOW, D.T.W. & R.J. ROBINSON. 1953. The forms of silicate available for colorimetric determination. *Anal. Chem.* 25:646.

4500-SiO₂ D. Heteropoly Blue Method

1. General Discussion

a. Principle: The principles outlined under Method C, ¶ 1a, also apply to this method. The yellow molybdosilicic acid is reduced by means of aminonaphtholsulfonic acid to heteropoly blue. The blue color is more intense than the yellow color of Method C and provides increased sensitivity.

b. Interference: See Section 4500-SiO₂.C.1b.

c. Minimum detectable concentration: Approximately 20 µg SiO₂/L can be detected in 50-mL nessler tubes and 50 µg SiO₂/L spectrophotometrically with a 1-cm light path at 815 nm.

2. Apparatus

a. Platinum dishes, 100-mL.

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

1) *Spectrophotometer,* for use at approximately 815 nm. The color system also obeys Beer's law at 650 nm, with appreciably reduced sensitivity. Use light path of 1 cm or longer. See Table 4500-SiO₂.I for light path selection.

2) *Filter photometer,* provided with a red filter exhibiting maximum transmittance in the wavelength range of 600 to 815 nm. Sensitivity improves with increasing wavelength. Use light path of 1 cm or longer.

3) *Nessler tubes,* matched, 50-mL, tall form.

3. Reagents

For best results, set aside and use batches of chemicals low in silica. Store all reagents in plastic containers to guard against high blanks. Use distilled water that does not contain detectable silica after storage in glass.

All of the reagents listed in Section 4500-SiO₂.C.3 are required, and in addition:

Reducing agent: Dissolve 500 mg 1-amino-2-naphthol-4-sulfonic acid and 1 g Na₂SO₃ in 50 mL distilled water, with gentle warming if necessary; add this to a solution of 30 g NaHSO₃ in 150 mL distilled water. Filter into a plastic bottle. Discard when solution becomes dark. Prolong reagent life by storing in a refrigerator and away from light. Do not use aminonaphtholsulfonic acid that is incompletely soluble or that produces reagents that are dark even when freshly prepared.*

4. Procedure

a. Color development: Proceed as in 4500-SiO₂.C.4a up to and including the words, "Add 2.0 mL oxalic acid solution and mix thoroughly." Measuring time from the moment of adding oxalic acid, wait at least 2 min but not more than 15 min, add 2.0 mL reducing agent, and mix thoroughly. After 5 min, measure blue color photometrically or visually. If NaHCO₃ pretreatment is used, follow 4500-SiO₂.C.4b.

b. Photometric measurement: Prepare a calibration curve from a series of approximately six standards to cover the optimum range indicated in Table 4500-SiO₂.I. Carry out the steps described above on suitable portions of standard silica solution diluted to 50.0 mL in nessler tubes; pretreat standards if NaHCO₃ digestion is used (see 4500-SiO₂.C.4b). Adjust photometer to zero absorbance with distilled water and read all standards, including a reagent blank, against distilled water. If

* Eastman No. 360 has been found satisfactory.

necessary to correct for color or turbidity in a sample, see 4500-SiO₂.C.4d. To the special blank add HCl and oxalic acid, but no molybdate or reducing agent. Plot micrograms silica in the final 55 mL developed solution against absorbance. Run a reagent blank and at least one standard with each group of samples to check the calibration curve.

c. *Visual comparison:* Prepare a series of not less than 12 standards, covering the range 0 to 120 µg SiO₂, by placing the calculated volumes of standard silica solution in 50-mL nessler tubes, diluting to mark with distilled water, and developing color as described in ¶ a preceding.

5. Calculation

$$\text{mg SiO}_2/\text{L} = \frac{\mu\text{g SiO}_2 \text{ (in 55 mL final volume)}}{\text{mL sample}}$$

Report whether NaHCO₃ digestion was used.

6. Precision and Bias

A synthetic sample containing 5.0 mg SiO₂/L, 10 mg Cl⁻/L, 0.200 mg NH₃-N/L, 1.0 mg NO₃⁻-N/L, 1.5 mg organic N/L, and 10.0 mg PO₄³⁻/L in distilled water was analyzed in 11 laboratories by the heteropoly blue method, with a relative standard deviation of 27.2% and a relative error of 3.0%.

A second synthetic sample containing 15 mg SiO₂/L, 200 mg Cl⁻/L, 0.800 mg NH₃-N/L, 1.0 mg NO₃⁻-N/L, 0.800 mg organic N/L, and 5.0 mg PO₄³⁻/L in distilled water was analyzed in 11 laboratories by the heteropoly blue method, with a relative standard deviation of 18.0% and a relative error of 2.9%.

A third synthetic sample containing 30.0 mg SiO₂/L, 400 mg Cl⁻/L, 1.50 mg NH₃-N/L, 1.0 mg NO₃⁻-N/L, 0.200 mg organic N/L, and 0.500 mg PO₄³⁻/L in distilled water was analyzed in 10 laboratories by the heteropoly blue method with a relative standard deviation of 4.9% and a relative error of 5.1%.

All results were obtained after sample digestion with NaHCO₃.

7. Bibliography

- BUNTING, W.E. 1944. Determination of soluble silica in very low concentrations. *Ind. Eng. Chem., Anal. Ed.* 16:612.
- STRAUB, F.G. & H. GRABOWSKI. 1944. Photometric determination of silica in condensed steam in the presence of phosphates. *Ind. Eng. Chem., Anal. Ed.* 16:574.
- BOLTZ, D.F. & M.G. MELLON. 1947. Determination of phosphorus, germanium, silicon, and arsenic by the heteropoly blue method. *Ind. Eng. Chem., Anal. Ed.* 19:873.
- MILTON, R.F. 1951. Estimation of silica in water. *J. Appl. Chem. (London)* 1:(Supplement No. 2) 126.
- CARLSON, A.B. & C.V. BANKS. 1952. Spectrophotometric determination of silicon. *Anal. Chem.* 24:472.

4500-SiO₂ E. Automated Method for Molybdate-Reactive Silica

1. General Discussion

a. *Principle:* This method is an adaptation of the heteropoly blue method (Method D) utilizing a continuous-flow analytical instrument.

b. *Interferences:* See Section 4500-SiO₂.C.1b. If particulate matter is present, filter sample or use a continuous filter as an integral part of the system.

c. *Application:* This method is applicable to potable, surface, domestic, and other waters containing 0 to 20 mg SiO₂/L. The range of concentration can be broadened to 0 to 80 mg/L by substituting a 15-mm flow cell for the 50-mm flow cell shown in Figure 4500-SiO₂.1.

2. Apparatus

Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-SiO₂.1.

3. Reagents

a. *Sulfuric acid,* H₂SO₄, 0.05M (0.1N).

b. *Ammonium molybdate reagent:* Dissolve 10 g (NH₄)₆Mo₇O₂₄ · 4H₂O in 1 L 0.05M H₂SO₄. Filter and store in an amber plastic bottle.

c. *Oxalic acid solution:* Dissolve 50 g oxalic acid in 900 mL distilled water and dilute to 1 L.

d. *Reducing agent:* Dissolve 120 g NaHSO₃ and 4 g Na₂SO₃ in 800 mL warm distilled water. Add 2 g 1-amino-2-naphthol-4-sulfonic acid, mix well, and dilute to 1 L. Filter into amber plastic bottle for storage.

To prepare working reagent, dilute 100 mL to 1 L with distilled water. Make working reagent daily.

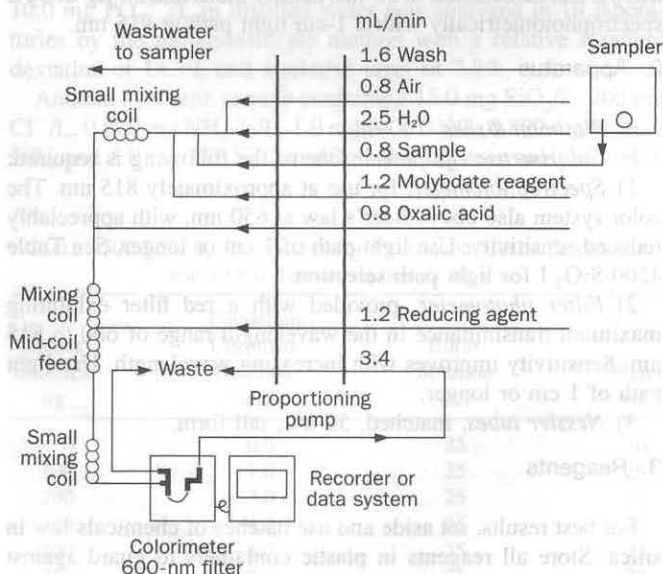


Figure 4500-SiO₂.1. Silica manifold.

e. *Standard silica solution:* See 4500-SiO₂-C.3g.

4. Procedure

Set up manifold as shown in Figure 4500-SiO₂:1 and follow the general procedure described by the manufacturer. Determine absorbance at 660 nm. Use quality control procedures given in Section 4020.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against SiO₂ concentration in

standards. Compute sample SiO₂ concentration by comparing sample response with standard curve.

6. Precision and Bias

For 0 to 20 mg SiO₂/L, when a 50-mm flow cell was used at 40 samples/h, the detection limit was 0.1 mg/L, sensitivity (concentration giving 0.398 absorbance) was 7.1 mg/L, and the coefficient of variation (95% confidence level at 7.1 mg/L) was 1.6%. For 0 to 80 mg SiO₂/L, when a 15-mm flow cell was used at 50 samples/h, detection limit was 0.5 mg/L, sensitivity was 31 mg/L, and coefficient of variation at 31 mg/L was 1.5%.

4500-SiO₂ F. Flow Injection Analysis for Molybdate-Reactive Silicate

1. General Discussion

Silicate reacts with molybdate under acidic conditions to form yellow beta-molybdosilicic acid. This acid is subsequently reduced with stannous chloride to form a heteropoly blue complex that has an absorbance maximum at 820 nm. Oxalic acid is added to reduce the interference from phosphate.

Collect samples in polyethylene or other plastic bottles and refrigerate at 4°C. Chemical preservation for silica is not recommended. Adding acid may cause polymerization of reactive silicate species. Freezing decreases silicate concentrations, especially at concentrations greater than 100 µg SiO₂/L. If filtration is required, preferably use a 0.45-µm TFE filter. Samples may be held for 28 d.

Also see Sections 4500-SiO₂-A, D, and E, and Section 4130, Flow Injection Analysis (FIA).

b. *Interferences:* Remove large or fibrous particulates by filtering sample through inert filter.

The interference due to phosphates is reduced by the addition of oxalic acid as a reagent on the flow injection manifold. By the following method, a solution of 1000 µg P/L was determined as 20 µg SiO₂/L. Verify extent of phosphate interference by determining a solution of phosphate at the highest concentration that is expected to occur.

Tannin and large amounts of iron or sulfides are interferences. Remove sulfides by boiling an acidified sample. Add disodium EDTA to eliminate interference due to iron. Treat with oxalic acid to decrease interference from tannin.

Sample color and turbidity can interfere. Determine presence of these interferences by analyzing samples without the presence of the molybdate.

Avoid silica contamination by storing samples, standards, and reagents in plastic. Do not use glass-distilled water for reagents or standards.

2. Apparatus

Flow injection analysis equipment consisting of:

- FIA injection valve with sample loop or equivalent.
- Multichannel proportioning pump.

c. *FIA manifold* (Figure 4500-SiO₂:2) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-SiO₂:2. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*

d. *Absorbance detector*, 820 nm, 10-nm bandpass.

e. *Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. All reagents can also be prepared on a weight/volume basis if desired.

a. *Molybdate:* To a tared 500-mL container add 20.0 g ammonium molybdate tetrahydrate [(NH₄)₆Mo₇O₂₄ · 4H₂O]. Add 486 g warm water and 14.8 g conc sulfuric acid (H₂SO₄). Stir or shake until dissolved. Store in plastic and refrigerate. Prepare fresh monthly and discard if precipitate or blue color is observed.

b. *Oxalic acid:* To a tared 500-mL container add 50.0 g oxalic acid (HO₂CCO₂H · 2H₂O) and 490 g water. Stir or shake until dissolved. Store in plastic.

* Teflon or equivalent.

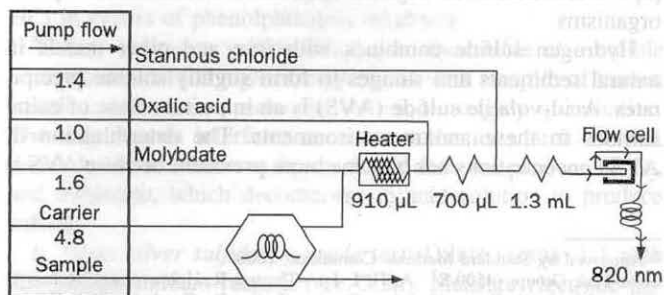


Figure 4500-SiO₂:2. FIA manifold.

c. *Stannous chloride*: To a tared 1-L container, add 978 g water. Add 40.0 g conc H_2SO_4 . Dissolve 2.0 g hydroxylamine hydrochloride in this solution. Then dissolve 0.30 g stannous chloride. Prepare fresh weekly.

d. *Stock silicate standard*, 100 mg SiO_2/L : In a 1-L volumetric flask dissolve 0.473 g sodium metasilicate nonahydrate ($\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$) in approximately 800 mL water. Dilute to mark and invert three times. Alternatively, use a commercially prepared standard solution, especially if nonstoichiometry of the solid metasilicate is suspected; the original degree of polymerization of the sodium metasilicate, which depends on storage time, can affect free silica concentration of the resulting solution.

e. *Standard silicate solutions*: Prepare silicate standards in the desired concentration range, using the stock standard (§ 3d), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-SiO₂:2 and follow method supplied by manufacturer or laboratory standard operating procedure.

4500-S²⁻—SULFIDE*

4500-S²⁻ A. Introduction

1. Occurrence and Significance

Sulfide is often present in groundwater and sediment. It is produced by decomposition of organic matter and bacterial reduction of sulfate. It is sometimes found in industrial or municipal wastewater. Hydrogen sulfide escaping into the air from sulfide-containing wastewater causes odor nuisances. The threshold odor concentration of H_2S in clean water is between 0.025 and 0.25 $\mu\text{g}/\text{L}$. Gaseous H_2S is very toxic and has claimed the lives of numerous workers. At levels toxic to humans it interferes with the olfactory system, giving a false sense of the safe absence of H_2S . It attacks metals directly, and indirectly has caused serious corrosion of concrete sewers because it is oxidized biologically in the presence of oxygen to H_2SO_4 on the pipe wall. Dissolved H_2S is toxic to fish and other aquatic organisms.

Hydrogen sulfide combines with iron and other metals in natural sediments and sludges to form slightly-soluble precipitates. Acid-volatile sulfide (AVS) is an important class of metal sulfides in these anoxic environments. The determination of AVS concentrations has become more prevalent because AVS is

5. Calculations

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

6. Precision and Bias

Twenty-one replicates of a 5.0- μg SiO_2/L standard were analyzed with a 780- μL sample loop by a published MDL method.¹ These gave a mean of 4.86 μg SiO_2/L , a standard deviation of 0.31 μg SiO_2/L , and an MDL of 0.78 μg SiO_2/L .

