

Nitrogen, nitrite plus nitrate, colorimetry, cadmium reduction-diazotization, automated-segmented flow

Parameters and Codes:

Nitrogen, nitrite plus nitrate, dissolved, I-2545-90 (mg/L as N): 00631

Nitrogen, nitrite plus nitrate, total-in-bottom-material, dry weight, I-6545-90 (mg/kg as N): 00633

1. Application

1.1 This method is used to analyze samples of surface, domestic, and industrial water and brines containing from 0.05 to 5.0 mg/L of nitrite- plus nitrate-nitrogen. Samples containing greater concentrations must be diluted. This modified method was implemented in the National Water Quality Laboratory in March 1988.

1.2 This method is used to determine the sum of nitrite- plus nitrate-nitrogen concentrations in samples of bottom material containing at least 2 mg/kg.

2. Summary of method

2.1 An acidified sodium chloride extraction procedure is used to extract nitrate and nitrite from samples of bottom material for this determination (Jackson, 1958).

2.2 Nitrate is reduced to nitrite by cadmium metal. Imidazole is used to buffer the analytical stream. The sample stream then is treated with sulfanilamide to yield a diazo compound, which couples with N-1-naphthylethylenediamine dihydrochloride to form an azo dye, the absorbance of which is measured colorimetrically. The result is the sum of the nitrite originally present plus that formed by the reduction of the nitrate (Morris and Riley, 1963; Brewer and Riley, 1965; Wood and others, 1967; Strickland and Parsons, 1972; Nydahl, 1976; Sherwood and Johnson, 1981; Patton, 1982; U.S. Environmental Protection Agency, 1983).

3. Interferences

3.1 Concentrations of potentially interfering substances generally are negligible in unpolluted surface and ground water. For specific details of inorganic and organic compounds that interfere, see Norwitz and Keliher (1985, 1986) as well as more general information from the American Society for Testing and Materials (1991).

3.2 Sulfides, often present in anoxic water, rapidly deactivate cadmium reactors by forming an insoluble layer of cadmium sulfide on the active metal surface (Strickland and Parsons, 1972).

3.3 The buffer capacity of the imidazole solution and the approximate 10:1 volume ratio of buffer to sample eliminate the possibility of erroneous results for moderately acidic ($\text{pH} \geq 1$) samples.

3.4 Mercury (II) does not interfere. It rapidly forms an amalgam on the inlet end of the cadmium reactor without detriment to the reduction reaction.

4. Apparatus

4.1 *Centrifuge*.

4.2 *Shaker*, wrist-action.

4.3 *Alpkem rapid flow analyzer (RFA)*, consisting of sampler, peristaltic pump, analytical cartridge (including copper-cadmium reduction column), colorimeter, data station, and printer.

4.4 With this equipment, the following operating conditions are satisfactory for the range from 0.1 to 5.0 mg/L ($\text{NO}_2 + \text{NO}_3$) as N:

Flow cell	10 mm
Wavelength	520 nm
Sample time	24 seconds
Sampling rate	90 per hour
Wash time	16 seconds
Pecking	ON
Damp (RC)	1 second

5. Reagents

5.1 *Brij-35 solution*, 30-percent aqueous solution.

5.2 *Cadmium powder*, 100 mesh: Weigh 10 g cadmium powder into a 50-mL Griffin beaker and wash with 1M HCl. Stir vigorously to break clumps of cadmium formed by the addition of HCl. Rinse with copper sulfate solution (20 g/L). Wash thoroughly with imidazole solution to remove colloidal copper which is visible as a blue color in the wash solution. A minimum of five washings usually is required to eliminate perceptible blue color. Store in imidazole solution.

