

# Standard Methods for the Examination of Water and Wastewater

## 4500-NO<sub>3</sub><sup>-</sup> NITROGEN (NITRATE)\*#(1)

### 4500-NO<sub>3</sub><sup>-</sup> A. Introduction

#### 1. Selection of Method

Determination of nitrate (NO<sub>3</sub><sup>-</sup>) is difficult because of the relatively complex procedures required, the high probability that interfering constituents will be present, and the limited concentration ranges of the various techniques.

An ultraviolet (UV) technique (Method B) that measures the absorbance of NO<sub>3</sub><sup>-</sup> at 220 nm is suitable for screening uncontaminated water (low in organic matter).

Screen a sample; if necessary, then select a method suitable for its concentration range and probable interferences. Nitrate may be determined by ion chromatography (Section 4110) or capillary ion electrophoresis (Section 4140). Applicable ranges for other methods are: nitrate electrode method (D), 0.14 to 1400 mg NO<sub>3</sub><sup>-</sup>-N/L; cadmium reduction method (E), 0.01 to 1.0 mg NO<sub>3</sub><sup>-</sup>-N/L; automated cadmium reduction methods (F and I), 0.001 to 10 mg NO<sub>3</sub><sup>-</sup>-N/L. For higher NO<sub>3</sub><sup>-</sup>-N concentrations, dilute into the range of the selected method.

Colorimetric methods (B, E) require an optically clear sample. Filter turbid sample through 0.45- $\mu$ m-pore-diam membrane filter. Test filters for nitrate contamination.

#### 2. Storage of Samples

Start NO<sub>3</sub><sup>-</sup> determinations promptly after sampling. If storage is necessary, store for up to 2 d at 4°C; disinfected samples are stable much longer without acid preservation. For longer storage of unchlorinated samples, preserve with 2 mL conc H<sub>2</sub>SO<sub>4</sub>/L and store at 4°C.

NOTE: When sample is preserved with acid, NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> cannot be determined as individual species.

### 4500-NO<sub>3</sub><sup>-</sup> B. Ultraviolet Spectrophotometric Screening Method

#### 1. General Discussion

*a. Principle:* Use this technique only for screening samples that have low organic matter contents, i.e., uncontaminated natural waters and potable water supplies. The NO<sub>3</sub><sup>-</sup> calibration curve follows Beer's law up to 11 mg N/L.

Measurement of UV absorption at 220 nm enables rapid determination of NO<sub>3</sub><sup>-</sup>. Because dissolved organic matter also may absorb at 220 nm and NO<sub>3</sub><sup>-</sup> does not absorb at 275 nm, a

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second measurement made at 275 nm may be used to correct the  $\text{NO}_3^-$  value. The extent of this empirical correction is related to the nature and concentration of organic matter and may vary from one water to another. Consequently, this method is not recommended if a significant correction for organic matter absorbance is required, although it may be useful in monitoring  $\text{NO}_3^-$  levels within a water body with a constant type of organic matter.

Correction factors for organic matter absorbance can be established by the method of additions in combination with analysis of the original  $\text{NO}_3^-$  content by another method.

Sample filtration is intended to remove possible interference from suspended particles. Acidification with 1N HCl is designed to prevent interference from hydroxide or carbonate concentrations up to 1000 mg  $\text{CaCO}_3/\text{L}$ . Chloride has no effect on the determination.

*b. Interference:* Dissolved organic matter, surfactants,  $\text{NO}_2^-$ , and  $\text{Cr}^{6+}$  interfere. Various inorganic ions not normally found in natural water, such as chlorite and chlorate, may interfere. Inorganic substances can be compensated for by independent analysis of their concentrations and preparation of individual correction curves. For turbid samples, see ¶ A.1.