7. Quality Control

Follow procedures outlined in Section 4020.

8. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY: 1989. Definition and Procedure for the Determination of Method Detection Limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

* Approved by Standard Methods Committee, 2000.

Joint Task Group: (4500-S²⁻—A, H, I, J)—Thomas R. Holm (chair), Robert P. Fisher, Martin S. Frant, Christian Gagnon, Lorne R. Goodwin; 20th Edition (4500-S²⁻—I)—Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

considered to be a key binding phase for controlling bioavailability of toxic metals in anoxic sediments.

AVS typically is determined by a purge-and-trap method in which hydrochloric acid is used to volatilize AVS at room temperature. The metals associated with the sulfides can be determined from the supernatant of the purged sample solution by using methods such as those in Part 3000. The hydrogen sulfide produced is trapped in zinc-acetate absorbing solution. AVS concentrations are measured by iodometric titration of the ZnS precipitated in the trap (Section 4500-S²⁻—F). Certain minerals, including iron pyrite, are partially digested by the AVS reagents at elevated temperatures, which may result in a significant overestimation of AVS. Iron pyrite can be partially digested to the extent of less than 10% of the total pyrite present. The addition of stannous chloride (SnCl_2) prevents the oxidation of sulfides by any liberated ferric iron.

2. Categories of Sulfides

Four categories of sulfide in water, wastewater, and sediment can be operationally defined:

a. *Total sulfide* includes dissolved H_2S and HS^- and acid-volatile metallic sulfides present in particulate matter. The $\text{pK}_{\text{a}2}$ of H_2S is so high that the concentration of S^{2-} is negligible at all pH values. Copper and silver sulfides are so insoluble that they do not respond in ordinary sulfide determinations; they can be ignored for practical purposes.

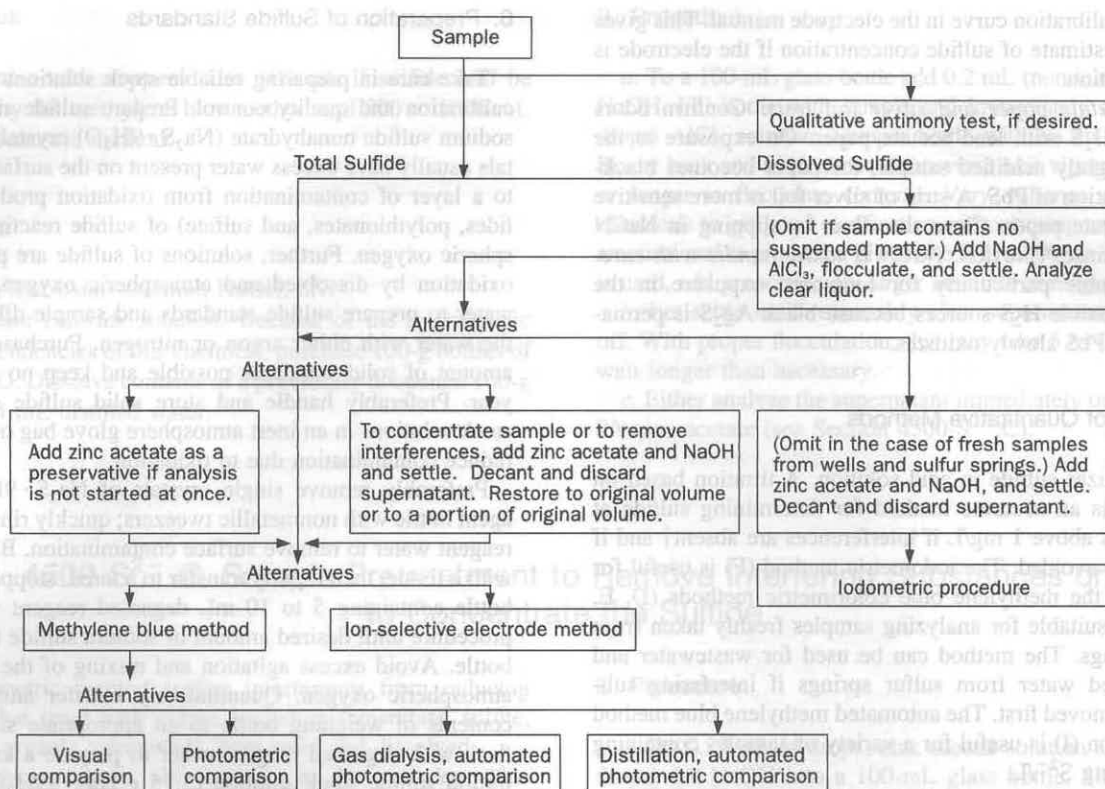


Figure 4500-S²⁻:1. Analytical flow paths for sulfide determination.

b. Dissolved sulfide is that remaining after suspended solids have been removed by flocculation and settling. Flocculation and settling are used to separate dissolved and particulate sulfide because sulfide may be oxidized during filtration. Centrifugation also may be used.

c. Acid-volatile sulfide includes amorphous iron monosulfides, including mackinawite (FeS), greigite (Fe₃S₄), and pyrrhotite (FeS), and amorphous monosulfides of other metals. Pyrite, another iron sulfide mineral, is not included in the acid-volatile sulfides.

d. Un-ionized hydrogen sulfide can be calculated from the concentration of dissolved sulfide, the sample pH, and the conditional ionization constant of H₂S.

Figure 4500-S²⁻:1 shows analytical flow paths for sulfide determinations under various conditions and options. None of the operationally-defined sulfide categories includes pyrite or marcasite (FeS₂).

3. Sampling and Storage

Collect water samples with minimum aeration. Either analyze samples immediately after collection or preserve with zinc acetate solution for later analysis. To preserve a sample for a total sulfide determination put zinc acetate and sodium hydroxide solutions into sample bottle before filling it with sample. Use 0.2 mL 2M zinc acetate solution per 100 mL sample. Increase volume of zinc acetate solution if the sulfide concentration is expected to be greater than 64 mg/L. The final pH should be at

least 9. Add more NaOH if necessary. Fill bottle completely and stopper.

Sample sediments and sludges under nitrogen atmosphere if possible. Store samples at 4°C or frozen, and analyze within 2 weeks (1 month for frozen samples) of collection. Do not freeze-dry because acid-volatile sulfide may decompose as a result of oxidation artifacts.

4. Qualitative Tests

A qualitative test for sulfide often is useful. It is advisable in the examination of industrial wastes containing interfering substances that may give a false negative result in the methylene blue method (D).

a. Antimony test: To about 200 mL sample, add 0.5 mL saturated solution of antimony potassium tartrate and 0.5 mL 6N HCl in excess of phenolphthalein alkalinity.

Yellow antimony sulfide (Sb₂S₃) is discernible at a sulfide concentration of 0.5 mg/L. Comparisons with samples of known sulfide concentration make the technique roughly quantitative. The only known interferences are metallic ions such as lead, which hold the sulfide so firmly that it does not produce Sb₂S₃, and dithionite, which decomposes in acid solution to produce sulfide.

b. Silver-silver sulfide electrode test: Dilute sample 1:1 with alkaline antioxidant reagent (see G.3a). Measure electrode potential relative to a double-junction reference electrode and estimate the sulfide concentration from an old calibration curve or

the example calibration curve in the electrode manual. This gives a reasonable estimate of sulfide concentration if the electrode is in good condition.

c. *Lead acetate paper and silver foil tests:* Confirm odors attributed to H_2S with lead acetate paper. On exposure to the vapor of a slightly acidified sample, the paper becomes blackened by formation of PbS . A strip of silver foil is more sensitive than lead acetate paper. Clean the silver by dipping in NaCN solution and rinse. CAUTION: *NaCN is toxic; handle with care.* Silver is suitable particularly for long-time exposure in the vicinity of possible H_2S sources because black Ag_2S is permanent whereas PbS slowly oxidizes.

5. Selection of Quantitative Methods

Iodine oxidizes sulfide in acid solution. A titration based on this reaction is an accurate method for determining sulfide at concentrations above 1 mg/L if interferences are absent† and if loss of H_2S is avoided. The iodometric method (F) is useful for standardizing the methylene blue colorimetric methods (D, E, and I) and is suitable for analyzing samples freshly taken from wells or springs. The method can be used for wastewater and partly oxidized water from sulfur springs if interfering substances are removed first. The automated methylene blue method with distillation (I) is useful for a variety of samples containing more than 1 mg S^{2-} /L.

The methylene blue method (D) is based on the reaction of sulfide, ferric chloride, and dimethyl-*p*-phenylenediamine to produce methylene blue. Ammonium phosphate is added after color development to remove ferric chloride color. The procedure is applicable at sulfide concentrations between 0.1 and 20.0 mg/L. The automated methylene blue method (E) is similar to Method D. A gas dialysis technique separates the sulfide from the sample matrix. Gas dialysis eliminates most interferences, including turbidity and color. The addition of the antioxidant ascorbic acid improves sulfide recoveries. The method is applicable at sulfide concentrations between 0.002 and 0.100 mg/L.

Potentiometric methods utilizing a silver/sulfide ion-selective electrode (G) may be suitable. The sulfide concentration can be estimated from the potential of the electrode relative to a reference electrode, but careful attention to details of procedures and frequent standardizations are needed to secure good results. The electrode is useful particularly as an end-point indicator for titration of dissolved sulfide with silver nitrate. The ion-selective electrode method is unaffected by sample color or turbidity and is applicable for concentrations greater than 0.03 mg/L.

† Many substances can reduce iodine; all of these are potential interferences in procedures using this chemistry.

4500-S²⁻ B. Separation of Soluble and Insoluble Sulfides

Unless the sample is entirely free from suspended solids (dissolved sulfide equals total sulfide), to measure dissolved sulfide first remove insoluble matter. This can be done by pro-

6. Preparation of Sulfide Standards

Take care in preparing reliable stock solutions of sulfide for calibration and quality control. Prepare sulfide standards from sodium sulfide nonahydrate ($\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$) crystals. These crystals usually have excess water present on the surface, in addition to a layer of contamination from oxidation products (polysulfides, polythionates, and sulfate) of sulfide reacting with atmospheric oxygen. Further, solutions of sulfide are prone to ready oxidation by dissolved and atmospheric oxygen. Use reagent water to prepare sulfide standards and sample dilutions. Degas the water with either argon or nitrogen. Purchase the smallest amount of solid standards possible and keep no longer than 1 year. Preferably handle and store solid sulfide standards and stock solutions in an inert atmosphere glove bag or glove box to reduce contamination due to oxidation.

Preferably remove single crystals of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ from reagent bottle with nonmetallic tweezers; quickly rinse in degassed reagent water to remove surface contamination. Blot crystal dry with a tissue, then rapidly transfer to a tared, stoppered weighing bottle containing 5 to 10 mL degassed reagent water. Repeat procedure until desired amount of sodium sulfide is in weighing bottle. Avoid excess agitation and mixing of the solution with atmospheric oxygen. Quantitatively transfer and dilute entire contents of weighing bottle to an appropriate size volumetric flask with degassed reagent water to prepare a known concentration sulfide stock solution (3.75 g $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ diluted to a final volume of 500 mL will give a stock solution of which 1.00 mL = 1.00 mg S^{2-}). Standardize stock solution using the iodometric method, Section 4500-S²⁻. F. Alternatively, purchase precertified stock solutions of sulfide. Verify concentration of stock solution daily using the iodometric method (F). Store stock solution with minimum headspace for no more than 1 week.

7. Bibliography

- CRUSE, H. & R.D. POMEROY. 1969. Hydrogen sulfide odor threshold. *J. Amer. Water Works Assoc.* 61:677.
- KARCHMER, J.H., ed. 1970. *The Analytical Chemistry of Sulfur and Its Compounds*. Wiley-Interscience, New York, N.Y.
- NICKLESS, G., ed. 1970. *Inorganic Sulphur Chemistry*. Elsevier Publ., Amsterdam, The Netherlands.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. 1974. *Process Design Manual for Sulfide Control in Sanitary Sewerage Systems*. Publ. 625/1-74-005.
- BAGARINAO, T. 1992. Sulfide as an environmental factor and toxicant: Tolerance and adaptations in aquatic organisms. *Aquat. Toxicol.* 24:21.
- BUTLER, I.B., M.A.A. SCHOONEN & D.T. RICKARD. 1994. Removal of dissolved oxygen from water: a comparison of four common techniques. *Talanta* 41:211.

ducing an aluminum hydroxide floc that is settled, leaving a clear supernatant for analysis.

1. Apparatus

Glass bottles with stoppers: Use 100 mL if sulfide will be determined by the methylene blue method and 500 to 1000 mL if by the iodometric method.

2. Reagents

- Sodium hydroxide solution, NaOH, 6N.**
- Aluminum chloride solution:** Because of the hygroscopic and caking tendencies of this chemical, purchase 100-g bottles of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$. Dissolve contents of a previously unopened 100-g bottle in 144 mL distilled water.

4500-S²⁻ C. Sample Pretreatment to Remove Interfering Substances or to Concentrate the Sulfide

The iodometric method suffers interference from reducing substances that react with iodine, including thiosulfate, sulfite, and various organic compounds, both solid and dissolved.

Strong reducing agents also interfere in the methylene blue method (D) by preventing formation of the blue color. Thiosulfate at concentrations about 10 mg/L may retard color formation or completely prevent it. Ferrocyanide produces a blue color. Sulfide itself prevents the reaction if its concentration is very high, in the range of several hundred milligrams per liter. To avoid the possibility of false negative results, use the antimony method to obtain a qualitative result in industrial wastes likely to contain sulfide but showing no color by the methylene blue method. Iodide, which is likely to be present in oil-field wastewaters, may diminish color formation if its concentration exceeds 2 mg/L. Many metals (e.g., Hg, Cd, Cu) form insoluble sulfides and give low recoveries.

Eliminate interferences due to sulfite, thiosulfate, iodide, and many other soluble substances, but not ferrocyanide, by first precipitating ZnS , removing the supernatant, and replacing it with distilled water. Use the same procedure, even when not needed for removal of interferences, to concentrate sulfide. The automated methylene blue method (E) is relatively free from interferences because gas dialysis separates the sulfide from the sample matrix.

1. Apparatus

Glass bottles with stoppers: See Section 4500-S²⁻.B.1.

2. Reagents

- Zinc acetate solution:** Dissolve 220 g $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$ in 870 mL water; this makes 1 L solution.
- Sodium hydroxide solution, NaOH, 6N.**

3. Procedure

- To a 100-mL glass bottle add 0.2 mL (nominally 4 drops) 6N NaOH. Fill bottle with sample and immediately add 0.2 mL (4 drops) AlCl_3 solution. Stopper bottle with no air under stopper. Rotate back and forth about a transverse axis vigorously for 1 min or longer to flocculate contents. Vary volumes of these added chemicals to get good clarification without using excessively large amounts and to produce a pH of 6 to 9. If a 500- or 1000-mL bottle is used, add proportionally larger amounts of reagents.
- Let settle until reasonably clear supernatant can be drawn off. With proper flocculation, this may take 5 to 15 min. Do not wait longer than necessary.
- Either analyze the supernatant immediately or preserve with 2N zinc acetate (see Section 4500-S²⁻.C).

