

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.

2. Selection of Method

The colorimetric method (4500-NO₂⁻.B) is suitable for concentrations of 5 to 1000 µg NO₂⁻-N/L (See 4500-NO₂⁻.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).

* Approved by Standard Methods Committee, 2000. Editorial revisions, 2011.

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.

d. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Table 4020:I.

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 0.05N 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 0.05N 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 0.05N KMnO₄ to the faint pink endpoint. 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 0.05N KMnO₄ consumed by the NaNO₂ solution corresponds to 1725 µg NaNO₂ or 350 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*, 0.05N: 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 endpoint 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.

$$\text{Normality of KMnO}_4 = \frac{\text{g Na}_2\text{C}_2\text{O}_4}{(A - B) \times 0.067}$$

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. 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.