5.3 *Cadmium reduction column*: The reduction column is 4 cm long, 1/8-in. OD standard Teflon tubing (about 3.0 mm OD x 1.5 mm ID). Plugs to retain the cadmium in the column are cut from a sheet of hydrophilic, porous plastic with an average pore size of 40 μm . The cadmium column is plugged at the bottom with a plastic frit and attached to a syringe filled with

imidazole solution. A custom-made funnel is attached to the top of the cadmium column, and the column and part of the funnel are filled with imidazole solution. The cadmium is added slowly to prevent air bubbles and pockets. Decant the solution in the funnel to facilitate the insertion of the top frit without introducing air to the column. The reduction efficiency of the column needs to be checked regularly by comparing the peak heights of nitrite and nitrate standards. Equal concentration standards give equal heights. Replace the column if the efficiency falls to less than 90 percent.

5.4 Color reagent: Add 200 mL concentrated phosphoric acid (sp gr 1.69) and 20 g sulfanilamide to about 1,500 mL demineralized water. Dissolve completely (warm if necessary). Add 1.0 g N-1-naphthylethylenediamine dihydrochloride and dissolve completely. Dilute to 2 L with demineralized water. Add 1 mL Brij-35 solution. Store in an amber bottle and refrigerate. This reagent is stable for about 1 month.

5.5. Copper sulfate solution, 20 g/L: Dissolve 20 g CuSO_4 in demineralized water and dilute to 1 L.

5.6 Hydrochloric acid, 1M: Add 83 mL concentrated HCl (sp gr 1.19) to demineralized water and dilute to 1 L.

5.7 Imidazole solution: Dissolve 6.8 g of imidazole in demineralized water. Stir and dilute to about 950 mL. Adjust pH to 7.5 ± 0.1 with concentrated HCl (about 4 mL). Add 0.5 mL copper sulfate solution and dilute to 1 L. Add 1 mL Brij-35 solution.

5.8 Nitrate-nitrogen standard solution I, 1.00 mL = 0.50 mg $\text{NO}_3\text{-N}$: Dissolve 3.609 g KNO_3 , dried overnight over concentrated H_2SO_4 , in demineralized water, and dilute to 1 L.

5.9 Nitrate-nitrogen standard solution II, 1.00 mL = 0.005 mg $\text{NO}_3\text{-N}$: Dilute 10.0 mL nitrate-nitrogen standard solution I to 1,000 mL with demineralized water.

5.10 Nitrate-nitrogen working solutions: Prepare a blank and 200 mL of a series of working solutions by appropriate dilution of nitrate-nitrogen standard solutions I and II, as listed in the following table. If the samples to be analyzed are preserved, the nitrate-nitrogen working solutions need to contain an equivalent concentration of the same preservative.

Working solution No.	Solution added (m L)	Solution used	Nitrate nitrogen concentration (mg/L)
1	2	Standard solution II	5.00
2	1	Standard solution I	2.50
3	40	Standard solution II	1.00

4	20	Standard solution II	0.50
5	10	Working solution II	0.25
6	4	Working solution II	0.10

5.11 *Sodium chloride solution*, 100 g/L, acidified: Dissolve 100 g NaCl in 950 mL demineralized water. Acidify with concentrated HCl (sp gr 1.19) to a pH of 2.5. Dilute to 1 L.

6. Procedure

6.1 Proceed to paragraph 6.2 for processing samples of water. For bottom material, begin with paragraph 6.1.1.

6.1.1 Weigh about 5 g of sample, prepared as directed in either method P-0520 or P-0810, and transfer to a 250-mL Erlenmeyer flask.

6.1.2 Add 50 mL NaCl solution (paragraph 5.11) and shake on the wrist-action shaker for 30 minutes.

6.1.3 Carefully transfer the entire sample, including all sediment particles, to a centrifuge tube. Centrifuge for 5 minutes; if the sample does not flocculate, add a drop of concentrated HCl (sp gr 1.19) and recentrifuge.

6.1.4 Transfer the supernatant solution to a 100-mL volumetric flask, taking care not to disturb the residue in the bottom of the centrifuge tube.