3. Procedure

- Put 0.20 mL (4 drops) zinc acetate solution and 0.10 mL (2 drops) 6N NaOH into a 100-mL glass bottle, fill with sample, and add 0.10 mL (2 drops) 6N NaOH solution. Stopper with no air bubbles under stopper and mix by rotating back and forth vigorously about a transverse axis. For the iodometric procedure, use a 500-mL bottle or other convenient size, with proportionally larger volumes of reagents. Vary volume of reagents added according to sample so that the resulting precipitate is not excessively bulky and settles readily. Add enough NaOH to raise the pH above 9. Let precipitate settle for 30 min. The treated sample is relatively stable and can be held for several hours. However, if much iron is present, oxidation may be fairly rapid.
- If the iodometric method is to be used, collect precipitate on a glass fiber filter and continue at once with titration according to the procedure of Method F. If the methylene blue method (D) is used, let precipitate settle for 30 min and decant as much supernatant as possible without loss of precipitate. Refill bottle with distilled water, shake to resuspend precipitate, and quickly withdraw a sample. If interfering substances are present in high concentration, settle, decant, and refill a second time. If sulfide concentration is known to be low, add only enough water to bring volume to one-half or one-fifth of original volume. Use this technique for analyzing samples of very low sulfide concentrations. After determining the sulfide concentration colorimetrically, multiply the result by the ratio of final to initial volume. No concentration or pretreatment steps to remove interferences are necessary for Method E.

4500-S²⁻ D. Methylene Blue Method

1. Apparatus

a. *Matched test tubes*, approximately 125 mm long and 15 mm OD.

b. *Droppers*, delivering 20 drops/mL methylene blue solution. To obtain uniform drops hold dropper in a vertical position and let drops form slowly.

c. If photometric rather than visual color determination will be used, either:

1) *Spectrophotometer*, for use at a wavelength of 664 nm with cells providing light paths of 1 cm and 1 mm, or other path lengths, or

2) *Filter photometer*, with a filter providing maximum transmittance near 660 nm.

2. Reagents

a. *Amine-sulfuric acid stock solution*: Dissolve 27 g *N,N*-dimethyl-*p*-phenylenediamine oxalate* in an iced mixture of 50 mL conc H₂SO₄ and 20 mL distilled water. Cool and dilute to 100 mL with distilled water. Use fresh oxalate because an old supply may be oxidized and discolored to a degree that results in interfering colors in the test. Store in a dark glass bottle. When this stock solution is diluted and used in the procedure with a sulfide-free sample, it first will be pink but then should become colorless within 3 min.

b. *Amine-sulfuric acid reagent*: Dilute 25 mL amine-sulfuric acid stock solution with 975 mL 1 + 1 H₂SO₄. Store in a dark glass bottle.

c. *Ferric chloride solution*: Dissolve 100 g FeCl₃ · 6H₂O in 40 mL water.

d. *Sulfuric acid solution*, H₂SO₄, 1 + 1.

e. *Diammonium hydrogen phosphate solution*: Dissolve 400 g (NH₄)₂HPO₄ in 800 mL distilled water.

f. *Methylene blue solution I*: Use USP grade dye or one certified by the Biological Stain Commission. The dye content should be reported on the label and should be 84% or more. Dissolve 1.0 g in distilled water and make up to 1 L. This solution will be approximately the correct strength, but because of variation between different lots of dye, standardize against sulfide solutions of known strength and adjust its concentration so that 0.05 mL (1 drop) = 1.0 mg sulfide/L.

Standardization—Prepare five known-concentration sulfide standards ranging from 1 to 8 mg/L as described in 4500-S²⁻.A.6, or proceed as follows: Put several grams of clean, washed crystals of Na₂S · 9H₂O into a small beaker. Add somewhat less than enough water to cover crystals. Stir occasionally for a few minutes, then pour solution into another vessel. This solution reacts slowly with oxygen but the change is insignificant if analysis is performed within a few hours. Prepare solution daily. To 1 L distilled water add 1 drop of Na₂S solution and mix. Immediately determine sulfide concentration by the meth-

ylene blue procedure and by the iodometric procedure. Repeat, using more than 1 drop Na₂S solution or smaller volumes of water, until at least five tests have been made, with a range of sulfide concentrations between 1 and 8 mg/L. Calculate average percent error of the methylene blue result as compared to the iodometric result. If the average error is negative, that is, methylene blue results are lower than iodometric results, dilute methylene blue solution by the same percentage, so that a greater volume will be used in matching colors. If methylene blue results are high, increase solution strength by adding more dye.

g. *Methylene blue solution II*: Dilute 10.00 mL of adjusted methylene blue solution I to 100 mL with reagent water.

3. Procedure

a. *Color development*: Transfer 7.5 mL sample to each of two matched test tubes, using a special wide-tip pipet or filling to marks on test tubes. If sample has been preserved with zinc acetate, shake vigorously before taking subsample. Add to Tube A 0.5 mL amine-sulfuric acid reagent and 0.15 mL (3 drops) FeCl₃ solution. Mix immediately by inverting slowly, only once. (Excessive mixing causes low results by loss of H₂S as a gas before it has had time to react). To Tube B add 0.5 mL 1 + 1 H₂SO₄ and 0.15 mL (3 drops) FeCl₃ solution and mix. The presence of S²⁻ will be indicated by the appearance of blue color in Tube A. Color development usually is complete in about 1 min, but a longer time often is required for fading out of the initial pink color. Wait 3 to 5 min and add 1.6 mL (NH₄)₂HPO₄ solution to each tube. Wait 3 to 15 min and make color comparisons. If zinc acetate was used, wait at least 10 min before making a visual color comparison.

b. *Color determination*:

1) *Visual color estimation*—Add methylene blue solution I or II, depending on sulfide concentration and desired accuracy, dropwise, to the second tube, until color matches that developed in first tube. If the concentration exceeds 20 mg/L, repeat test with a portion of sample diluted tenfold.

With methylene blue solution I, adjusted so that 0.05 mL (1 drop) = 1.0 mg S²⁻/L when 7.5 mL of sample are used:

$$\text{mg S}^{2-}/\text{L} = \text{no. drops solution I} + 0.1 (\text{no. drops solution II})$$

2) *Photometric color measurement*—A cell with a light path of 1 cm is suitable for measuring sulfide concentrations from 0.1 to 2.0 mg/L. Use shorter or longer light paths for higher or lower concentrations. This method is suitable for sample concentrations up to 20 mg/L. Zero instrument with a portion of treated sample from Tube B. Prepare calibration curves on basis of colorimetric tests made on Na₂S solutions simultaneously analyzed by the iodometric method, plotting concentration vs. absorbance. A linear relationship between concentration and absorbance can be assumed from 0 to 1.0 mg/L.

Read sulfide concentration from calibration curve.

* Eastman catalog No. 5672 has been found satisfactory for this purpose.

4. Precision and Bias

In a study by two chemists working in the same laboratory, the standard deviation estimated from 34 sets of duplicate sulfide measurements was 0.04 mg/L for concentrations between 0.2 and 1.5 mg/L. The average recoveries of known additions were 92% for 40 samples containing 0.5 to 1.5 mg/L and 89% for samples containing less than 0.1 mg/L.

5. Bibliography

POMEROY, R.D. 1936. The determination of sulfides in sewage. *Sewage Works J.* 8:572.
NUSBAUM, I. 1965. Determining sulfides in water and waste water. *Water Sewage Works* 112:113.

4500-S²⁻ E. Gas Dialysis, Automated Methylene Blue Method

1. Apparatus

a. Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-S²⁻:2.
The sampler is equipped with a mixer to stir samples before analysis and the gas dialysis membrane, which is maintained at room temperature, separates H₂S from the sample matrix.

2. Reagents

a. N,N-dimethyl-p-phenylenediamine stock solution: Dissolve 1 g N,N-dimethyl-p-phenylenediamine dihydrochloride in 500 mL 6N HCl. Prepare fresh monthly. Store in an amber bottle.
b. N,N-dimethyl-p-phenylenediamine working solution: Dilute 190 mL N,N-dimethyl-p-phenylenediamine stock solution to 1 L. Store in an amber bottle. Prepare weekly.

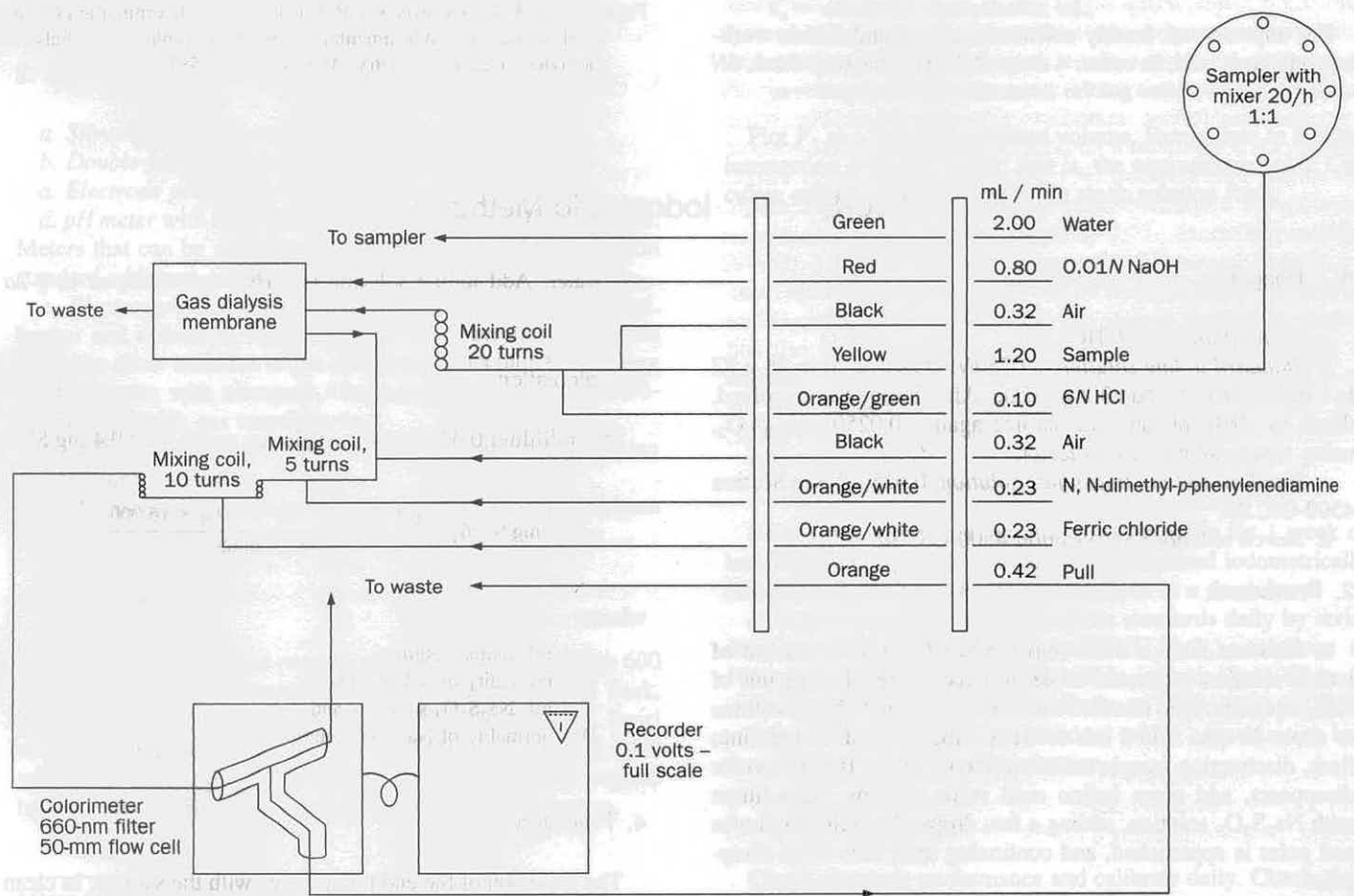


Figure 4500-S²⁻:2. Sulfide manifold.

c. *Ferric chloride stock solution*: Dissolve 13.5 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 500 mL 5*N* HCl. Store in an amber bottle. Prepare fresh monthly.

d. *Working ferric chloride solution*: Dilute 190 mL ferric chloride stock solution to 1 L. Store in an amber bottle. Prepare fresh weekly.

e. *Hydrochloric acid*, HCl, 6*N*:

f. *Sodium hydroxide stock solution*, NaOH, 1*N*.

g. *Sodium hydroxide*, NaOH, 0.01*N*: Dilute 10 mL NaOH stock solution to 1 L.

h. *Sulfide stock solution*, 1.00 mg S^{2-} /1.00 mL: See 4500-S²⁻.A.6.

i. *Sulfide intermediate standard solution*: Dilute 10 mL sulfide stock solution to 1 L with water. Prepare fresh daily. Standardize by iodometric titration method, 4500-S²⁻.F. 1 mL $\approx$ 0.01 mg S^{2-} .

j. *Sulfide tertiary standard solution*: Dilute 50 mL sulfide intermediate solution to 500 mL with 0.01*N* NaOH. Prepare fresh daily. Use standardization value from ¶ 2i to determine exact concentration. 1.00 mL $\approx$ 0.001 mg S^{2-} .

k. *Working sulfide standard solutions*: Prepare a suitable series of standards by diluting appropriate volumes of sulfide tertiary standing solutions with 0.01*N* NaOH. Prepare fresh daily.

l. *Zinc acetate preservative solution*: Dissolve 220 g $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$ in 870 mL water (this makes 1 L solution).

3. Procedure

For unpreserved, freshly collected samples and sulfide working standards, add, in order, 4 drops 2*N* zinc acetate, 0.5 mL 6*N*

NaOH, and 400 mg ascorbic acid/100 mL. For preserved samples, add 0.5 mL 6*N* NaOH and 400 mg ascorbic acid/100 mL. Shake well.

Let precipitate settle for at least 30 min. Pour a portion of well-mixed sample or working standard into a sample cup. Set up manifold as shown in Figure 4500-S²⁻.2 and follow the general procedure described by the manufacturer. Determine absorbance at 660 nm.

4. Calculation

Prepare standard curves by plotting peak heights of standards processed through the manifold against S^{2-} concentration in the standards. Compute S^{2-} sample concentration by comparing sample response with standard curve.

5. Precision and Bias

In a single laboratory, samples with S^{2-} concentrations of 0.012, 0.015, 0.034, and 0.085 mg/L had standard deviations of 0.001, 0.001, 0.001, and 0.001 mg/L, respectively, with coefficients of variation of 8.3%, 6.3%, 2.9%, and 1.2%, respectively. In two environmental samples with added S^{2-} , recoveries were 104.2% and 97.6%.