### 2. Apparatus

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

### 3. Reagents

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

*b. Stock nitrate solution:* Dry potassium nitrate ( $\text{KNO}_3$ ) in an oven at 105°C for 24 h. Dissolve 0.7218 g in water and dilute to 1000 mL; 1.00 mL = 100  $\mu\text{g}$   $\text{NO}_3^-$ -N. Preserve with 2 mL  $\text{CHCl}_3/\text{L}$ . 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  $\mu\text{g}$   $\text{NO}_3^-$ -N. Preserve with 2 mL  $\text{CHCl}_3/\text{L}$ . 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  $\text{NO}_3^-$  calibration standards in the range 0 to 7 mg  $\text{NO}_3^-$ -N/L by diluting to 50 mL the following volumes of intermediate nitrate solution: 0, 1.00, 2.00, 4.00, 7.00 . . . 35.0 mL. Treat  $\text{NO}_3^-$  standards in same manner as samples.

*c. Spectrophotometric measurement:* Read absorbance or transmittance against redistilled water set at zero absorbance or 100% transmittance. Use a wavelength of 220 nm to obtain  $\text{NO}_3^-$  reading and a wavelength of 275 nm to determine interference due to

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dissolved organic matter.

### 5. Calculation

For samples and standards, subtract two times the absorbance reading at 275 nm from the reading at 220 nm to obtain absorbance due to  $\text{NO}_3^-$ . Construct a standard curve by plotting absorbance due to  $\text{NO}_3^-$  against  $\text{NO}_3^-$ -N concentration of standard. Using corrected sample absorbances, obtain sample concentrations directly from standard curve. NOTE: If correction value is more than 10% of the reading at 220 nm, do not use this method.

### 6. Bibliography

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### 4500- $\text{NO}_3^-$ C. (Reserved)

### 4500- $\text{NO}_3^-$ D. Nitrate Electrode Method

#### 1. General Discussion

*a. Principle:* The  $\text{NO}_3^-$  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  $\text{NO}_3^-$  ion activity between about  $10^{-5}$  and  $10^{-1}$  M (0.14 to 1400 mg  $\text{NO}_3^-$ -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  $\text{NO}_3^-$ -N are >10 or >5, respectively. Ions that are potential interferences but do not normally occur at significant levels in potable waters are  $\text{NO}_2^-$ ,  $\text{CN}^-$ ,  $\text{S}^{2-}$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{ClO}_3^-$ , and  $\text{ClO}_4^-$ . 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  $\text{NO}_3^-$  activity rather than concentration, ionic strength must be constant in all samples and standards. Minimize these problems by using a buffer solution containing  $\text{Ag}_2\text{SO}_4$  to remove  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{S}^{2-}$ , and  $\text{CN}^-$ , sulfamic acid to remove  $\text{NO}_2^-$ , a buffer at pH 3 to eliminate  $\text{HCO}_3^-$  and to maintain a constant pH and ionic strength, and  $\text{Al}_2(\text{SO}_4)_3$  to complex organic

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acids.

### 2. Apparatus

- a. *pH meter, expanded-scale or digital*, capable of 0.1 mV resolution.
- b. *Double-junction reference electrode*. (2) Fill outer chamber with  $(\text{NH}_4)_2\text{SO}_4$  solution.
- c. *Nitrate ion electrode*: (3) 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  $\text{NO}_3^-$ -N/L, respectively.
- d. *Buffer solution*: Dissolve 17.32 g  $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ , 3.43 g  $\text{Ag}_2\text{SO}_4$ , 1.28 g  $\text{H}_3\text{BO}_3$ , and 2.52 g sulfamic acid ( $\text{H}_2\text{NSO}_3\text{H}$ ), in about 800 mL water. Adjust to pH 3.0 by slowly adding 0.10N NaOH. Dilute to 1000 mL and store in a dark glass bottle.
- e. *Sodium hydroxide*, NaOH, 0.1N.
- f. *Reference electrode filling solution*: Dissolve 0.53 g  $(\text{NH}_4)_2\text{SO}_4$  in water and dilute to 100 mL.

