**METHOD #: 353.3**  Approved for NPDES and SDWA (Issued 1974)

**TITLE:**  Nitrogen, Nitrate-Nitrite (Spectrophotometric, Cadmium Reduction)

**ANALYTE:**  CAS # N  Nitrogen  7727-37-9
$NO_3$  Nitrate
$NO_2$  Nitrite

**INSTRUMENTATION:**  Spectrophotometer

**STORET No.**  Total 00630


1.0  Scope and Application

1.1  This method is applicable to the determination of nitrite singly, or nitrite and nitrate combined in drinking, surface and saline waters, domestic and industrial wastes. The applicable range of this method is 0.01 to 1.0 mg/L nitrate-nitrite nitrogen. The range may be extended with sample dilution.

2.0  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, spectrophotometrically. 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.

3.0  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°C. When samples must be stored for more than 24 hours, they should be preserved with sulfuric acid (2 mL $H_2SO_4$ per liter) and refrigeration.

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

4.0  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 through a glass fiber filter or a 0.45 *u* membrane filter. Highly turbid samples may be pretreated with zinc sulfate before filtration to remove the bulk of particulate matter present in the sample.

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.

4.4 This procedure determines both nitrate and nitrite. If only nitrate is desired, a separate determination must be made for nitrite and subsequent corrections made. The nitrite may be determined by the procedure below without the reduction step.

5.0 Apparatus

5.1 Reduction column: The column in Figure 1 was constructed from a 100 mL pipet by removing the top portion. This column may also be constructed from two pieces of tubing joined end to end. A 10 mm length of 3 cm I.D. tubing is joined to a 25 cm length of 3.5 mm I.D. tubing.

5.2 Spectrophotometer for use at 540 nm, providing a light path of 1 cm or longer.

6.0 Reagents

6.1 Granulated cadmium: 40-60 mesh (MCB Reagents).

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

    6.2.1 Wash the cadmium with dilute HCl (6.10) and rinse with distilled water. The color of the cadmium should be silver.

    6.2.2 Swirl 25 g cadmium in 100 mL portions of a 2% solution of copper sulfate (6.11) for 5 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 copper-cadmium 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 reaction column: Insert a glass wool plug into the bottom of the reduction column and fill with distilled water. Add sufficient copper-cadmium granules to produce a column 18.5 cm in length. Maintain a level of distilled water above the copper- cadmium granules to eliminate entrapment of air. Wash the column with 200 mL of dilute ammonium chloride solution (6.5). The column is then activated by passing through the column 100 mL of a solution composed of 25 mL of a 1.0 mg/L $NO_3$-N standard and 75 mL of ammonium chloride - EDTA solution (6.4). Use a flow rate between 7 and 10 mL per minute.

6.4 Ammonium chloride- EDTA solution: Dissolve 13 g ammonium chloride and 1.7 g disodium ethylenediamine tetraacetate in 900 mL of distilled water. Adjust the pH to 8.5 with conc. ammonium hydroxide (6.9) and dilute to 1 liter.

6.5 Dilute ammonium chloride-EDTA solution: Dilute 300 mL of ammonium chloride- EDTA solution (6.4) to 500 mL with distilled water.

6.6 Color reagent: Dissolve 10 g sulfanilamide and 1 g N-(1-naphthyl) ethylene diamine dihydrochloride in a mixture of 100 mL conc. phosphoric acid and 800

mL of distilled water and dilute to 1 liter with distilled water.

6.7 Zinc sulfate solution: Dissolve 100 g $ZnSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 liter.

6.8 Sodium hydroxide solution, 6N: Dissolve 240 g NaOH in 500 mL distilled water, cool and dilute to 1 liter.

6.9 Ammonium hydroxide, conc.

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

6.11 Copper sulfate solution, 2%: Dissolve 20 g of $CuSO_4 \cdot 5H_2O$ in 500 mL of distilled water and dilute to 1 liter.

6.12 Stock nitrate solution: Dissolve 7.218 g $KNO_3$ in distilled water and dilute to 1000 mL. Preserve with 2 mL of chloroform per liter. This solution is stable for at least 6 months. 1.0 mL = 1.00 mg $NO_3$-N.

80-85 ml

10 cm

3 cm I.D.