6.1.5 Wash the sediment in the centrifuge tube with 20 mL sodium chloride solution, recentrifuge, and transfer the clear wash solution to a volumetric flask. Adjust to volume with

sodium chloride solution (5.11). Proceed to paragraph 6.2.

6.2 Set up manifold (fig. 1).

6.3 Allow the color reagent to come to room temperature.

6.4 Allow colorimeter and recorder to warm for at least 10 minutes.

6.5 Begin pumping reagents but do not connect the reduction column to the analytical cartridge until all air has been removed from the reagent and sample tubes (NOTE 1).

NOTE 1. It is important to avoid introduction of air bubbles to the reduction column because they adversely affect sample contact with the cadmium powder and decrease the reduction efficiency. Column needs to be replaced if air bubbles are introduced.

6.6 After all reagents are on line, adjust the sample output of the photometer to 5 V. Then switch the photometer to "absorbance" mode and use the reference detector "fine gain" control to adjust the baseline absorbance to about 0.2 V. See operation manuals for complete details (Alpkem Corp., 1986).

6.7 Place the most concentrated working solution in two cups before analysis. As the peaks appear on the recorder, adjust the STD CAL control until the peak obtains 95 percent of full scale.

6.8 When the system is clear of all working solutions, determine a dwell time using the most concentrated working solution.

6.9 Place a complete set of working solutions and a blank in the first positions of the sample tray, beginning with the most concentrated working solution. Place individual working solutions of differing concentrations in about every eighth position on the tray following the accepted protocol. Fill remainder of each tray with unknown samples.

