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NITROGEN, NITRATE-NITRITE

Method 353.2 (Colorimetric, Automated, Cadmium Reduction)

STORET NO.: Total 00630

1. Scope and Application

1.1 This method pertains to the determination of nitrite singly, or nitrite and nitrate combined in surface and saline waters, and domestic and industrial wastes. The applicable range of this method is 0.05 to 10.0 mg/l nitrate-nitrite nitrogen. The range may be extended with sample dilution.

2. Summary of Method

2.1 A filtered sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (that originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured colorimetrically. Separate, rather than combined nitrate-nitrite, values are readily obtained by carrying out the procedure first with, and then without, the Cu-Cd reduction step.

3. Sample Handling and Preservation

3.1 Analysis should be made as soon as possible. If analysis can be made within 24 hours, the sample should be preserved by refrigeration at 4 degrees C. When samples must be stored for more than 24 hours, they should be preserved with sulfuric acid (2 ml conc. H₂SO₄ per liter) and refrigeration.

Caution: Samples for reduction column must not be preserved with mercuric chloride.

4. Interferences

4.1 Build up of suspended matter in the reduction column will restrict sample flow. Since nitrate-nitrogen is found in a soluble state, the sample may be pre-filtered.

4.2 Low results might be obtained for samples that contain high concentrations of iron, copper or other metals. EDTA is added to the samples to eliminate this interference.

4.3 Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.

5. Apparatus

5.1 Technicon AutoAnalyzer (AAI or AAI) consisting of the following components:

5.1.1 Sampler.

5.1.2 Manifold (AAI) or analytical cartridge (AAI).

5.1.3 Proportioning Pump

5.1.4 Colorimeter equipped with a 15 mm or 50 mm tubular flow cell and 540 nm filters.

5.1.5 Recorder.

5.1.6 Digital printer for AAI (Optional).

6. Reagents

6.1 Granulated cadmium: 40-60 mesh (E M Laboratories, Inc., 500 Exec. Blvd., Elmsford, NY 10523, Cat. 2001 Cadmium, Coarse Powder).

6.2 Copper-cadmium: The cadmium granules (new or used) are cleaned with dilute HCl (6.7) and copperized with 2% solution of copper sulfate (6.8) in the following manner:

6.2.1 Wash the cadmium with HCl (6.7) and rinse with distilled water. The color of the cadmium so treated should be silver.

6.2.2 Swirl 10 g cadmium in 100 ml portions of 2% solution of copper sulfate (6.8) for five minutes or until blue color partially fades, decant and repeat with fresh copper sulfate until a brown colloidal precipitate forms.

6.2.3 Wash the cadmium-copper with distilled water (at least 10 times) to remove all the precipitated copper. The color of the cadmium so treated should be black.

6.3 Preparation of reduction column AAI: The reduction column is an 8 by 50 mm glass tube with the ends reduced in diameter to permit insertion into the system. Copper-cadmium granules (6.2) are placed in the column between glass wool plugs. The packed reduction column is placed in an up-flow 20 degree incline to minimize channeling. See Figure 1.

6.4 Preparation of reduction column AAI: The reduction column is a U-shaped, 35 cm length, 2 mm I.D. glass tube (Note 1). Fill the reduction column with distilled water to prevent entrapment of air bubbles during the filling operations. Transfer the copper- cadmium granules (6.2) to the reduction column and place a glass wool plug in each end. To prevent entrapment of air bubbles in the reduction column be sure that all pump tubes are filled with reagents before putting the column into the analytical system. NOTE 1: A 0.081 I.D. pump tube (purple) can be used in place of the 2 mm glass tube.

6.5 Distilled water: Because of possible contamination, this should be prepared by passage through an ion exchange column comprised of a mixture of both strongly acidic-cation and strongly basic-anion exchange resins. The regeneration of the ion exchange column should be carried out according to the manufacturer's instructions.

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

6.7 Dilute hydrochloric acid, 6N: Dilute 50 ml of conc. HCl to 100 ml with distilled water.

6.8 Copper sulfate solution, 2%: Dissolve 20 g of $\text{CuSO}_4 \times 5\text{H}_2\text{O}$ in 500 ml of distilled water and dilute to 1 liter.

6.9 Wash solution: Use distilled water for unpreserved samples. For samples preserved with H_2SO_4 , use 2 ml H_2SO_4 per liter of wash water.

6.10 Ammonium chloride-EDTA solution: Dissolve 85 g of reagent grade ammonium chloride and 0.1 g of disodium ethylenediamine tetracetate in 900 ml of distilled water. Adjust the pH to 8.5 with conc. ammonium hydroxide and dilute to 1 liter. Add 1/2 ml Brij-35 (available from Technicon Corporation).