### 4. Procedure

- a. *Preparation of calibration curve*: Transfer 10 mL of 1 mg  $\text{NO}_3^-$ -N/L standard to a 50-mL beaker, add 10 mL buffer, and stir with a magnetic stirrer. Immerse tips of electrodes and record millivolt reading when stable (after about 1 min). Remove electrodes, rinse, and blot dry. Repeat for 10-mg  $\text{NO}_3^-$ -N/L and 50-mg  $\text{NO}_3^-$ -N/L standards. Plot potential measurements against  $\text{NO}_3^-$ -N concentration on semilogarithmic graph paper, with  $\text{NO}_3^-$ -N concentration on the logarithmic axis (abscissa) and potential (in millivolts) on the linear axis (ordinate). A straight line with a slope of  $+57 \pm 3$  mV/decade at 25°C should result. Recalibrate electrodes several times daily by checking potential reading of the 10 mg  $\text{NO}_3^-$ -N standard and adjusting the calibration control until the reading plotted on the calibration curve is displayed again.
- b. *Measurement of sample*: Transfer 10 mL sample to a 50-mL beaker, add 10 mL buffer solution, and stir (for about 1 min) with a magnetic stirrer. Measure standards and samples at about the same temperature. Immerse electrode tips in sample and record potential reading when stable (after about 1 min). Read concentration from calibration curve.

### 5. Precision

Over the range of the method, precision of  $\pm 0.4$  mV, corresponding to 2.5% in

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concentration, is expected.

### 6. Bibliography

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### 4500-NO<sub>3</sub><sup>-</sup> E. Cadmium Reduction Method

#### 1. General Discussion

*a. Principle:* NO<sub>3</sub><sup>-</sup> is reduced almost quantitatively to nitrite (NO<sub>2</sub><sup>-</sup>) in the presence of cadmium (Cd). This method uses commercially available Cd granules treated with copper sulfate (CuSO<sub>4</sub>) and packed in a glass column.

The NO<sub>2</sub><sup>-</sup> 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 NO<sub>2</sub><sup>-</sup> present in the sample by analyzing without the reduction step. The applicable range of this method is 0.01 to 1.0 mg NO<sub>3</sub><sup>-</sup>-N/L. The method is recommended especially for NO<sub>3</sub><sup>-</sup> levels below 0.1 mg N/L where other methods lack adequate sensitivity.

*b. Interferences:* Suspended matter in the column will restrict sample flow. For turbid samples, see ¶ A.1. Concentrations of iron, copper, or other metals above several milligrams per liter lower reduction efficiency. Add EDTA to samples to eliminate this interference. Oil and grease will coat the Cd surface. Remove by pre-extraction with an organic solvent (see Section 5520). Residual chlorine can interfere by oxidizing the Cd column, reducing its efficiency. Check samples for residual chlorine (see DPD methods in Section 4500-Cl). Remove residual chlorine by adding sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) solution (Section 4500-NH<sub>3</sub>.B.3d). Sample color that absorbs at about 540 nm interferes.

#### 2. Apparatus

*a. Reduction column:* Purchase or construct the column\*#(4) (Figure 4500-NO<sub>3</sub><sup>-</sup>:1) from a 100-mL volumetric pipet by removing the top portion. The column also can be constructed from two pieces of tubing joined end to end: join a 10-cm length of 3-cm-ID tubing to a 25-cm length of 3.5-mm-ID tubing. Add a TFE stopcock with metering valve<sup>1</sup> to control flow

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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†#(5) with 6N HCl and rinse with water. Swirl Cd with 100 mL 2% CuSO<sub>4</sub> solution for 5 min or until blue color partially fades. Decant and repeat with fresh CuSO<sub>4</sub> 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-NO<sub>2</sub><sup>-</sup>.B.3b.

d. *Ammonium chloride-EDTA solution:* Dissolve 13 g NH<sub>4</sub>Cl and 1.7 g disodium ethylenediamine tetraacetate in 900 mL water. Adjust to pH 8.5 with conc NH<sub>4</sub>OH and dilute to 1 L.

e. *Dilute ammonium chloride-EDTA solution:* Dilute 300 mL NH<sub>4</sub>Cl-EDTA solution to 500 mL with water.