2 cm

3.5 mm I.D.

25 cm

18.5 cm

Cu/cd

GLASS WOOL PLUG

CLAMP

TYGON TUBING
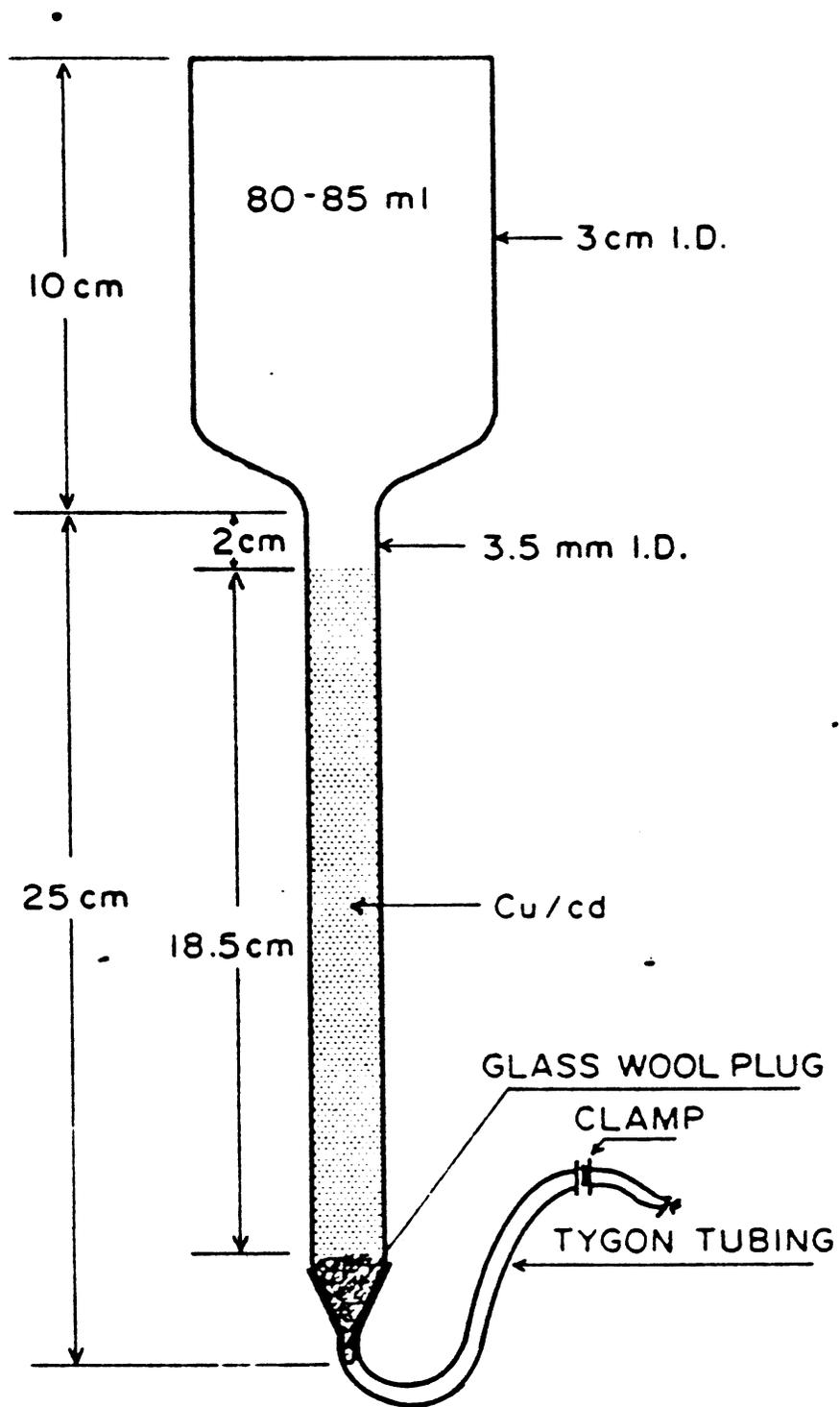
FIGURE 1. REDUCTION COLUMN

6.13 Standard nitrate solution: Dilute 10.0 mL of nitrate stock solution (6.12) to 1000 mL with distilled water. $1.0mL = 0.01$ $mgNO_3$-N.