6. Bibliography

FRANCOM, D., L.R. GOODWIN & F.P. DIEKEN. 1989. Determination of low level sulfides in environmental waters by automated gas dialysis/methylene blue colorimetry. *Anal. Lett.* 22:2587.

4500-S²⁻ F. Iodometric Method

1. Reagents

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

b. *Standard iodine solution*, 0.0250*N*: Dissolve 20 to 25 g KI in a little water and add 3.2 g iodine. After iodine has dissolved, dilute to 1000 mL and standardize against 0.0250*N* $\text{Na}_2\text{S}_2\text{O}_3$, using starch solution as indicator.

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

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

2. Procedure

a. Measure from a buret into a 500-mL flask an amount of iodine solution estimated to be an excess over the amount of sulfide present. Add distilled water, if necessary, to bring volume to about 20 mL. Add 2 mL 6*N* HCl. Pipet 200 mL sample into flask, discharging sample under solution surface. If iodine color disappears, add more iodine until color remains. Back-titrate with $\text{Na}_2\text{S}_2\text{O}_3$ solution, adding a few drops of starch solution as end point is approached, and continuing until blue color disappears.

b. If sulfide was precipitated with zinc and ZnS filtered out, return filter with precipitate to original bottle and add about 100

mL water. Add iodine solution and HCl and titrate as in ¶ 2a above.

3. Calculation

One milliliter 0.0250*N* iodine solution reacts with 0.4 mg S^{2-} :

$$\text{mg S}^{2-}/\text{L} = \frac{[(A \times B) - (C \times D)] \times 16\,000}{\text{mL sample}}$$

where:

A = mL iodine solution,

B = normality of iodine solution,

C = mL $\text{Na}_2\text{S}_2\text{O}_3$ solution, and

D = normality of $\text{Na}_2\text{S}_2\text{O}_3$ solution.

4. Precision

The precision of the end point varies with the sample. In clean waters it should be determinable within 1 drop, which is equivalent to 0.1 mg/L in a 200-mL sample.

4500-S²⁻ G. Ion-Selective Electrode Method

1. General Discussion

a. Principle: The potential of a silver/sulfide ion-selective electrode (ISE) is related to the sulfide ion activity. An alkaline antioxidant reagent (AAR) is added to samples and standards to inhibit oxidation of sulfide by oxygen and to provide a constant ionic strength and pH. Use of the AAR allows calibration in terms of total dissolved sulfide concentration. All samples and standards must be at the same temperature. Sulfide concentrations between 0.032 mg/L ($1 \times 10^{-6} M$) and 100 mg/L can be measured without preconcentration. For lower concentrations, preconcentration is necessary.

b. Interferences: Humic substances may interfere with Ag/S-ISE measurements. For highly colored water (high concentration of humic substances), use the method of standard additions to check results. Sulfide is oxidized by dissolved oxygen. Sulfide oxidation may cause potential readings to drift in the direction of decreasing concentration, i.e., to more positive values. Flush surface of samples and standards with nitrogen to minimize contact with atmospheric oxygen for low-level measurements. Temperature changes may cause potentials to drift either upward or downward. Therefore, let standards and samples come to the same temperature. If samples cannot be analyzed immediately, preserve dissolved sulfide by precipitating with zinc acetate (4500-S²⁻.C).

2. Apparatus

*a. Silver/sulfide electrode.**

b. Double-junction reference electrode.

c. Electrode polishing strips.†

d. pH meter with millivolt scale, capable of 0.1-mV resolution. Meters that can be calibrated in concentration and that perform standard-additions calculations are available.

e. Electrochemical cell: Make suitable cell from a 150-mL beaker and a sheet of rigid plastic (PVC or acrylic) with holes drilled to allow insertion of the electrodes and a tube for flushing the headspace with nitrogen. Alternatively, purchase a polarographic cell with gas transfer tube.‡

f. Gas dispersion tube: Use to deaerate water for preparing reagents and standards.

g. Magnetic stirrer and stirring bar: Use a piece of styrofoam or cardboard to insulate the cell from the magnetic stirrer.

3. Reagents

a. Alkaline antioxidant reagent (AAR): To approximately 600 mL deaerated reagent water (DRW) in a 1-L volumetric flask, add 80 g NaOH, 35 g ascorbic acid, and 67 g Na₂H₂EDTA. Swirl to dissolve and dilute to 1 L. The color of freshly prepared AAR will range from colorless to yellow. Store in a tightly capped brown glass bottle. Discard when solution becomes brown.

b. Lead perchlorate, 0.1M: Dissolve 4.60 g Pb(ClO₄)₂ · 3H₂O in 100 mL reagent water. Standardize by titrating with Na₂H₂EDTA. Alternatively, use commercially available 0.1M Pb(ClO₄)₂ solutions.

c. Sulfide stock solution, 130 mg/L: See 4500-S²⁻.A.6, and dilute 13.0 mL of 1.00 mg S²⁻/mL stock to 100.0 mL with AAR. Alternatively, add 500 mL AAR and 1 g Na₂S · 9H₂O to a 1-L volumetric flask; dissolve. Dilute to 1 L with DRW. Use deaerated artificial seawater (DASW), Table 8010:III, or 0.7M NaCl if sulfide concentrations are to be determined in seawater. Standardize stock solution by titrating with 0.1M Pb(ClO₄)₂. Pipet 50 mL sulfide stock solution into the electrochemical cell. (Use 10 mL with a small-volume polarographic cell.) Insert Ag/S electrode and reference electrode and read initial potential. Titrate with 0.1M Pb(ClO₄)₂. Let electrode potential stabilize and record potential after each addition. Locate equivalence point as in Section 4500-Cl⁻.D.4a. Alternatively, linearize the titration curve.¹ Calculate the function F_1 for points before the equivalence point.

$$F_1 = (V_o + V)10^{\frac{E}{m}}$$

where:

V_o = volume of stock solution, mL,

V = titrant volume, mL,

E = potential, mV, and

m = slope of calibration curve, mV/log unit.

Plot F_1 as a function of titrant volume. Extrapolate to find the intersection with the x-axis; that is, the equivalence point. Calculate sulfide concentration in the stock solution from:

$$C = \frac{V_{eq}[Pb]}{V_o}$$

where:

C = sulfide concentration, mg/L,

V_{eq} = equivalence volume, mL,

$[Pb]$ = concentration of Pb in titrant, mg/L, and

V_o = volume of stock solution, mL.

Store stock solution in a tightly capped bottle for 1 week or less. The stock solution also can be standardized iodometrically (see Section 4500-S²⁻.E). CAUTION: Store in a fume hood.

d. Sulfide standards: Prepare sulfide standards daily by serial dilution of stock. Add AAR and Zn(C₂H₃O₂)₂ solutions to 100-mL volumetric flasks. Add sulfide solutions and dilute to volume with DRW (or DASW). Refer to Table 4500-S²⁻.I for volumes. Prepare at least one standard with a concentration less than the lowest sample concentration.

4. Procedure

Check electrode performance and calibrate daily. Check electrode potential in a sulfide standard every 2 h. The procedure depends on the sulfide concentration and the time between

* Orion 941600 or equivalent.

† Orion 948201 or equivalent.

‡ EG&G Princeton Applied Research K0066, K0060, G0028, or equivalent.

TABLE 4500-S²⁻:I. DILUTION OF SULFIDE STOCK SOLUTION FOR PREPARATION OF STANDARDS (100 mL TOTAL VOLUME)

Dilution	Alkaline Antioxidant Reagent mL	Sulfide Solution	Sulfide Solution mL	1M Zinc Acetate mL
1:10	45	Stock	10	0.15
1:100	50	Stock	1	0.15
1:1 000	45	1:100	10	0.14
1:10 000	50	1:100	1	0.15

sample collection and sulfide determination. If the total sulfide concentration is greater than 0.03 mg/L ($1 \times 10^{-6}M$) and the time delay is only a few minutes, sulfide can be determined directly. Otherwise, precipitate ZnS and filter as described in Section 4500-S²⁻.C.

a. *Check electrode performance:* Pipet 50 mL AAR, 50 mL DWR, and 1 mL sulfide stock solution into the measurement cell. Place Ag/S and reference electrodes in the solution and read potential. Add 10 mL stock solution and read potential. The change in potential should be -28 ± 2 mV. If it is not, follow the troubleshooting procedure in the electrode manual.

b. *Calibration:* Place electrodes in the most dilute standard but use calibration standards that bracket the sulfide concentrations in the samples. Record potential when the rate of change is less than 0.3 mV/min. (This may take up to 30 min for very low sulfide concentrations, i.e., less than 0.03 mg/L.) Rinse electrodes, blot dry with a tissue, and read potential of the next highest standard. For a meter that can be calibrated directly in concentration, follow manufacturer's directions. For other meters, plot potential as a function of the logarithm (base 10) of the sulfide concentration. For potentials in the linear range, calculate the slope and intercept of the linear portion of the calibration plot.

c. *Sulfide determination by comparison with calibration curve, no ZnS precipitation:* Add 40 mL AAR, 0.15 mL (3 drops) zinc acetate, and 50 mL sample to a 100-mL volumetric flask. Dilute to 100 mL with AAR. Pour into the electrochemical cell and insert the electrodes. Record potential when the rate of change is less than 0.3 mV/min. Read sulfide concentration from the calibration curve. Alternatively, for potentials in the linear range, calculate the sulfide concentration from:

$$S_{Tot} = 10^{\frac{E-b}{m}}$$

where:

E = electrode potential and

b and m are the intercept and slope of the calibration curve. For a meter that can be calibrated directly in concentration, follow the manufacturer's directions.

d. *Sulfide determination by comparison with calibration curve, with ZnS precipitation:* Place filter with ZnS precipitate in a 150-mL beaker containing a stir bar. Wash sample bottle with 50

mL AAR and 20 mL DRW and pour the washings into the beaker. Stir to dissolve precipitate. Remove filter with forceps while rinsing it into the beaker with a minimum amount of DRW. Quantitatively transfer to a 100-mL volumetric flask and dilute to mark with DRW. Pour into the electrochemical cell and place the electrodes in the solution. Measure potential as in ¶ 4c above. Calculate sulfide concentration (¶ 4c).

e. *Sulfide determination by standard addition with or without ZnS precipitation:* Measure the Ag/S-ISE electrode potential as in ¶ c or d above. Add sulfide stock solution and measure potential again. Calculate sulfide concentration as follows:

$$C_o = \frac{fC_s}{(1+f)10^{\frac{E_s-E_o}{m}} - 1}$$

where:

C_o and C_s = sulfide concentrations in sample and known addition,

E_o and E_s = potentials measured for sample and known addition,

m = slope of calibration curve (approximately 28 mV/log S²⁻, and

f = ratio of known-addition volume to sample volume.

f. *Sulfide determination by titration:* Use the same procedure as for standardizing the sulfide stock solution (¶ 3c). The minimum sulfide concentration for determination by titration is 0.3 mg/L ($10^{-5}M$).

5. Precision

For sulfide determination by comparison with the calibration curve, the relative standard deviation varies with the sulfide concentration. RSD values of 23% for 0.0091 mg/L and 5% for 0.182 mg/L have been reported.² (0.0091 µg/L was below the range for which the potential varied linearly with the logarithm of the sulfide concentration, i.e., the Nernstian range.) For sulfide determination by standard addition, the precision is greatest if the amount of sulfide added is as large as possible while staying within the linear range.³

6. References

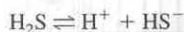
1. GRAN, G. 1952. Determination of the equivalence point in potentiometric titrations. Part II. *Analyst* 77:661.
2. BAUMANN, E. 1974. Determination of parts per billion sulfide in water with the sulfide-selective electrode. *Anal. Chem.* 46:1345.
3. RATZLAFF, K.L. 1979. Optimizing precision in standard addition measurement. *Anal. Chem.* 51:232.

7. Bibliography

- ORION RESEARCH, INC. 1980. Instruction Manual for Silver-Sulfide Electrode.
- VIVIT, D.V., J.W. BALL & E.A. JENNE. 1984. Specific-ion electrode determinations of sulfide preconcentrated from San Francisco Bay waters. *Environ. Geol. Water Sci.* 6:79.

4500-S²⁻ H. Calculation of Un-ionized Hydrogen Sulfide

Hydrogen sulfide (H₂S) and bisulfide ion (HS⁻), which together constitute dissolved sulfide, are in equilibrium with hydrogen ions:



The fraction of sulfide present as un-ionized H₂S can be estimated with an error of less than 40% from Figure 4500-S²⁻:3. If more accuracy is needed, use the methods given below. For both fresh water and seawater, it is convenient to define "conditional" dissociation constants, which are valid for the temperature and ionic strength of the water of interest. In the following mass-action equation for fresh water, K'_{FW} is a mixed equilibrium constant that relates the hydrogen ion activity (calculated from the pH) and the concentrations of H₂S and HS⁻:

$$K'_{FW} = \frac{[\text{H}^+][\text{HS}^-]}{[\text{H}_2\text{S}]}$$

The square brackets indicate concentrations and the braces indicate activity. The value of pK'_{FW} for H₂S is approximately 7.0 ± 0.3 for the ionic strengths and temperatures likely to be encountered in water-quality monitoring. For seawater, it is convenient to use a stoichiometric equilibrium constant (K'_{SW}), which relates the concentrations of H⁺, HS⁻, and H₂S:

$$K'_{SW} = \frac{[\text{H}^+][\text{HS}^-]}{[\text{H}_2\text{S}]}$$

The mass-action equations can be rearranged to give:

$$\text{pH} - pK' = \log \frac{[\text{HS}^-]}{[\text{H}_2\text{S}]}$$

In this equation, pK' can be either pK'_{FW} or pK'_{SW} . The fraction of un-ionized H₂S can either be read from Figure 4500-S²⁻:3 or calculated with the following equation:

$$\alpha_{\text{H}_2\text{S}} = \frac{[\text{H}_2\text{S}]}{S_T} = \frac{1}{10^{\text{pH} - pK'} + 1}$$

where:

S_T = total dissolved sulfide concentration.

1. Calculation for Fresh Water ($I \leq 0.01M$)

Calculate ionic strength I as in Table 2330:I. Read value of pK'_{FW} from Table 4500-S²⁻:II.

Sample calculation: Total sulfide concentration, 1.5 mg S²⁻/L; pH, 6.87; temperature, 10°C; ionic strength, 0.04. From Table 4500-S²⁻:II, $pK'_{FW} = 7.11$.

$$\text{pH} - pK'_{FW} = -0.24$$

$$10^{\text{pH} - pK'_{FW}} = 10^{-0.24} = 0.575$$

$$\alpha_{\text{H}_2\text{S}} = \frac{1}{1 + 0.575} = 0.63$$

$$0.63 \times 1.5 = 0.95$$

The concentration of un-ionized H₂S is 0.95 mg S²⁻/L.