6.11 Stock nitrate solution: Dissolve 7.218 g KNO_3 and dilute to 1 liter in a volumetric flask with distilled water. Preserve with 2 ml of chloroform per liter. Solution is stable for 6 months. 1 ml = 1.0 mg $\text{NO}_3\text{-N}$.

6.12 Stock nitrite solution: Dissolve 6.072 g KNO_2 in 500 ml of distilled water and dilute to 1 liter in a volumetric flask. Preserve with 2 ml of chloroform and keep under refrigeration. 1.0 ml = 1.0 mg $\text{NO}_2\text{-N}$.

6.13 Standard nitrate solution: Dilute 10.0 ml of stock nitrate solution (6.11) to 1000 ml. 1.0 ml = 0.01 mg NO₃-N. Preserve with 2 ml of chloroform per liter. Solution is stable for 6 months.

6.14 Standard nitrite solution: Dilute 10.0 ml of stock nitrite (6.12) solution to 1000 ml. 1.0 ml = 0.01 mg NO₂-N. Solution is unstable; prepare as required.

6.15 Using standard nitrate solution (6.13), prepare the following standards in 100.0 ml volumetric flasks. At least one nitrite standard should be compared to a nitrate standard at the same concentration to verify the efficiency of the reduction column.

Concentration, mg NO ₂ -N or NO ₃ -N/l -----	ml Standard Solution/100 ml -----
0.0	0
0.05	0.5
0.10	1.0
0.20	2.0
0.50	5.0
1.00	10.0
2.00	20.0
4.00	40.0
6.00	60.0

NOTE 2: When the samples to be analyzed are saline waters, Substitute Ocean Water (SOW) should be used for preparing the standards; otherwise, distilled water is used. A tabulation of SOW composition follows:

NaCl - 24.53 g/l	MgCl ₂ - 5.20 g/l	Na ₂ SO ₄ - 4.09 g/l
CaCl ₂ - 1.16 g/l	KCl - 0.70 g/l	NaHCO ₃ - 0.20 g/l
KBr - 0.10 g/l	H ₃ BO ₃ - 0.03 g/l	SrCl ₂ - 0.03 g/l
NaF - 0.003 g/l		

7. Procedure

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

7.2 Set up the manifold as shown in Figure 2 (AAI) or Figure 3 (AAII). Note that reductant column should be in 20 degree incline position (AAI). Care should be taken not to introduce air into reduction column on the AAII.

7.3 Allow both colorimeter and recorder to warm up for 30 minutes. Obtain a stable baseline with all reagents, feeding distilled water through the sample line. NOTE 3: Condition column by running 1 mg/l standard for 10 minutes if a

new reduction column is being used. Subsequently wash the column with reagents for 20 minutes.

7.4 Place appropriate nitrate and/or nitrite standards in sampler in order of decreasing concentration of nitrogen. Complete loading of sampler tray with unknown samples.

7.5 For the AAI system, sample at a rate of 30/hr, 1:1. For the AAI, use a 40/hr, 4:1 cam and a common wash.

7.6 Switch sample line to sampler and start analysis.

8. Calculations

8.1 Prepare appropriate standard curve or curves derived from processing NO₂ and/or NO₃ standards through manifold. Compute concentration of samples by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 Three laboratories participating in an EPA Method Study, analyzed four natural water samples containing exact increments of inorganic nitrate, with the following results:

Increment as Nitrate Nitrogen mg N/liter	Precision as Standard Deviation mg N/liter	Accuracy as	
		Bias, %	Bias, mg N/liter
0.29	0.012	+ 5.75	+0.017
0.35	0.092	+18.10	+0.063
2.31	0.318	+ 4.47	+0.103
2.48	0.176	- 2.69	-0.067

Bibliography

1. Fiore, J., and J. E. O'Brien. 1962.
Automation in Sanitary Chemistry - parts 1 & 2, Determination of Nitrates and Nitrites. *Wastes Engineering* v.33 p.128 & 238.
2. Armstrong, F. A., C. R. Stearns, and J. D. Strickland. 1967.
The Measurement of Upwelling and Subsequent Biological Processes by Means of the Technicon AutoAnalyzer and Associated Equipment. *Deep Sea Research* v.14 p.381-389.
3. **Annual Book of ASTM Standards.** 1976.
Part 31, Water, Standard D1254, p.366.
4. **Chemical Analyses for Water Quality Manual.** 1966.
Department of the Interior, FWPCA, R. A. Taft Sanitary Engineering Center Training Program, Cincinnati, Ohio 45226.
5. **Annual Book of ASTM Standards.** 1976.

Part 31, Water, Standard D1141-75, Substitute Ocean Water, p.48.