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

g. *Copper sulfate solution, 2%:* Dissolve 20 g CuSO<sub>4</sub>·5H<sub>2</sub>O 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-NO<sub>2</sub><sup>-</sup>.B.3e.

k. *Intermediate nitrite solution:* See Section 4500-NO<sub>2</sub><sup>-</sup>.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 NO<sub>2</sub><sup>-</sup>-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 NH<sub>4</sub>Cl-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 NO<sub>3</sub><sup>-</sup>-N/L standard and 75% NH<sub>4</sub>Cl-EDTA solution.

b. *Treatment of sample:*

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- 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  $\text{NH}_4\text{Cl}$ -EDTA solution.
- 3) Sample reduction—To 25.0 mL sample or a portion diluted to 25.0 mL, add 75 mL  $\text{NH}_4\text{Cl}$ -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  $\text{NH}_4\text{Cl}$ -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  $\text{NO}_3^-$  concentration exceeds the standard curve range (about 1 mg N/L), use remainder of reduced sample to make an appropriate dilution and analyze again.

*c. Standards:* Using the intermediate  $\text{NO}_3^-$ -N solution, prepare standards in the range 0.05 to 1.0 mg  $\text{NO}_3^-$ -N/L by diluting the following volumes to 100 mL in volumetric flasks: 0.5, 1.0, 2.0, 5.0, and 10.0 mL. Carry out reduction of standards exactly as described for samples. Compare at least one  $\text{NO}_2^-$  standard to a reduced  $\text{NO}_3^-$  standard at the same concentration to verify reduction column efficiency. Reactivate Cu-Cd granules as described in ¶ 3b above when efficiency of reduction falls below about 75%.

### 5. Calculation

Obtain a standard curve by plotting absorbance of standards against  $\text{NO}_3^-$ -N concentration. Compute sample concentrations directly from standard curve. Report as milligrams oxidized N per liter (the sum of  $\text{NO}_3^-$ -N plus  $\text{NO}_2^-$ -N) unless the concentration of  $\text{NO}_2^-$ -N is separately determined and subtracted.

### 6. Precision and Bias

In a single laboratory using wastewater samples at concentrations of 0.04, 0.24, 0.55, and 1.04 mg  $\text{NO}_3^- + \text{NO}_2^-$ -N/L, the standard deviations were  $\pm 0.005$ ,  $\pm 0.004$ ,  $\pm 0.005$ , and  $\pm 0.01$ , respectively. In a single laboratory using wastewater with additions of 0.24, 0.55, and 1.05 mg  $\text{NO}_3^- + \text{NO}_2^-$ -N/L, the recoveries were 100%, 102%, and 100%, respectively.<sup>2</sup>

### 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.
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NYDAHL, F. 1976. On the optimum conditions for the reduction of nitrate by cadmium. *Talanta* 23:349.

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## 4500-NO<sub>3</sub><sup>-</sup> 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-NO<sub>3</sub><sup>-</sup>:2.

### 3. Reagents

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

b. *Copper sulfate solution*: Dissolve 20 g CuSO<sub>4</sub>·5H<sub>2</sub>O in 500 mL water and dilute to 1 L.

c. *Wash solution*: Use water for unpreserved samples. For samples preserved with H<sub>2</sub>SO<sub>4</sub>, add 2 mL conc H<sub>2</sub>SO<sub>4</sub>/L wash water.