2. Calculation for Seawater, Estuarine Water, and Brackish Water

This procedure is the same as that for fresh water. A potential source of error is the determination of the hydrogen ion concentration. If the pH electrode is calibrated using NIST buffers as in Section 4500-H⁺, then a correction factor² must be determined. Add acid (HNO₃, HCl, or HClO₄, not H₂SO₄) to artificial seawater diluted to the salinity of interest and at the temperature of interest to give an acid concentration of 0.001N. (Prepare artificial seawater as in Table 8010:III, substituting NaCl for Na₂SO₄ on an equimolar basis and omitting NaF, SrCl₂ · 6H₂O, H₃BO₃, KBr, Na₂SiO₃ · 9H₂O, Na₄EDTA, and NaHCO₃.) Measure the pH. The difference between the negative logarithm of the known acid concentration and the measured pH is the correction factor. For example, if the acid concentration is 0.001N and the measured pH is 3.15, the correction factor is 0.15. Subtract 0.15 from measured pH values to get p^cH, the negative logarithm of the hydrogen ion concentration. (The pH in fresh water corresponds to the negative logarithm of the hydrogen ion activity.) Alternatively, calibrate the pH electrode with Tris* buffer in artificial seawater diluted to the salinity of interest and at the temperature of interest.³ Read pK'_{SW} from Table 4500-S²⁻:III. Calculate the fraction of un-ionized H₂S as for fresh water.

Sample calculation: Total sulfide concentration, 1.5 mg S²⁻/L; temperature 10°C; pH, 7.15; salinity 25‰. From Table 4500-S²⁻: III, $pK'_{SW} = 6.87$.

$$\text{pH} - pK'_{SW} = 0.28$$

$$10^{\text{pH} - pK'_{SW}} = 10^{0.28} = 1.91$$

$$\alpha_{\text{H}_2\text{S}} = \frac{1}{1 + 1.91} = 0.34$$

$$0.34 \times 1.5 = 0.51$$

The concentration of un-ionized H₂S is 0.51 mg S²⁻/L.

3. References

1. MILLERO, F. J. 1986. The thermodynamics and kinetics of the hydrogen sulfide system in natural waters. *Mar. Chem.* 18:121.
2. SIGEL, H., A. D. ZUBERBUHLER & O. YAMAUCHI. 1991. Comments on potentiometric pH titrations and the relationship between pH-meter reading and hydrogen ion concentration. *Anal. Chim. Acta.* 255:63.
3. MILLERO, F. J. 1986. The pH of estuarine waters. *Limnol. Oceanogr.* 31:839.

* Trishydroxymethylaminomethane.

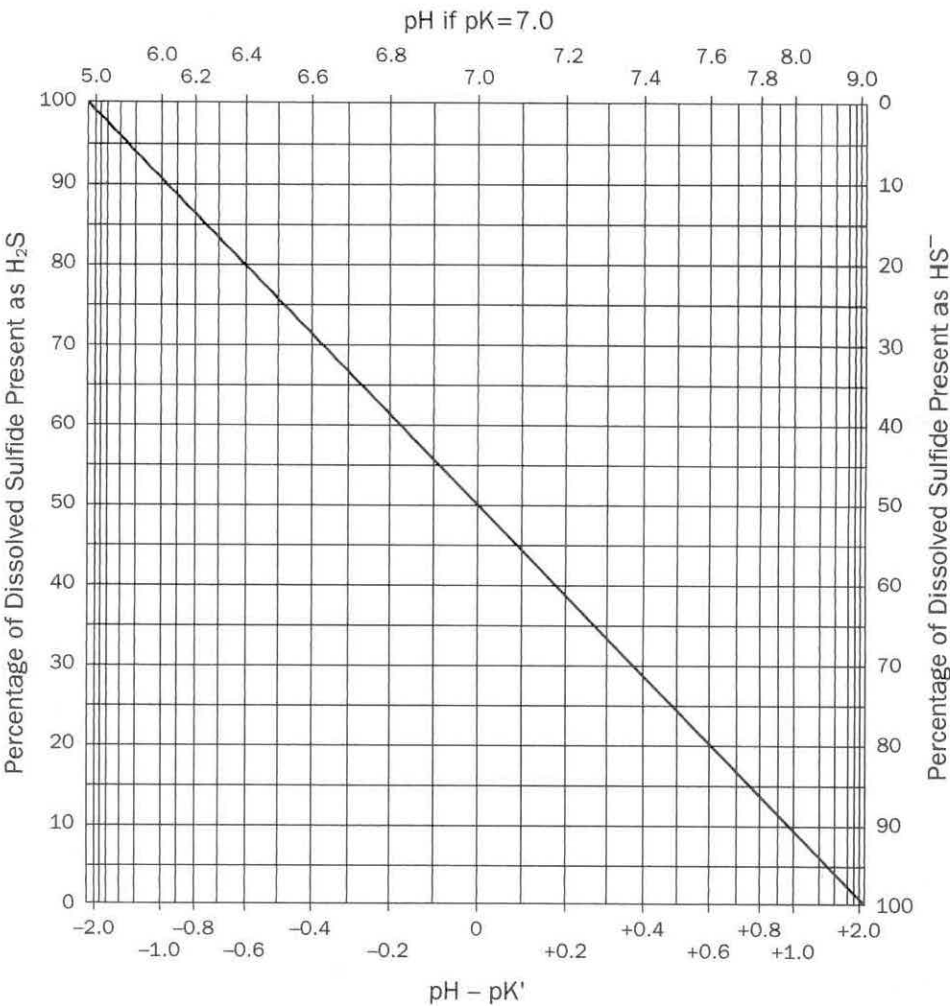


Figure 4500-S²⁻:3. Proportion of H_2S and HS^- in dissolved sulfide.

TABLE 4500-S²⁻:II. CONDITIONAL FIRST DISSOCIATION CONSTANT OF HYDROGEN SULFIDE, FRESH WATER*

Temperature °C	pK'_{FW} at Given Ionic Strength						
	0.00 mol/L	0.005 mol/L	0.01 mol/L	0.02 mol/L	0.03 mol/L	0.05 mol/L	0.10 mol/L
0	7.36	7.33	7.32	7.30	7.29	7.27	7.24
5	7.28	7.25	7.23	7.22	7.21	7.19	7.16
10	7.20	7.16	7.15	7.13	7.12	7.10	7.07
15	7.12	7.09	7.08	7.06	7.05	7.03	7.00
20	7.05	7.02	7.00	6.99	6.97	6.96	6.92
25	6.98	6.95	6.94	6.92	6.91	6.89	6.86
30	6.92	6.89	6.87	6.86	6.84	6.83	6.79

* Values calculated according to Millero¹.

TABLE 4500-S²⁻:III. CONDITIONAL FIRST DISSOCIATION CONSTANT OF HYDROGEN SULFIDE, SEAWATER*

Temperature °C	pK'_{sw} at Given Salinity						
	5‰	10‰	15‰	20‰	25‰	30‰	35‰
0	7.17	7.12	7.09	7.07	7.06	7.06	7.06
5	7.08	7.02	6.99	6.97	6.96	6.96	6.96
10	6.99	6.93	6.90	6.88	6.87	6.86	6.86
15	6.91	6.85	6.82	6.80	6.78	6.78	6.77
20	6.83	6.77	6.74	6.72	6.70	6.69	6.69
25	6.76	6.70	6.66	6.64	6.63	6.62	6.61
30	6.70	6.63	6.60	6.57	6.56	6.55	6.54

* Values calculated according to Millero¹.

4. Bibliography

D'YACHKOVA, I. B. & I. L. KHODAKOVSKIY. 1968. Thermodynamic equilibria in the systems S-H₂O, Se-H₂O and Te-H₂O in the 25–300°C temperature range and their geochemical interpretations. *Geochem. Internat.* 1108.

ARCHER, D. G. & P. WANG. 1990. The dielectric constant of water and Debye-Huckel limiting law slopes. *J. Phys. Chem. Ref. Data* 12: 817.

STUMM, W. & J. J. MORGAN. 1991. *Aquatic Chemistry*, 3rd ed. John Wiley & Sons, New York, N.Y.

4500-S²⁻ I. Distillation, Methylene Blue Flow Injection Analysis Method

1. General Discussion

a. Principle: Water and wastewater samples are distilled into a sodium hydroxide trapping solution and the distillate is analyzed. Hydrogen sulfide (H₂S) reacts in acid media and in the presence of ferric chloride with two molecules of *N, N*-dimethyl-*p*-phenylenediamine to form methylene blue. The resulting color is read at 660 nm.

b. Sample preservation: Because H₂S oxidizes rapidly, analyze samples and standards without delay. To preserve samples, add 4 drops 2M zinc acetate to 100 mL sample and adjust pH to >9 with 6M NaOH, then cool to 4°C. Samples are distilled into a trapping solution resulting in 0.25M NaOH matrix.

Also see Sections 4500-S²⁻.A, B, and E, and Section 4130, Flow Injection Analysis (FIA).

c. Interferences: This method measures total sulfide, which is defined as the acid-soluble sulfide fraction of a sample. Total sulfide includes both acid-soluble sulfides such as H₂S, and acid-soluble metal sulfides present in suspended matter. This method does not measure acid-insoluble sulfides such as CuS.

Most nonvolatile interferences are eliminated by distillation. Strong reducing agents inhibit color formation at concentrations of several hundred milligrams per liter. Iodide interferes at concentrations greater than 2 mg IL.

Also see Section 4500-S²⁻.A and B.

2. Apparatus

a. Distillation apparatus consisting of a glass or polypropylene micro-distillation device* capable of distilling 6 mL or

more of sample into a 0.25M NaOH final concentration trapping solution.

b. Flow injection analysis equipment consisting of:

1) *FIA injection valve* with sample loop or equivalent.

2) *Multichannel proportioning pump*.

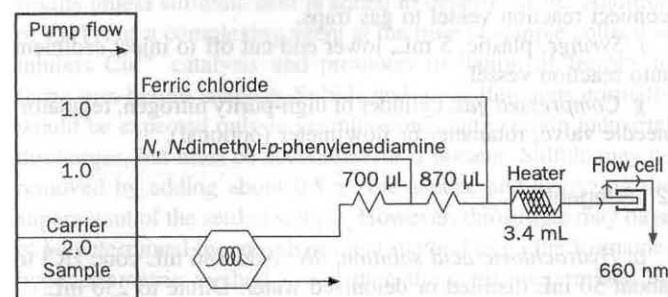
3) *FIA manifold* (Figure 4500-S²⁻:4) with cation exchange column and flow cell. Relative flow rates only are shown in Figure 4500-S²⁻:4. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

4) *Absorbance detector*, 660 nm, 10-nm bandpass.

5) *Injection valve control and data acquisition system*.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier 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.

Figure 4500-S²⁻:4. FIA sulfide manifold.

* Lachat Instruments MICRO DIST or equivalent.

a. *Sodium hydroxide diluent*, NaOH, 0.25M: In a 2-L volumetric flask, dissolve 20 g NaOH in approximately 1800 mL water. Dilute to mark and mix with a magnetic stirrer until dissolved. Store in a plastic container.

b. *Hydrochloric acid*, HCl, 3M: To a tared 1-L container, add 752 g water and then slowly add 295 g conc HCl. Invert to mix.

c. *Hydrochloric acid*, HCl, 0.20M: To a tared 1-L container, add 983.5 g water. Then add 19.7 g conc HCl. Invert to mix.

d. *N,N*-dimethyl-*p*-phenylenediamine: In a 1-L volumetric flask dissolve 1.0 g *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride, $(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{NH}_2 \cdot 2\text{HCl}$, in about 800 mL 3M HCl (¶ 3b). Dilute to mark and invert to mix. If solution appears dark, it is likely that the *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride is decomposed; discard, and use fresh reagent.

e. *Ferric chloride*: In a 500-mL volumetric flask dissolve 6.65 g ferric chloride hexahydrate, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, in about 450 mL 0.20M HCl (¶ 3c). Dilute to mark with water and invert to mix.

f. *Stock sulfide standard*, 100 mg S^{2-}/L : In a 1-L volumetric flask dissolve 0.750 g sodium sulfide nonahydrate, $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, in approximately 900 mL NaOH diluent (¶ 3a). Dilute to mark and invert to mix. Standardize as in 4500-S²⁻.F; also see 4500-S²⁻.A.6.

g. *Standard solutions*: Prepare sulfide standards in desired concentration range, using stock standard (¶ 3f), and diluting with NaOH diluent (¶ 3a).

h. *Sulfuric acid distillation releasing solution*, H_2SO_4 , 9M: To a tared 500-mL container, add 150.0 g water, then add slowly while swirling, in increments of 40 g, 276 g conc H_2SO_4 . CAUTION: Solution will become very hot. Allow to cool before using.

4. Procedure

a. *Distillation*: This procedure is designed for the determination of sulfides in aqueous solutions, solid waste materials, or

effluents. To preserve and remove sulfide from interfering substances, distill samples immediately after collection.

Follow manufacturer's instructions for use of distillation apparatus. Add sufficient 9M H_2SO_4 (¶ 3h) to sample to dissolve ZnS (s), digest total sulfides, and release the sulfide as hydrogen sulfide gas. Immediately place sample on-line with the receiving vessel or collector tube and distill hydrogen sulfide and water in the sample into a 0.25M trapping solution.

b. *Flow injection analysis*: Set up a manifold equivalent to that in Figure 4500-S²⁻.4 and follow method supplied by the manufacturer or laboratory standard operating procedure. Follow quality control protocols outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus sulfide concentration.

6. Precision and Bias (MDL)

a. *Method detection level (MDL)*: A 200- μL sample loop was used in the method described above. Using a published method¹, analysts ran 21 replicates of 0.02-mg S^{2-}/L standard. These gave a mean of 0.024 mg S^{2-}/L , a standard deviation of 0.0021 mg S^{2-}/L , and MDL of 0.006 mg S^{2-}/L .

b. *Precision*: Ten injections of a distilled 0.8 mg S^{2-}/L standard gave a mean of 0.82 mg S^{2-}/L , a standard deviation of 0.0054 mg S^{2-}/L , and percent relative standard deviation of 0.66.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY, 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 Amended June 30, 1986. 49CFR 43430.

4500-S²⁻.J. Acid-Volatile Sulfide

1. Apparatus

See Figure 4500-S²⁻.5.

a. *Reaction vessel*, 250-mL, 3-neck flask, standard taper.

b. *Gas traps*, 125-mL gas scrubbers.

c. *Dropping funnel*, standard taper to fit reaction vessel.

d. *Purge-gas tube*, standard taper to fit reaction vessel.

e. *Tubing*, TFE or polypropylene, 0.635 cm (0.25-in.) OD, to connect reaction vessel to gas traps.

f. *Syringe*, plastic, 5 mL, lower end cut off to inject sediment into reaction vessel.

g. *Compressed gas*, cylinder of high-purity nitrogen, regulator, needle valve, rotameter or flow meter (optional).

2. Reagents

a. *Hydrochloric acid solution*, 9N: Add 186 mL conc HCl to about 50 mL distilled or deionized water. Dilute to 250 mL.

b. *Stannous chloride solution*, 0.53M: Dissolve 50 g SnCl_2 in 250 mL 9N HCl solution.

c. *Sodium hydroxide solution*, NaOH, 2N.

d. *Zinc acetate solution*, 1M, 22%: Dissolve 220 g $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ in 1 L distilled or deionized water.

e. *Alkaline zinc solution*: Add 150 mL 22% zinc acetate solution to 850 mL 2N NaOH solution.