6.10 Begin analysis.

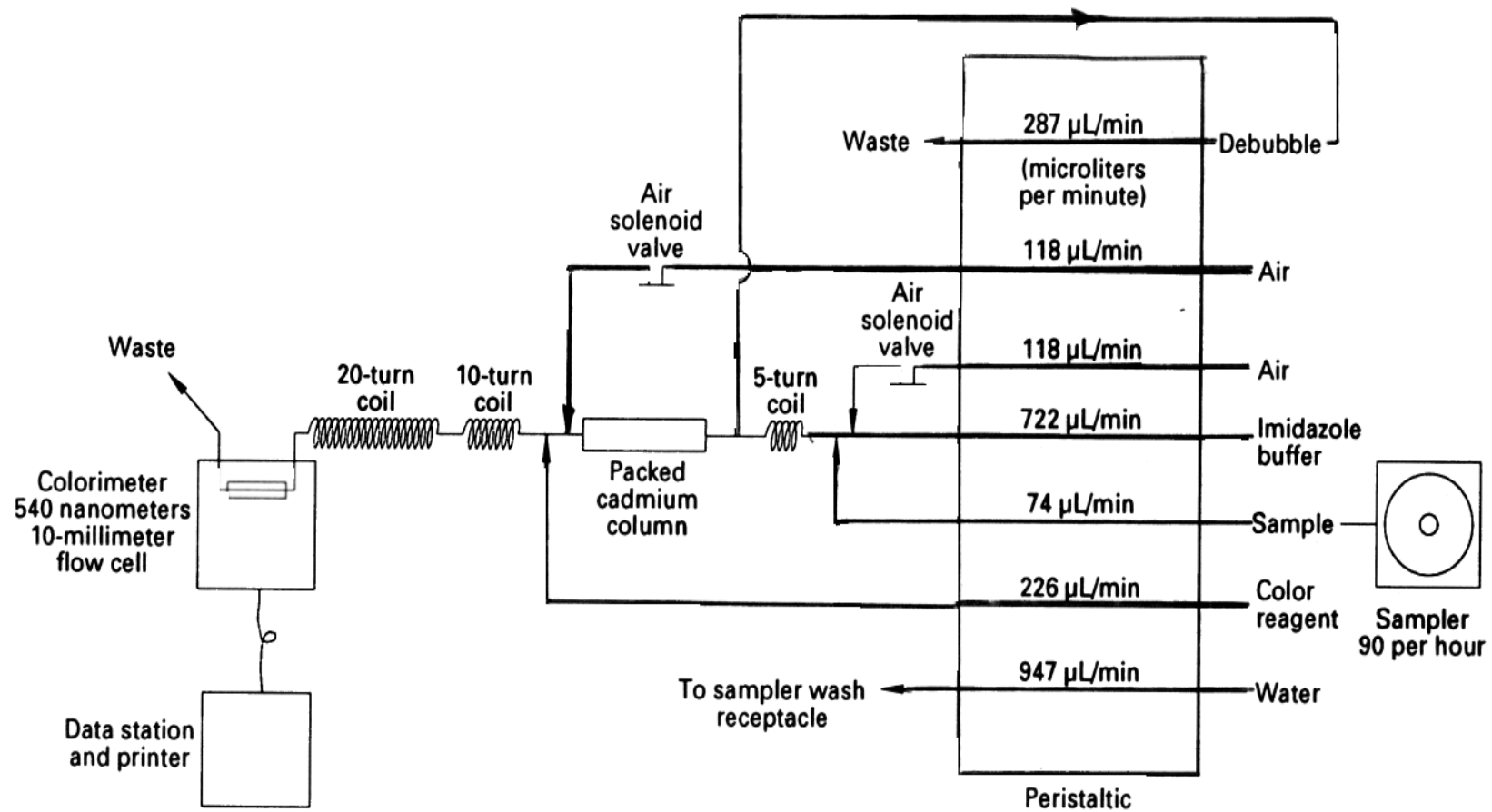


Figure 1. - Nitrogen, nitrite plus nitrate, cadmium reduction-diazotization manifold.

7. Calculations

7.1 Prepare an analytical curve by plotting the voltage of each working solution peak in relation to its respective nitrite- plus nitrate-nitrogen concentration, or by using the RFA Softpac data reduction package. See operation manuals for complete details (Alpkem Corp., 1986).

7.2 Compute the concentration of dissolved nitrite- plus nitrate-nitrogen in milligrams per liter in each sample either by comparing its voltage to the analytical curve or by using the software. Any baseline drift needs to be accounted for when computing the voltage of a sample or working solution peak; the RFA software automatically corrects for baseline drift.

7.3 Compute the concentration of nitrite- plus nitrate-nitrogen in each bottom material sample in milligrams per liter either by comparing its voltage to the analytical curve or by using the software. Any baseline drift needs to be accounted for when computing the voltage of a sample or standard peak.

$$\text{NO}_3\text{-N} + \text{NO}_2\text{-N (mg/kg)} = \frac{C_N \times 100}{\text{wt of sample (g)}}$$

where $C_N = \text{NO}_3\text{-N} + \text{NO}_2\text{-N}$ concentration in sample, in milligrams per liter.

8. Report

8.1 Report concentrations of nitrogen, nitrite plus nitrate, dissolved (00631), as follows: 0.1 to 1.0 mg/L, two decimals; 1.0 mg/L and greater, two significant figures.

8.2 Report concentrations of nitrogen, nitrite plus nitrate, total-inbottom-material (00633), as follows: less than 10 mg/kg, one decimal; 10 mg/kg and greater, two significant figures.

9. Precision and accuracy

9.1 Within-analytical run precision (repeatability) data for natural-water samples with "large", "medium", and "small" concentrations of dissolved nitrite plus nitrate-nitrogen are shown in the following table. In each case, the mean, standard deviation, and percentage of relative deviation result from five replicate determinations of each sample, which were performed at an analysis rate of 90 samples per hour. Sample interaction was 1 percent.

During this analytical run, four different samples in the medium concentration range were spiked at a level of 1 mg/L. The average recovery was 96.2 percent.