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

e. *Hydrochloric acid*, HCl, conc.

f. *Ammonium hydroxide*, NH<sub>4</sub>OH, conc.

g. *Color reagent*: To approximately 800 mL water, add, while stirring, 100 mL conc H<sub>3</sub>PO<sub>4</sub>, 40 g sulfanilamide, and 2 g N-(1-naphthyl)-ethylenediamine dihydrochloride. Stir until dissolved and dilute to 1 L. Store in brown bottle and keep in the dark when not in use. This solution is stable for several months.

h. *Ammonium chloride solution*: Dissolve 85 g NH<sub>4</sub>Cl in water and dilute to 1 L. Add 0.5 mL polyoxyethylene 23 lauryl ether.\*#(6)



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

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

k. *Standard nitrate solutions:* Using intermediate  $\text{NO}_3^-$ -N solution and water, prepare standards for calibration curve in appropriate nitrate range. Compare at least one  $\text{NO}_2^-$  standard to a  $\text{NO}_3^-$  standard at the same concentration to verify column reduction efficiency. To examine saline waters prepare standard solutions with the substitute ocean water described in Section 4500-NH<sub>3</sub>.G.3g.

l. *Standard nitrite solution:* See Section 4500-NO<sub>2</sub><sup>-</sup>.B.3g.

### 4. Procedure

Set up manifold as shown in Figure 4500-NO<sub>3</sub><sup>-</sup>: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 NH<sub>4</sub>OH.

### 5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against  $\text{NO}_3^-$ -N concentration in standards. Compute sample  $\text{NO}_3^-$ -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 $\text{NO}_3^-$ -N µg/L	Standard Deviation µg N/L	Bias %	Bias µg N/L
290	12	+5.75	+17
350	92	+18.10	+63
2310	318	+4.47	+103
2480	176	-2.69	-67

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

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

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### 4500-NO<sub>3</sub><sup>-</sup> G. (Reserved)

### 4500-NO<sub>3</sub><sup>-</sup> H. Automated Hydrazine Reduction Method

#### 1. General Discussion

a. *Principle:* NO<sub>3</sub><sup>-</sup> is reduced to NO<sub>2</sub><sup>-</sup> with hydrazine sulfate. The NO<sub>2</sub><sup>-</sup> (originally present) plus reduced NO<sub>3</sub><sup>-</sup> 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 NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> concentrations of ±10%.

c. *Application:* NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup> 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-NO<sub>3</sub><sup>-</sup>:3.

#### 3. Reagents

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

b. *Copper sulfate stock solution:* Dissolve 2.5 g CuSO<sub>4</sub>·5H<sub>2</sub>O in water and dilute to 1 L.

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

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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  $\text{N}_2\text{H}_4\cdot\text{H}_2\text{SO}_4$  in 900 mL water and dilute to 1 L. This solution is stable for approximately 6 months. CAUTION: *Toxic if ingested. Mark container with appropriate warning.*

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

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

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

j. *Standard nitrate solutions*: Prepare  $\text{NO}_3^-$  calibration standards in the range 0 to 10 mg/L by diluting to 100 mL the following volumes of stock nitrate solution: 0, 0.5, 1.0, 2.0 . . . 10.0 mL. For standards in the range of 0.01 mg/L use intermediate nitrate solution. Compare at least one nitrite standard to a nitrate standard at the same concentration to verify the efficiency of the reduction.

k. *Standard nitrite solution*: See Section 4500- $\text{NO}_2^-$ .B.3e, Section 4500- $\text{NO}_2^-$ .B.3 f, and Section 4500- $\text{NO}_2^-$ .B.3g.

### 4. Procedure

Set up manifold as shown in Figure 4500- $\text{NO}_3^-$ :3 and follow general procedure described by manufacturer. Run a 2.0-mg  $\text{NO}_3^-$ -N/L and a 2.0-mg  $\text{NO}_2^-$ -N/L standard through the system to check for 100% reduction of nitrate to nitrite. The two peaks should be of equal height; if not, adjust concentration of the hydrazine sulfate solution: If the  $\text{NO}_3^-$  peak is lower than the  $\text{NO}_2^-$  peak, increase concentration of hydrazine sulfate until they are equal; if the  $\text{NO}_3^-$  peak is higher than the  $\text{NO}_2^-$  reduce concentration of hydrazine sulfate. When correct concentration has been determined, no further adjustment should be necessary.