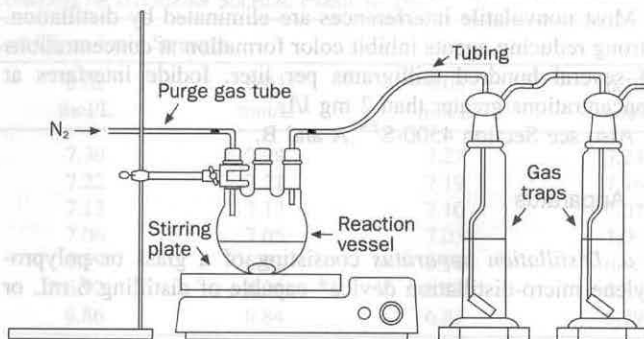


Figure 4500-S²⁻.5: Apparatus for acid-volatile sulfide analysis.

f. *Reagents for the iodometric titration method:* See Section 4500-S²⁻.F.

3. Procedure

Add 15 mL stannous chloride/hydrochloric acid solution to digestion vessel and 100 mL alkaline zinc solution to each trap. Adjust nitrogen flow rate to approximately 40 to 60 mL/min and flush system for 10 min. Add approximately 6 g fresh wet sediment to digestion vessel using a 5-mL syringe with its distal ends removed. Close outlets with clamps and gently stir the suspension. Allow H₂S generation to take place at room temperature (25±3°C) for 3 h while stirring and with the nitrogen flowing. Combine the solutions from the two traps and titrate as described in Section 4500-S²⁻.F.

4. Precision and Bias

For marine sediment samples (n=6), an average of 97% of the added ferrous monosulfide was recovered by the diffusion method. Reproducibility of this measurement performed on four

samples with concentrations of around 40 to 150 μmols/g dry weight (1.3 to 4.8 mg S/g) was better than 5%.

5. Bibliography

- CHANTON, J.P. & C.S. MARTENS. 1985. The effects of heat and stannous chloride on the active distillation of acid volatile sulfide from pyrite-rich marine sediment samples. *Biogeochemistry* 1:375.
- MORSE, J.W. & J.C. CORNWELL. 1987. Analysis and distribution of iron sulfide minerals in recent anoxic marine sediments. *Mar. Chem.* 22:55.
- HSIEH, Y.P. & C.H. YANG. 1989. Diffusion methods for the determination of reduced inorganic sulfur species in sediments. *Limnol. Oceanogr.* 34:1126.
- ALLEN, H.E., G.-M. FU & B.-L. DENG. 1993. Analysis of acid-volatile sulfide (AVS) and simultaneously extracted metals (SEM) for the estimation of potential toxicity in aquatic sediments. *Environ. Toxicol. Chem.* 12:1441.
- LASORSA, B. & A. CASAS. 1996. A comparison of sample handling and analytical methods for determination of acid volatile sulfides in sediment. *Mar. Chem.* 52:211.
- LEONARD, E.N., A.M. COTTER & G.T. ANKLEY. 1996. Modified diffusion method for analysis of acid volatile sulfides and simultaneously extracted metals in freshwater sediment. *Environ. Toxicol. Chem.* 15:1479.

4500-SO₃²⁻ SULFITE*

4500-SO₃²⁻ A. Introduction

water treatment and discharge may be important environmentally, principally because of its toxicity to fish and other aquatic life and its rapid oxygen demand.

2. Selection of Method

The iodometric titration method is suitable for relatively clean waters with concentrations above 2 mg SO₃²⁻/L. The phenanthroline colorimetric determination, following evolution of sulfite from the sample matrix as SO₂, is preferred for low levels of sulfite.

4500-SO₃²⁻ B. Iodometric Method

SO₃²⁻ in the acidic reaction medium and lead to low sulfite results unless sulfamic acid is added to destroy nitrite. Addition of EDTA as a complexing agent at the time of sample collection inhibits Cu²⁺ catalysis and promotes oxidation of ferrous to ferric iron before analysis. Sulfide and thiosulfate ions normally would be expected only in samples containing certain industrial discharges, but must be accounted for if present. Sulfide may be removed by adding about 0.5 g zinc acetate and analyzing the supernatant of the settled sample. However, thiosulfate may have to be determined by an independent method (e.g., the formaldehyde/iodometric method¹), and then the sulfite determined by difference.

c. *Minimum detectable concentration:* 2 mg SO₃²⁻/L.

1. Occurrence

Sulfite ions (SO₃²⁻) may occur in boilers and boiler feedwaters treated with sulfite for dissolved oxygen control, in natural waters or wastewaters as a result of industrial pollution, and in treatment plant effluents dechlorinated with sulfur dioxide (SO₂). Excess sulfite ion in boiler waters is deleterious because it lowers the pH and promotes corrosion. Control of sulfite ion in waste-

*Approved by Standard Methods Committee, 2000.

1. General Discussion

a. *Principle:* An acidified sample containing sulfite (SO₃²⁻) is titrated with a standardized potassium iodide-iodate titrant. Free iodine, liberated by the iodide-iodate reagent, reacts with SO₃²⁻. The titration endpoint is signalled by the blue color resulting from the first excess of iodine reacting with a starch indicator.

b. *Interferences:* The presence of other oxidizable materials, such as sulfide, thiosulfate, and Fe²⁺ ions, can cause apparently high results for sulfite. Some metal ions, such as Cu²⁺, may catalyze the oxidation of SO₃²⁻ to SO₄²⁻ when the sample is exposed to air, thus leading to low results. NO₂⁻ will react with

2. Reagents

- a. *Sulfuric acid*: H_2SO_4 , 1 + 1.
- b. *Standard potassium iodide-iodate titrant*, 0.002083M: Dissolve 0.4458 g primary-grade anhydrous KIO_3 (dried for 4 h at 120°C), 4.35 g KI, and 310 mg sodium bicarbonate (NaHCO_3) in distilled water and dilute to 1000 mL; $1.00\text{ mL} = 500\text{ }\mu\text{g SO}_3^{2-}$.
- c. *Sulfamic acid*, $\text{NH}_2\text{SO}_3\text{H}$, crystalline.
- d. *EDTA reagent*: Dissolve 2.5 g disodium EDTA in 100 mL distilled water.
- e. *Starch indicator*: To 5 g starch (potato, arrowroot, or soluble) in a mortar, add a little cold distilled water and grind to a paste. Add mixture to 1 L boiling distilled water, stir, and let settle overnight. Use clear supernatant. Preserve by adding either 1.3 g salicylic acid, 4 g ZnCl_2 , or a combination of 4 g sodium propionate and 2 g sodium azide to 1 L starch solution.

3. Procedure

- a. *Sample collection*: Collect a fresh sample, taking care to minimize contact with air. Fix cooled samples ($<50^\circ\text{C}$) immediately by adding 1 mL EDTA solution/100 mL sample. Cool hot samples to 50°C or below. Do not filter.
- b. *Titration*: Add 1 mL H_2SO_4 and 0.1 g $\text{NH}_2\text{SO}_3\text{H}$ crystals to a 250-mL erlenmeyer flask or other suitable titration vessel. Accurately measure 50 to 100 mL EDTA-stabilized sample into flask, keeping pipet tip below liquid surface. Add 1 mL starch indicator solution. Titrate immediately with standard KI- KIO_3 titrant, while swirling flask, until a faint permanent blue color develops. Analyze a reagent blank using distilled water instead of sample.

4. Calculation

$$\text{mg SO}_3^{2-}/\text{L} = \frac{(A - B) \times M \times 6 \times 40\,000}{\text{mL sample}}$$

where:

- A = mL titrant for sample,
 B = mL titrant for blank, and
 M = molarity of KI- KIO_3 titrant.

5. Precision and Bias

Three laboratories analyzed five replicate portions of a standard sulfite solution and of secondary treated wastewater effluent to which sulfite was added. The data are summarized below. Individual analyst's precision ranged from 0.7 to 3.6% standard deviation ($N = 45$).

Sample	$\bar{X}$ mg/L	Standard Deviation, σ mg/L	Relative Standard Deviation %
Standard, 6.3 mg $\text{SO}_3^{2-}/\text{L}$	4.5	0.25	5.5
Secondary effluent with 2.0 mg $\text{SO}_3^{2-}/\text{L}$	2.1	0.28	13.4
Secondary effluent with 4.0 mg $\text{SO}_3^{2-}/\text{L}$	3.6	0.17	4.8

6. Reference

1. KURTENACKER, A. 1924. The aldehyde-bisulfite reaction in mass analysis. *Z. Anal. Chem.* 64:56.

4500-SO₃²⁻ C. Phenanthroline Method

1. General Discussion

a. *Principle*: An acidified sample is purged with nitrogen gas and the liberated SO_2 is trapped in an absorbing solution containing ferric ion and 1,10-phenanthroline. Ferric iron is reduced to the ferrous state by SO_2 , producing the orange tris(1,10-phenanthroline) iron(II) complex. After excess ferric iron is removed with ammonium bifluoride, the phenanthroline complex is measured colorimetrically at 510 nm.¹

b. *Interferences*: See Section 4500-SO₃²⁻.B.1b.

c. *Minimum detectable concentration*: 0.01 mg $\text{SO}_3^{2-}/\text{L}$.

2. Apparatus

a. *Apparatus for evolution of SO_2* : Figure 4500-SO₃²⁻:1 shows the following components:

- 1) *Gas flow meter*, with a capacity to measure 2 L/min of pure nitrogen gas.
- 2) *Gas washing bottle*, 250-mL, with coarse-porosity, 12-mm-diam fritted cylinder gas dispersion tube.

- 3) *Tubing connectors*, quick-disconnect, polypropylene.
- 4) *Tubing*, flexible PVC, for use in all connections.
- 5) *Nessler tube*, 100-mL.

b. *Colorimetric equipment*: One of the following is required:

- 1) *Spectrophotometer*, for use at 510 nm, providing a light path of 1 cm or longer.
- 2) *Filter photometer*, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 510 nm.

3. Reagents

a. *1,10-phenanthroline solution*, 0.03M: Dissolve 5.95 g 1,10-phenanthroline in 100 mL 95% ethanol. Dilute to 1 L with distilled water. Discard if solution becomes colored.

b. *Ferric ammonium sulfate solution*, 0.01M: Dissolve 4.82 g $\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ in 1 L distilled water to which has been added 1 mL conc H_2SO_4 to suppress ferric hydrolysis. Filter through a glass fiber filter if insoluble matter is visible. If necessary, adjust volume of acid so that a mixture of 10 parts of

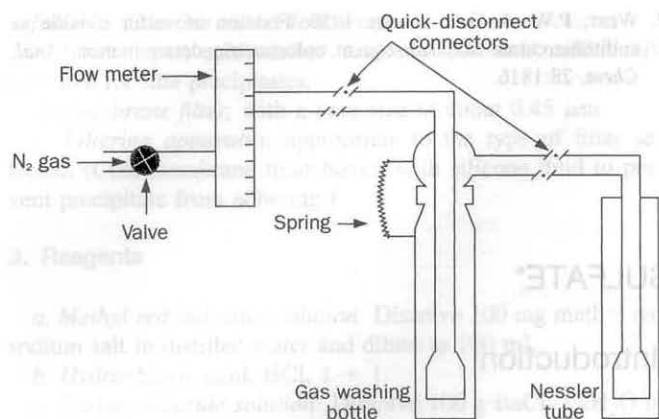


Figure 4500-SO₃²⁻.1. Apparatus for evolution of SO₂ from samples for colorimetric analysis.

phenanthroline solution and one part of ferric ammonium sulfate solution will have a pH between 5 and 6.

c. *Ammonium bifluoride, 5%:* Dissolve 25 g NH₄HF₂ in 500 mL distilled water. Store in a polyethylene bottle and dispense with a plastic pipet.

d. *Potassium tetrachloromercurate (TCM), K₂HgCl₄, 0.04M:* Dissolve 10.86 g HgCl₂, 5.96 g KCl, and 0.066 g disodium EDTA in distilled water and dilute to 1 L. Adjust pH to 5.2. This reagent normally is stable for 6 months, but discard if a precipitate forms.²

e. *Dilute TCM-stabilized sulfite standard:* Dissolve 0.5 g Na₂SO₃ in 500 mL distilled water. Standardize on the day of preparation, but wait at least 30 min to allow the rate of oxidation to slow. Determine molarity by titrating with standard 0.0125M potassium iodide-iodate titrant using starch indicator (see Section 4500-SO₃²⁻.B). Calculate molarity of working standard as follows:

$$\text{Molarity of SO}_3^{2-}\text{ standard} = \frac{(A - B) \times M}{\text{mL sample}}$$

where:

A = titrant for sample, mL,

B = titrant for blank, mL,

M = molarity of potassium iodide-iodate titrant.

Because stock Na₂SO₃ solution is unstable, immediately after standardization, pipet 10 mL into a 500-mL volumetric flask partially filled with TCM and dilute to mark with TCM. Calculate the concentration of this dilute sulfite solution by multiplying the stock solution concentration by 0.02. This TCM-stabilized standard is stable for 30 d if stored at 5°C. Discard as soon as any precipitate is noticed at the bottom.

f. *Standard potassium iodide-iodate titrant, 0.0125M:* See Section 4500-SO₃²⁻.B.2b.

g. *Hydrochloric acid, 1 + 1.*

h. *Octyl alcohol, reagent-grade.*

i. *Sulfamic acid, 10%:* Dissolve 10 g NH₂SO₃H in 100 mL distilled water. This reagent can be kept for a few days if protected from air.

j. *EDTA reagent:* See Section 4500-SO₃²⁻.B.2d.

4. Procedure

a. *Sample collection:* Collect a fresh sample taking care to minimize contact with air. Fix cooled samples (<50°C) immediately by adding 1 mL EDTA solution for each 100 mL sample.

b. *SO₂ evolution:* Prepare the absorbing solution by adding 5 mL 1,10-phenanthroline solution, 0.5 mL ferric ammonium sulfate solution, 25 mL distilled water, and 5 drops octyl alcohol (to act as defoamer) to a 100-mL nessler tube; insert a gas dispersion tube. Add 1 mL sulfamic acid solution to the gas washing bottle and 100 mL of sample or a portion containing less than 100 μg SO₃²⁻ diluted to 100 mL. Add 10 mL 1 + 1 HCl and immediately connect the gas washing bottle to the gas train as shown in Figure 4500-SO₃²⁻.1. Place a spring or rubber band on the gas washing bottle to keep the top securely closed during gas flow. Adjust nitrogen flow to 2.0 L/min and purge for 60 min.

c. *Colorimetric measurement:* After exactly 60 min, turn off nitrogen flow, disconnect nessler tube, and immediately add 1 mL ammonium bifluoride solution. Remove gas dispersion tube after rinsing it with distilled water into the tube and forcing the rinse water into the nessler tube with a rubber bulb. Dilute to 50 mL in the nessler tube and mix by rapidly moving the tube in a circular motion. Do not let rubber stoppers or PVC tubing come in contact with the absorbing solution. After at least 5 min from the time of adding ammonium bifluoride, read the absorbance versus distilled water at 510 nm using either a 5-cm cell for a range of 0 to 30 μg SO₃²⁻ per portion or a 1-cm cell for a range of 0 to 100 μg SO₃²⁻. Avoid transferring octyl alcohol into the cell by letting it rise to the surface of the absorbing solution and transferring the clear lower solution to the cell with a pipet. Make a calibration curve by analyzing a procedure blank and at least three standards. Run at least one standard with each set of samples. For maximum accuracy hold samples and standards at the same temperature and keep the time interval from start of purging to the addition of ammonium bifluoride constant. This is easier to do if several gas trains are used simultaneously in parallel. If ambient temperatures are subject to frequent fluctuation, a water bath may be used to control color development at a fixed temperature.