Sample No.	Mean (mg/L)	Standard deviation (mg/L)	Relative standard deviation (percent)
1	0.55	0.004	0.7
2	2.66	0.016	0.6
3	4.82	0.014	0.3

9.2 Between-day precision was estimated from dissolved nitrite- plus nitrate-nitrogen concentrations determined for standard reference water samples submitted as blind samples from March 1990 to March 1992. Data listed in the following table indicate long-term accuracy of the method with typical between-day precision in the range from 3 to 6 percent:

Standard reference water sample	Number of replicates	Concentration found (mg/L)	Most probable concentration (mg/L)
N-21	24	0.56 + 0.03	0.58 + 0.02
N-22	8	1.47 ± 0.04	1.54 ± 0.03
N-23	15	0.92 ± 0.08	0.97 ± 0.03
N-24	8	2.05 ± 0.70	1.82 ± 0.05
N-26	9	0.27 ± 0.06	0.29 ± 0.02
N-27	15	1.51 ± 0.16	1.66 ± 0.04
N-28	30	0.34 ± 0.02	0.36 ± 0.01
N-29	41	1.15 ± 0.04	1.23 ± 0.02
N-30	33	0.39 ± 0.02	0.41 ± 0.05
N-31	32	1.41 ± 0.04	1.42 ± 0.10
N-32	5	0.13 ± 0.00	0.14 ± 0.04
N-33	10	0.59 ± 0.02	0.59 ± 0.06

9.3 It is estimated that the percentage relative standard deviation for total nitrite- plus nitrate-nitrogen in bottom material will be greater than that reported for dissolved nitrite- plus nitrate-nitrogen.

References

Alpkem Corp., 1986, Rapid flow analyzer operator's manual: ALPKEM, methodology section.

- American Society for Testing and Materials, 1991, Annual book of ASTM standards, Section 11, Water: Philadelphia, American Society for Testing and Materials, v. 11.01, p. 508-15.
- Brewer, P.G., and Riley, J.P., 1965, The automatic determination of nitrate in sea water: Deep Sea Research, v. 12, p. 765-772.
- Jackson, M.L., 1958, Soil chemical analysis: Englewood Cliffs, N.J., Prentice-Hall, p. 193.
- Morris, A.W., and Riley, J.P., 1963, The determination of nitrate in sea water: Analytica Chimica Acta, v. 29, p. 272-279.
- Norwitz, George, and Keliher, P.N., 1985, Study of interferences in the spectrophotometric determination of nitrite using composite diazotization-coupling reagents: Analyst, v. 110, p. 689-694.
- _____, 1986, Study of organic interferences in the spectrophotometric determination of nitrite using composite diazotization-coupling reagents: Analyst, v. 111, p. 1033-1037.
- Nydahl, Folke, 1976, On the optimum conditions for the reduction of nitrate to nitrite by cadmium: Talanta, v. 23, p. 349-357.
- Patton, C.J., 1982, Novel cadmium reactors for determination of nitrate in water and seawater by segmented, continuous flow colorimetry, chap. 4 of Design, characterization, and applications of a miniature continuous flow analysis system: East Lansing, Michigan, Michigan State University, Ph.D. dissertation, p. 60-121.
- Sherwood, G.A., and Johnson, D.C., 1981, Electrocatalysis of the reduction of nitrate ion at cadmium electrodes by electrodeposited copper: Analytica Chimica Acta, v. 129, p. 87-100.
- Strickland, J.D.H., and Parsons, T.R., 1972, A manual of sea water analysis: Canada Fisheries Research Board Bulletin 167, p. 310.
- U.S. Environmental Protection Agency, 1983, Methods for the chemical analysis of water and wastes: Washington, D.C., U.S. Government Printing Office, p. 353.2-1.
- Wood, E.D., Armstrong, F.A.J., and Richards, F.A., 1967, Determination of nitrate in sea water by cadmium-copper reduction to nitrite: Journal Marine Biology Association, U.K., v. 47, p. 23-31.