6.14 Stock nitrite solution: Dissolve 6.072 g $KNO_2$ in 500 mL of distilled water and dilute to 1000 mL. Preserve with 2 mL of chloroform and keep under refrigeration. Stable for approximately 3 months. $1.0$ mL $= 1.00$ mg $NO_2$-N.

6.15 Standard nitrite solution: Dilute 10.0 mL of stock nitrite solution (6.14) to 1000 mL with distilled water. $1.0$ mL $= 0.01$ mg $NO_2$-N.

6.16 Using standard nitrate solution (6.13) prepare the following standards in 100 mL volumetric flasks:

| Conc., mg-NO3-N/L | mL of Standard Solution/100.0 mL |
|---|---|
| 0.00 | 0.0 |
| 0.05 | 0.5 |
| 0.10 | 1.0 |
| 0.20 | 2.0 |
| 0.50 | 5.0 |
| 1.00 | 10.0 |

7.0 Procedure

7.1 Turbidity removal: One of the following methods may be used to remove suspended matter.
   7.1.1 Filter sample through a glass fiber filter or a 0.45u membrane filter.
   7.1.2 Add 1 mL zinc sulfate solution (6.7) to 100 mL of sample and mix thoroughly. Add 0.4-0.5 mL sodium hydroxide solution (6.8) to obtain a pH of 10.5 as determined with a pH meter. Let the treated sample stand a few minutes to allow the heavy flocculent precipitate to settle. Clarify by filtering through a glass fiber filter or a 0.45u membrane filter.

7.2 Oil and grease removal: Adjust the pH of 100 mL of filtered sample to 2 by addition of conc. HCl. Extract the oil and grease from the aqueous solution with two 25 mL portions of a non-polar solvent (Freon, chloroform or equivalent).

7.3 If the pH of the sample is below 5 or above 9, adjust to between 5 and 9 with either conc. HCl or conc. $NH_4OH$. This is done to insure a sample pH of 8.5 after step 7.4.

7.4 To 25.0 mL of sample or an aliquot diluted to 25.0 ml, add 75 mL of ammonium chloride- EDTA solution (6.4) and mix.

7.5 Pour sample into column and collect sample at a rate of 7-10 mL per minute.

7.6 Discard the first 25 ml, collect the rest of the sample (approximately 70 ml) in the original sample flask. Reduced samples should not be allowed to stand longer than 15 minutes before addition of color reagent, step 7.7.

7.7 Add 2.0 mL of color reagent (6.6) to 50.0 mL of sample. Allow 10 minutes for color development. Within 2 hours measure the absorbance at 540 nm against a reagent blank. NOTE: If the concentration of sample exceeds 1.0 mg $NO_3$-N/L, the remainder of the reduced sample may be used to make an appropriate dilution before proceeding with step

7.8 Standards: Carry out the reduction of standards exactly as described for the

samples. At least one nitrite standard should be compared to a reduced nitrate standard at the same concentration to verify the efficiency of the reduction column.

8.0    Calculation

8.1    Obtain a standard curve by plotting the absorbance of standards run by the above procedure against $NO_3$-N mg/L. Compute concentration of samples by comparing sample absorbance with standard curve.

8.2    If less than 25 mL of sample is used for the analysis the following equation should be used:

$$\text{mg } NO_2 + NO_3 - N/L = \frac{A \times 25}{\text{mL sample used}}$$

where:

A = Concentration of nitrate from standard curve.

9.0    Precision and Accuracy

9.1    In a single laboratory (EMSL), using sewage samples at concentrations of 0.04, 0.24, 0.55 and 1.04 mg $NO_3 + NO_2$-N/L, the standard deviations were ± 0.005, ± 0.004, ± 0.005 and ± 0.01, respectively.

9.2    In a single laboratory (EMSL), using sewage samples at concentrations of 0.24, 0.55, and 1.05 mg $NO_3 + NO_2$-N/L, the recoveries were 100%, 102% and 100%, respectively.

## Bibliography

1.    Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 423, Method 419C (1975).
2.    Henrikson, A., and Selmer-Olsen, "Automatic Methods for Determining Nitrate and Nitrite in Water and Soil Extracts". Analyst, May 1970, Vol. 95, p 514-518.
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