5. Calculation

$$\text{mg SO}_3^{2-}/\text{L} = \frac{\mu\text{g SO}_3^{2-} \text{ from calibration curve}}{\text{mL sample}}$$

6. Precision and Bias

Three laboratories analyzed five replicate portions of a standard sulfite solution and of secondary treated wastewater effluent to which sulfite was added. The data are summarized below. Individual analyst's precision ranged from 4.1 to 10.5% standard deviation (N = 45).

Sample	$\bar{X}$ mg/L	Standard Deviation, σ mg/L	Relative Standard Deviation %
Standard, 4.7 mg SO ₃ ²⁻ /L	3.7	0.78	21
Secondary effluent with 0.12 mg SO ₃ ²⁻ /L	0.12	0.03	25
Secondary effluent with 4.0 mg SO ₃ ²⁻ /L	3.7	0.30	8.0

7. References

1. STEPHENS, B.G. & F. LINDSTROM. 1964. Spectrophotometric determination of sulfur dioxide suitable for atmospheric analysis. *Anal. Chem.* 36:1308.

2. WEST, P.W. & G.C. GAEKE. 1956. Fixation of sulfur dioxide as sulfitomercurate and subsequent colorimetric determination. *Anal. Chem.* 28:1816.

4500-SO₄²⁻ SULFATE*4500-SO₄²⁻ A. Introduction

1. Occurrence

Sulfate (SO₄²⁻) is widely distributed in nature and may be present in natural waters in concentrations ranging from a few to several thousand milligrams per liter. Mine drainage wastes may contribute large amounts of SO₄²⁻ through pyrite oxidation. Sodium and magnesium sulfate exert a cathartic action.

2. Selection of Method

The ion chromatographic method (4110) and capillary ion electrophoresis (CIE—see Section 4140) are suitable for sulfate

concentrations above 0.1 mg/L. The gravimetric methods (C and D) are suitable for SO₄²⁻ concentrations above 10 mg/L. The turbidimetric method (E) is applicable in the range of 1 to 40 mg SO₄²⁻ /L. The automated methylthymol blue methods (F and G) are the procedures for analyzing large numbers of samples for sulfate alone when the equipment is available; over 30 samples can be analyzed per hour. Methods C, D, F, G, 4110, or CIE (4140) are preferred for accurate results.

3. Sampling and Storage

In the presence of organic matter certain bacteria may reduce SO₄²⁻ to S²⁻. To avoid this, store samples at 4°C.

4500-SO₄²⁻ B. (Reserved)4500-SO₄²⁻ C. Gravimetric Method with Ignition of Residue

1. General Discussion

a. Principle: Sulfate is precipitated in a hydrochloric acid (HCl) solution as barium sulfate (BaSO₄) by the addition of barium chloride (BaCl₂).

The precipitation is carried out near the boiling temperature, and after a period of digestion the precipitate is filtered, washed with water until free of Cl⁻, ignited or dried, and weighed as BaSO₄.

b. Interference: The gravimetric determination of SO₄²⁻ is subject to many errors, both positive and negative. In potable waters where the mineral concentration is low, these may be of minor importance.

1) Interferences leading to high results—Suspended matter, silica, BaCl₂ precipitant, NO₃⁻, SO₃²⁻ and occluded mother liquor in the precipitate are the principal factors in positive errors. Suspended matter may be present in both the sample and the precipitating solution; soluble silicate may be rendered insoluble and SO₃²⁻ may be oxidized to SO₄²⁻ during analysis. Barium nitrate [Ba(NO₃)₂], BaCl₂, and water are occluded to some extent with the BaSO₄ although water is driven off if the temperature of ignition is sufficiently high.

2) Interferences leading to low results—Alkali metal sulfates frequently yield low results. This is true especially of alkali hydrogen sulfates. Occlusion of alkali sulfate with BaSO₄ causes substitution of an element of lower atomic weight than barium in the precipitate. Hydrogen sulfates of alkali metals act similarly and, in addition, decompose on being heated. Heavy metals, such as chromium and iron, cause low results by interfering with the complete precipitation of SO₄²⁻ and by formation of heavy metal sulfates. BaSO₄ has small but significant solubility, which is increased in the presence of acid. Although an acid medium is necessary to prevent precipitation of barium carbonate and phosphate, it is important to limit its concentration to minimize the solution effect.

2. Apparatus

- a. Steam bath.*
- b. Drying oven, equipped with thermostatic control.*
- c. Muffle furnace, with temperature indicator.*
- d. Desiccator.*
- e. Analytical balance, capable of weighing to 0.1 mg.*

* Approved by Standard Methods Committee, 1997.

Joint Task Group: 20th Edition (4500-SO₄²⁻.G)—Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

f. *Filter*: Use one of the following:

1) *Filter paper*, acid-washed, ashless hard-finish, sufficiently retentive for fine precipitates.

2) *Membrane filter*, with a pore size of about 0.45 μm .

g. *Filtering apparatus*, appropriate to the type of filter selected. (Coat membrane filter holder with silicone fluid to prevent precipitate from adhering.)

3. Reagents

a. *Methyl red indicator solution*: Dissolve 100 mg methyl red sodium salt in distilled water and dilute to 100 mL.

b. *Hydrochloric acid*, HCl, 1 + 1.

c. *Barium chloride solution*: Dissolve 100 g BaCl₂ · 2H₂O in 1 L distilled water. Filter through a membrane filter or hard-finish filter paper before use; 1 mL is capable of precipitating approximately 40 mg SO₄²⁻.

d. *Silver nitrate-nitric acid reagent*: Dissolve 8.5 g AgNO₃ and 0.5 mL conc HNO₃ in 500 mL distilled water.

e. *Silicone fluid*.*

4. Procedure

a. *Removal of silica*: If the silica concentration exceeds 25 mg/L, evaporate sample nearly to dryness in a platinum dish on a steam bath. Add 1 mL HCl, tilt, and rotate dish until the acid comes in complete contact with the residue. Continue evaporation to dryness. Complete drying in an oven at 180°C and if organic matter is present, char over flame of a burner. Moisten residue with 2 mL distilled water and 1 mL HCl, and evaporate to dryness on a steam bath. Add 2 mL HCl, take up soluble residue in hot water, and filter. Wash insoluble silica with several small portions of hot distilled water. Combine filtrate and washings. Discard residue.

b. *Precipitation of barium sulfate*: Adjust volume of clarified sample to contain approximately 50 mg SO₄²⁻ in a 250-mL volume. Lower concentrations of SO₄²⁻ may be tolerated if it is impracticable to

concentrate sample to the optimum level, but in such cases limit total volume to 150 mL. Adjust pH with HCl to pH 4.5 to 5.0, using a pH meter or the orange color of methyl red indicator. Add 1 to 2 mL HCl. Heat to boiling and, while stirring gently, slowly add warm BaCl₂ solution until precipitation appears to be complete; then add about 2 mL in excess. If amount of precipitate is small, add a total of 5 mL BaCl₂ solution. Digest precipitate at 80 to 90°C, preferably overnight but for not less than 2 h.

c. *Filtration and weighing*: Mix a small amount of ashless filter paper pulp with the BaSO₄, quantitatively transfer to a filter, and filter at room temperature. The pulp aids filtration and reduces the tendency of the precipitate to creep. Wash precipitate with small portions of warm distilled water until washings are free of Cl⁻ as indicated by testing with AgNO₃-HNO₃ reagent. Place filter and precipitate in a weighed platinum crucible and ignite at 800°C for 1 h. Do not let filter paper flame. Cool in desiccator and weigh.

5. Calculation

$$\text{mg SO}_4^{2-}/\text{L} = \frac{\text{mg BaSO}_4 \times 411.6}{\text{mL sample}}$$

6. Precision and Bias

A synthetic sample containing 259 mg SO₄²⁻/L, 108 mg Ca²⁺/L, 82 mg Mg²⁺/L, 3.1 mg K⁺/L, 19.9 mg Na⁺/L, 241 mg Cl⁻/L, 0.250 mg NO₂⁻-N/L, 1.1 mg NO₃⁻-N/L, and 42.5 mg total alkalinity/L (contributed by NaHCO₃) was analyzed in 32 laboratories by the gravimetric method, with a relative standard deviation of 4.7% and a relative error of 1.9%.

7. Bibliography

- HILLEBRAND, W.F. et al. 1953. *Applied Inorganic Analysis*, 2nd ed. John Wiley & Sons, New York, N.Y.
KOLTHOFF, I.M., E.J. MEEHAN, E.B. SANDELL & S. BRUCKENSTEIN. 1969. *Quantitative Chemical Analysis*, 4th ed. Macmillan Co., New York, N.Y.

* "Desicote" (Beckman), or equivalent.

4500-SO₄²⁻ D. Gravimetric Method with Drying of Residue

1. General Discussion

See Method C, preceding.

2. Apparatus

With the exception of the filter paper, all of the apparatus cited in Section 4500-SO₄²⁻.C.2 is required, plus the following:

a. *Filters*: Use one of the following:

1) *Fritted-glass filter*, fine ("F") porosity, with a maximum pore size of 5 μm .

2) *Membrane filter*, with a pore size of about 0.45 μm .

b. *Vacuum oven*.

3. Reagents

All the reagents listed in Section 4500-SO₄²⁻.C.3 are required.

4. Procedure

a. *Removal of interference*: See Section 4500-SO₄²⁻.C.4a.

b. *Precipitation of barium sulfate*: See Section 4500-SO₄²⁻.C.4b.

c. *Preparation of filters*:

1) Fritted glass filter—Dry to constant weight in an oven maintained at 105°C or higher, cool in desiccator, and weigh.

2) Membrane filter—Place filter on a piece of filter paper or a watch glass and dry to constant weight* in a vacuum oven at 80°C, while maintaining a vacuum of at least 85 kPa or in a conventional oven at a temperature of 103 to 105°C. Cool in desiccator and weigh membrane only.

d. Filtration and weighing: Filter BaSO_4 at room temperature. Wash precipitate with several small portions of warm distilled water until washings are free of Cl^- , as indicated by testing with $\text{AgNO}_3\text{-HNO}_3$ reagent. If a membrane filter is used add a few drops of silicone fluid

* Constant weight is defined as a change of not more than 0.5 mg in two successive operations consisting of heating, cooling in desiccator, and weighing.

4500-SO₄²⁻ E. Turbidimetric Method

1. General Discussion

a. Principle: Sulfate ion (SO_4^{2-}) is precipitated in an acetic acid medium with barium chloride (BaCl_2) so as to form barium sulfate (BaSO_4) crystals of uniform size. Light absorbance of the BaSO_4 suspension is measured by a photometer and the SO_4^{2-} concentration is determined by comparison of the reading with a standard curve.

b. Interference: Color or suspended matter in large amounts will interfere. Some suspended matter may be removed by filtration. If both are small in comparison with the SO_4^{2-} concentration, correct for interference as indicated in § 4d below. Silica in excess of 500 mg/L will interfere, and in waters containing large quantities of organic material it may not be possible to precipitate BaSO_4 satisfactorily.

In potable waters there are no ions other than SO_4^{2-} that will form insoluble compounds with barium under strongly acid conditions. Make determination at room temperature; variation over a range of 10°C will not cause appreciable error.

c. Minimum detectable concentration: Approximately 1 mg SO_4^{2-} /L.

2. Apparatus

a. Magnetic stirrer: Use a constant stirring speed. It is convenient to incorporate a fixed resistance in series with the motor operating the magnetic stirrer to regulate stirring speed. Use magnets of identical shape and size. The exact speed of stirring is not critical, but keep it constant for each run of samples and standards and adjust it to prevent splashing.

b. Photometer: One of the following is required, with preference in the order given:

1) *Nephelometer.*
2) *Spectrophotometer,* for use at 420 nm, providing a light path of 2.5 to 10 cm.

3) *Filter photometer,* equipped with a violet filter having maximum transmittance near 420 nm and providing a light path of 2.5 to 10 cm.

c. Stopwatch or electric timer.

d. Measuring spoon, capacity 0.2 to 0.3 mL.

to the suspension before filtering, to prevent adherence of precipitate to holder. Dry filter and precipitate by the same procedure used in preparing filter. Cool in a desiccator and weigh.

5. Calculation

$$\text{mg SO}_4^{2-}/\text{L} = \frac{\text{mg BaSO}_4 \times 411.6}{\text{mL sample}}$$

6. Bibliography

See Section 4500-SO₄²⁻.C.7.

3. Reagents

a. Buffer solution A: Dissolve 30 g magnesium chloride, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5 g sodium acetate, $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$, 1.0 g potassium nitrate, KNO_3 , and 20 mL acetic acid, CH_3COOH (99%), in 500 mL distilled water and make up to 1000 mL.

b. Buffer solution B (required when the sample SO_4^{2-} concentration is less than 10 mg/L): Dissolve 30 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5 g $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$, 1.0 g KNO_3 , 0.111 g sodium sulfate, Na_2SO_4 , and 20 mL acetic acid (99%) in 500 mL distilled water and make up to 1000 mL.

c. Barium chloride, BaCl_2 , crystals, 20 to 30 mesh. In standardization, uniform turbidity is produced with this mesh range and the appropriate buffer.

d. Standard sulfate solution: Prepare a standard sulfate solution as described in 1) or 2) below; 1.00 mL = 100 $\mu\text{g SO}_4^{2-}$.

1) Dilute 10.4 mL standard 0.0200N H_2SO_4 titrant specified in Alkalinity, Section 2320B.3c, to 100 mL with distilled water.

2) Dissolve 0.1479 g anhydrous Na_2SO_4 in distilled water and dilute to 1000 mL.

4. Procedure

a. Formation of barium sulfate turbidity: Measure 100 mL sample, or a suitable portion made up to 100 mL, into a 250-mL erlenmeyer flask. Add 20 mL buffer solution and mix in stirring apparatus. While stirring, add a spoonful of BaCl_2 crystals and begin timing immediately. Stir for 60 ± 2 s at constant speed.

b. Measurement of barium sulfate turbidity: After stirring period has ended, pour solution into absorption cell of photometer and measure turbidity at 5 ± 0.5 min.

c. Preparation of calibration curve: Estimate SO_4^{2-} concentration in sample by comparing turbidity reading with a calibration curve prepared by carrying SO_4^{2-} standards through the entire procedure. Space standards at 5-mg/L increments in the 0- to 40-mg/L SO_4^{2-} range. Above 40 mg/L accuracy decreases and BaSO_4 suspensions lose stability. Check reliability of calibration curve by running a standard with every three or four samples.

d. *Correction for sample color and turbidity:* Correct for sample color and turbidity by running blanks to which BaCl₂ is not added.