### 5. Calculation

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

### 6. Precision and Bias

In a single laboratory using drinking water, surface water, and industrial waste at concentrations of 0.39, 1.15, 1.76, and 4.75 mg  $\text{NO}_3^-$ -N/L, the standard deviations were  $\pm 0.02$ ,  $\pm 0.01$ ,  $\pm 0.02$ , and  $\pm 0.03$ , respectively. In a single laboratory using drinking water at concentrations of 0.75 and 2.97 mg  $\text{NO}_3^-$ -N/L, the recoveries were 99% and 101%.<sup>1</sup>

### 7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical

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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-NO<sub>3</sub><sup>-</sup> I. Cadmium Reduction Flow Injection Method (PROPOSED)

#### 1. General Discussion

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

Nitrite alone can be determined by removing the cadmium column, recalibrating the method, and repeating the sample analyses. A TON and a nitrite FIA method also can be run in parallel for a set of samples. In this arrangement, the concentrations determined in the nitrite method can be subtracted from the corresponding concentrations determined in the TON method to give the resulting nitrate concentrations of the samples.

Also see Section 4500-NO<sub>2</sub><sup>-</sup> 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-NO<sub>2</sub><sup>-</sup>.B.1*b* and *c* and Section 4500-NO<sub>3</sub><sup>-</sup>.A.2 and Section 4500-NO<sub>3</sub><sup>-</sup>.B.1*b*.

#### 2. Apparatus

*Flow injection analysis equipment* consisting of:

- a. FIA injection valve* with sample loop or equivalent.
- b. Multichannel proportioning pump.*
- c. FIA manifold* (Figure 4500-NO<sub>3</sub><sup>-</sup>:4) with flow cell. Relative flow rates only are shown

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in Figure 4500-NO<sub>3</sub><sup>-</sup>:4. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

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

e. *Injection valve control and data acquisition system*.

### 3. Reagents

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

a. *Ammonium chloride buffer*: CAUTION: *Fumes*. Use a hood. To a tared 1-L container add 800.0 g water, 126 g conc hydrochloric acid, HCl, 55.6 g ammonium hydroxide, NH<sub>4</sub>OH, 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, H<sub>3</sub>PO<sub>4</sub>, 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, CuSO<sub>4</sub>·5H<sub>2</sub>O, 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, KNO<sub>3</sub>, 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, NaNO<sub>2</sub>, or 1.214 g potassium nitrite, KNO<sub>2</sub>, 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

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range, using the stock standards (¶ 3 f or g), and diluting with water.

### 4. Procedure

Set up a manifold equivalent to that in Figure 4500-NO<sub>3</sub><sup>-</sup>: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:

$$\text{Column efficiency} = 100\% \times (\text{slope of nitrate curve/slope of nitrite curve})$$

Determine column efficiency at least weekly.

### 6. Precision and Bias

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

*a. Recovery and relative standard deviation:* Table 4500-NO<sub>3</sub><sup>-</sup>: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,<sup>1</sup> analysts ran 21 replicates of a 2.00-μg N/L standard. These gave a mean of 1.82 μg N/L, a standard deviation of 0.098 μg N/L, and MDL of 0.25 μg N/L. A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagent flow rate.

### 7. Reference

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

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## Endnotes

### **1 (Popup - Footnote)**

\* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

### **2 (Popup - Footnote)**

\* Orion Model 90-02, or equivalent.

### **3 (Popup - Footnote)**

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

### **4 (Popup - Footnote)**

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

### **5 (Popup - Footnote)**

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

### **6 (Popup - Footnote)**

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