5. Calculation

$$\text{mg SO}_4^{2-}/\text{L} = \frac{\text{mg SO}_4^{2-} \times 1000}{\text{mL sample}}$$

If buffer solution A was used, determine SO₄²⁻ concentration directly from the calibration curve after subtracting sample absorbance before adding BaCl₂. If buffer solution B was used subtract SO₄²⁻ concentration of blank from apparent SO₄²⁻ concentration as determined above; because the calibration curve is not a straight line, this is not equivalent to subtracting blank absorbance from sample absorbance.

4500-SO₄²⁻ F. Automated Methylthymol Blue Method

1. General Discussion

a. *Principle:* Barium sulfate is formed by the reaction of the SO₄²⁻ with barium chloride (BaCl₂) at a low pH. At high pH excess barium reacts with methylthymol blue to produce a blue chelate. The uncomplexed methylthymol blue is gray. The amount of gray uncomplexed methylthymol blue indicates the concentration of SO₄²⁻.

b. *Interferences:* Because many cations interfere, use an ion-exchange column to remove interferences.

Molybdenum, often used to treat cooling waters, has been shown to cause a strong positive bias with this method, even with as little as 1 mg Mo/L.

c. *Application:* This method is applicable to potable, ground, surface, and saline waters as well as domestic and industrial wastewaters over a range from about 10 to 300 mg SO₄²⁻/L.

2. Apparatus

a. *Automated analytical equipment:* An example of the required continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-SO₄²⁻:1.

b. *Ion-exchange column:* Fill a piece of 2-mm-ID glass tubing about 20 cm long with the ion-exchange resin.* To simplify filling column put resin in distilled water and aspirate it into the tubing, which contains a glass-wool plug. After filling, plug other end of tube with glass wool. Avoid trapped air in the column.

6. Precision and Bias

With a turbidimeter,* in a single laboratory with a sample having a mean of 7.45 mg SO₄²⁻/L, a standard deviation of 0.13 mg/L and a coefficient of variation of 1.7% were obtained. Two samples dosed with sulfate gave recoveries of 85 and 91%.

7. Bibliography

- SHEEN, R.T., H.L. KAHLER & E.M. ROSS. 1935. Turbidimetric determination of sulfate in water. *Ind. Eng. Chem., Anal. Ed.* 7:262.
THOMAS, J.F. & J.E. COTTON. 1954. A turbidimetric sulfate determination. *Water Sewage Works* 101:462.
ROSSUM, J.R. & P. VILLARRUZ. 1961. Suggested methods for turbidimetric determination of sulfate in water. *J. Amer. Water Works Assoc.* 53:873.

* Hach 2100 A.

3. Reagents

a. *Barium chloride solution:* Dissolve 1.526 g BaCl₂ · 2H₂O in 500 mL distilled water and dilute to 1 L. Store in a polyethylene bottle.

b. *Methylthymol blue reagent:* Dissolve 118.2 mg methylthymol blue† in 25 mL BaCl₂ solution. Add 4 mL 1N HCl and 71 mL distilled water and dilute to 500 mL with 95% ethanol. Store in a brown glass bottle. Prepare fresh daily.

c. *Buffer solution, pH 10.1:* Dissolve 6.75 g NH₄Cl in 500 mL distilled water. Add 57 mL conc NH₄OH and dilute to 1 L with

† Eastman Organic Chemicals, Rochester, NY 14615. No. 8068 3',3'-bis [N,N-bis(carboxymethyl)-aminomethyl] thymolsulfonphthalein pentasodium salt.

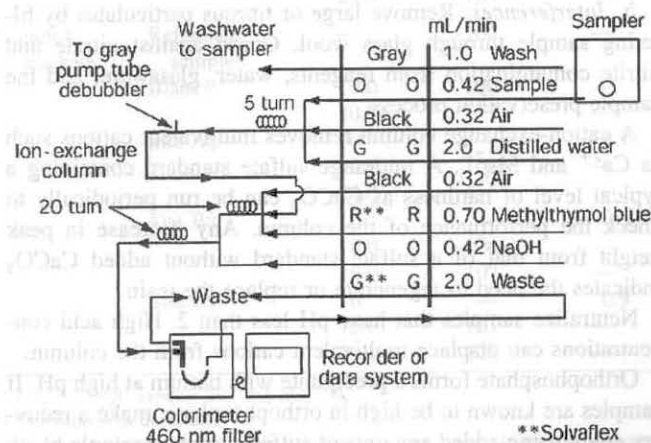


Figure 4500-SO₄²⁻:1. Sulfate manifold.

* Ion-exchange resin Bio-Rex 70, 20-50 mesh, sodium form, available from Bio-Rad Laboratories, Richmond, CA 94804, or equivalent.

distilled water. Adjust pH to 10.1 and store in a polyethylene bottle. Prepare fresh monthly.

d. *EDTA reagent:* Dissolve 40 g tetrasodium ethylenediaminetetraacetate in 500 mL pH 10.1 buffer solution. Dilute to 1 L with pH 10.1 buffer solution and store in a polyethylene bottle.

e. *Sodium hydroxide solution, 0.36N:* Dissolve 7.2 g NaOH in 250 mL distilled water. Cool and make up to 500 mL with distilled water.

f. *Stock sulfate solution:* Dissolve 1.479 g anhydrous Na_2SO_4 in 500 mL distilled water and dilute to 1000 mL; 1.00 mL = 1.00 mg SO_4^{2-} .

g. *Standard sulfate solutions:* Prepare in appropriate concentrations from 10 to 300 mg SO_4^{2-} /L, using the stock sulfate solution.

4. Procedure

Set up the manifold as shown in Figure 4500-SO₄²⁻:1 and follow the general procedure described by the manufacturer.

After use, rinse methylthymol blue and NaOH reagent lines in water for a few minutes, rinse them in the EDTA solution for 10 min, and then rinse in distilled water.

4500-SO₄²⁻ G. Methylthymol Blue Flow Injection Analysis

1. General Discussion

a. *Principle:* At pH 13.0 barium forms a blue complex with methylthymol blue (MTB). This gives a dark blue base line. The sample is injected into a low, but known, concentration of sulfate. The sulfate from the sample then reacts with the ethanolic barium-MTB solution and displaces the MTB from the barium to give barium sulfate and uncomplexed MTB. Uncomplexed MTB has a grayish color. The pH is raised with NaOH and the color of the gray uncomplexed MTB is measured at 460 nm. The intensity of gray color is proportional to the sulfate concentration.

Also see Sections 4500-SO₄²⁻.A and F, and Section 4130, Flow Injection Analysis (FIA).

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

A cation-exchange column removes multivalent cations such as Ca^{2+} and Mg^{2+} . A midrange sulfate standard containing a typical level of hardness as CaCO_3 can be run periodically to check the performance of the column. Any decrease in peak height from that of a sulfate standard without added CaCO_3 indicates the need to regenerate or replace the resin.

Neutralize samples that have pH less than 2. High acid concentrations can displace multivalent cations from the column.

Orthophosphate forms a precipitate with barium at high pH. If samples are known to be high in orthophosphate, make a recovery study using added amounts of sulfate, or run a sample blank containing only the orthophosphate matrix.

Also see Section 4500-SO₄²⁻.F.1b.

5. Calculation

Prepare standard curves by plotting peak heights of standards processed through the manifold against SO_4^{2-} concentrations in standards. Compute sample SO_4^{2-} concentration by comparing sample peak height with standard curve.

6. Precision and Bias

In a single laboratory a sample with an average concentration of about 28 mg SO_4^{2-} /L had a standard deviation of 0.68 mg/L and a coefficient of variation of 2.4%. In two samples with added SO_4^{2-} , recoveries were 91% and 100%.

7. Bibliography

LAZRUS, A.L., K.C. HILL & J.P. LODGE. 1965. A new colorimetric microdetermination of sulfate ion. In *Automation in Analytical Chemistry*. Technicon Symposium.

COLOROS, E., M. R. PANESAR & F.P. PERRY. 1976. Linearizing the calibration curve in determination of sulfate by the methylthymol blue method. *Anal. Chem.* 48:1693.

2. Apparatus

Flow injection analysis equipment consisting of:

a. *FIA injection valve* with sample loop or equivalent.

b. *Multichannel proportioning pump.*

c. *FIA manifold* (Figure 4500-SO₄²⁻:2) with cation-exchange column and flow cell. Relative flow rates only are shown in Figure 4500-SO₄²⁻:2. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

d. *Absorbance detector*, 460 nm, 10-nm bandpass.

e. *Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He

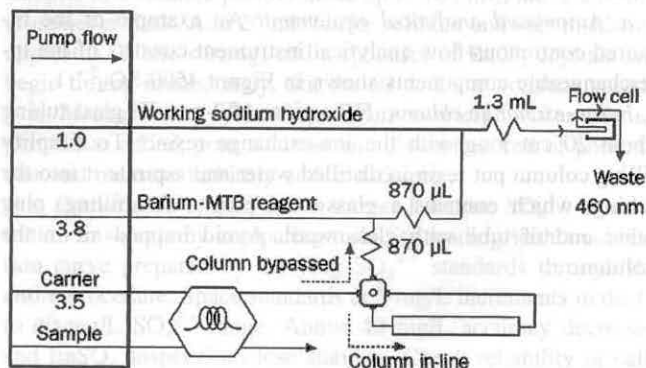


Figure 4500-SO₄²⁻:2. FIA manifold.

at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. *Carrier solution*, 0.30 mg SO₄²⁻/L: To a tared 1-L container, add 0.30 g 1000 mg/L stock sulfate standard (§ 3h) and 999.7 g water. Shake or stir to mix. Degas with helium.

b. *Barium chloride solution*, 6.24mM: To a tared 1-L container, add 1.526 g barium chloride dihydrate, BaCl₂ · 2H₂O, and 995 g water. Shake or stir until dissolved. Degas with helium.

c. *Hydrochloric acid*, HCl, 1.0M: To a tared 1-L container, add 913 g water and 99.6 g conc HCl (specific gravity 1.20, 37%). CAUTION: *Fumes*. Shake or stir to mix well. Degas with helium.

d. *Barium—MTB color reagent*: NOTE: The purity of the methylthymol blue and the denaturants in the alcohol are critical. Use the sources specified below, or test the material from alternative sources for suitability before using.

To a tared 500-mL dry brown plastic bottle, place 0.236 g methylthymol blue, 3,3'-bis[*N,N*-di(carboxymethyl)amino-methyl]-thymol-sulfonephthalein, pentasodium salt. Add 50 g barium chloride solution (§ 3b), which may be used to aid in transfer of the dye. Swirl to dissolve. Add 4.0 g of 1.0M HCl (§ 3c) and mix. The solution should turn orange. Add 71 g water and 321 g ethanol (ethyl alcohol, specially denatured anhydrous alcohol*). Stir or shake to mix well. The pH should be 2.5. Prepare solution the day before use and store, refrigerated, in a brown plastic bottle. Let warm to room temperature before using, then degas with helium.

e. *Stock sodium hydroxide solution*, NaOH, 50% (w/v) solution: To a glass 1-L container add 500 g water and 500.0 g NaOH. Dilute to 1 L. CAUTION: *The solution becomes very hot*. Shake or stir until dissolved. Cool to ambient. Store in a plastic bottle.

f. *Working sodium hydroxide solution*, NaOH, 0.18M: To a tared plastic 1-L container add 982 g water and 19.8 g stock NaOH solution (§ 3e). Shake or stir to mix. Degas with helium.

g. *Cation exchange column preparation*: Prepare approximately 0.5 g ion exchange resin,† 50 to 100 mesh, by mixing with sufficient water to make a slurry. Remove one end fitting from the threaded glass column. Fill column with water and aspirate slurry into column or let it settle into column by gravity. Take care to avoid trapping air bubbles in column or fittings at this point and during all subsequent operations. When resin has settled, replace end fitting. To ensure a good seal, remove any resin particles from the threads of glass, column end, and end fitting. To store column, join ends of the TFE tubing.

To test column effectiveness, make up two midrange standards, one of only sodium sulfate and the other with an identical concentration of sodium sulfate but with hardness typical of the samples. If the column is depleted, the standard with hardness will give a lower response because the divalent Mg²⁺ and Ca²⁺ cations are complexing with the free MTB. If depletion has occurred, repack column with fresh resin.

h. *Stock sulfate standard*, 1000 mg SO₄²⁻/L: Dry approximately 2 g sodium sulfate, Na₂SO₄, at 105° overnight. Cool in a

desiccator. In a 1-L volumetric flask, add 1.479 g of dried sodium sulfate to about 800 mL water. Dissolve by swirling, dilute to mark, and mix by inversion.

i. *Standard solutions*: Prepare sulfate standards in desired concentration range, using the stock standard (§ 3h), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-SO₄²⁻:2 and follow method supplied by manufacturer or laboratory stan-

TABLE 4500-SO₄²⁻:I. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg SO ₄ ²⁻ /L	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	99	—
	Blank†	10.0	99	—
		20.0	99	—
	Site A‡	0	—	0.7
		10.0	109	—
		20.0	110	—
	Site B‡	0	—	0.7
		10.0	106	—
		20.0	112	—
	Site C‡	0	—	1.9
		10.0	104	—
		20.0	107	—
Wastewater treatment plant effluent	Reference sample*	—	99	—
	Blank†	10.0	95	—
		20.0	99	—
	Site A‡	0	—	0.9
		10.0	108	—
		20.0	108	—
	Site B‡	0	—	2.4
		10.0	107	—
		20.0	107	—
	Site C‡	0	—	0.6
		10.0	97	—
		20.0	104	—
Landfill leachate	Reference sample*	—	100	—
	Blank†	10.0	100	—
		20.0	99	—
	Site A‡	0	—	0.7
		10.0	106	—
		20.0	110	—
	Site B‡	0	—	0.5
		10.0	106	—
		20.0	107	—
	Site C‡	0	—	0.9
		10.0	101	—
		20.0	103	—

* U.S. EPA QC sample, 20.0 mg SO₄²⁻/L.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate. Typical relative difference between duplicates 1%. Sample dilutions: Influent and effluent, all sites - 5-fold; leachate A - 100-fold; B - 50-fold; C - 10-fold.

* Aldrich 24, 511-9, or equivalent.

† BioRex 70 or equivalent.

dard operating procedure. Follow quality control protocols outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting adsorbance of standards processed through the manifold versus sulfate concentration.

6. Precision and Bias

a. Recovery and relative standard deviation: Table 4500-SO₄²⁻:I gives results of single-laboratory studies.

b. MDL: A 180- μ L sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 5.00-mg SO₄²⁻/L standard. These gave a mean of 4.80 mg SO₄²⁻/L, a standard deviation of 0.69 mg SO₄²⁻/L, and MDL of 1.8 mg SO₄²⁻/L.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

