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4500-NO_2^-

Nitrogen (Nitrite)

Approved by Standard Methods Committee, 2000. Editorial revisions, 2021.

4500-NO_2^- 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-NO_2^- B) is suitable for concentrations of 5 to 1000 µg/L NO_2^--N (See 4500-NO_2^- B.1*a*). Nitrite values can be obtained by the automated method given in Section 4500-NO_3^- 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-NO_3^- I).

4500-NO_2^- B. Colorimetric Method (Griess Assay)

1. General Discussion

a. Principle: Nitrite (NO_2^-) 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/L NO_2^--N. Photometric measurements can be made in the range 5 to 50 µg/L N if a 5-cm light path and a green color filter are used. The color system obeys the Beer–Lambert law up to 180 µg/L N with a 1-cm light path at 543 nm. Higher NO_2^- concentrations can be determined by diluting a sample.

b. Interferences: Chemical incompatibility makes it unlikely that nitrite, free chlorine, and nitrogen trichloride (NCl_3) will coexist. NCl_3 imparts a false red color when color reagent is added. The following ions interfere because of precipitation under test conditions and should be absent: Sb^{3+}, Au^{3+}, Bi^{3+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Ag^+, chloroplatinate ($PtCl_6^{2-}$), and metavanadate (VO_3^{2-}). 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 NO_2^-. Make the determination promptly on fresh samples to prevent bacterial conversion of NO_2^- to NO_3^- or NH_3. 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 4020:1.

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 reagent water is free from NO_2^-, use either of the following procedures to prepare nitrite-free water:

1) Add to 1 L reagent water one small crystal each of $KMnO_4$ and either $Ba(OH)_2$ or $Ca(OH)_2$. Distill 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.2*b*) indicates the presence of permanganate.

2) Add 1 mL conc H_2SO_4 and 0.2 mL $MnSO_4$ solution (36.4 g $MnSO_4 \cdot H_2O$/100 mL reagent water) to each 1 L reagent water, and make pink with 1 to 3 mL $KMnO_4$ solution (400 mg $KMnO_4$ per liter of reagent water). Distill 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. The solution is stable for about one month when stored in a dark bottle in refrigerator.

c. Sodium oxalate, 0.025 M (0.05 N): Dissolve 3.350 g $Na_2C_2O_4$, primary standard grade, in water and dilute to 1000 mL.

d. Ferrous ammonium sulfate, 0.05 M (0.05 N): Dissolve 19.607 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ plus 20 mL conc H_2SO_4 in water and dilute to 1000 mL. Standardize as in Section 5220 B.3*d*.

e. Stock nitrite solution: Commercial reagent-grade $NaNO_2$ assays at less than 99%. Because NO_2^- 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 $NaNO_2$ content, add a known excess of standard 0.05 N $KMnO_4$ solution (see ¶ *h* below), discharge permanganate color with a known quantity of standard reductant, such as 0.025 M $Na_2C_2O_4$ or 0.05 M $Fe(NH_4)_2(SO_4)_2$, and back-titrate with standard permanganate solution.

1) Preparation of stock solution—Dissolve 1.232 g $NaNO_2$ in water and dilute to 1000 mL; 1.00 mL = 250 = µg/L NO_2^--N. Preserve with 1 mL $CHCl_3$.

2) Standardization of stock nitrite solution—Pipet, in order, 50.00 mL standard 0.05 N $KMnO_4$, 5 mL conc H_2SO_4, and 50.00 mL stock NO_2^- solution into a glass-stoppered flask or bottle. Submerge the pipet tip well below the surface of the permanganate acid solution while adding the stock NO_2^- 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 0.025 M $Na_2C_2O_4$. Titrate excess $Na_2C_2O_4$ with 0.05 N $KMnO_4$ 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 0.05 M ferrous ammonium sulfate solution is substituted for $Na_2C_2O_4$, omit heating and extend reaction period between $KMnO_4$ and Fe^{2+} to 5 min before making final $KMnO_4$ titration. Calculate NO_2^--N content of stock solution by the following equation:

$$A = \frac{[(B \times C) - (D \times E)] \times 7}{F}$$

where:

A = mg/mL NO_2^--N in stock $NaNO_2$ solution,
B = total mL standard $KMnO_4$ used,
C = normality of standard $KMnO_4$,
D = total mL standard reductant added,
E = normality of standard reductant, and
F = mL stock $NaNO_2$ solution taken for titration.

Each 1.00 mL 0.05 N $KMnO_4$ consumed by the $NaNO_2$ solution corresponds to 1725 µg $NaNO_2$ or 350 µg NO_2^--N.

f. Intermediate nitrite solution: Calculate the volume, G, of stock NO_2^- solution required for the intermediate NO_2^- 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 NO_2^- solution to 1000 mL with water; 1.00 mL = 0.500 µg N. Prepare daily.

h. Standard potassium permanganate titrant, 0.05 N: Dissolve 1.6 g $KMnO_4$ in 1 L reagent water. Keep in a brown glass-stoppered bottle and age for at least 1 week. Carefully decant or pipet the 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 $Na_2C_2O_4$ into 400-mL beakers. To each beaker, in turn, add 100 mL reagent water and stir to dissolve. Add 10 mL 1 + 1 H_2SO_4 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 the temperature fall below 85 °C. If necessary, warm the beaker contents during titration; 100 mg will consume about 6 mL solution. Run a blank on reagent water and H_2SO_4.

$$\text{Normality of } KMnO_4 = \frac{\text{g } Na_2C_2O_4}{(A - B) \times 0.067}$$

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 a sample contains suspended solids, filter through a 0.45-µm-pore-diam membrane filter.

b. Color development: If the sample pH is not between 5 and 9, adjust to that range with 1 N HCl or NH_4OH 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 NO_2^--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 NO_2^--N concentration. Compute the 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/L NO_3^- + NO_2^--N, 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/L NO_3^- + NO_2^--N, the recoveries were 100%, 102%, and 100%, respectively.[1]

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4500-N_{org} NITROGEN (ORGANIC)

Approved by Standard Methods Committee, 1997. Editorial revisions, 2021. Joint Task Group: Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

4500-N_{org} A. INTRODUCTION

1. Selection of Method

The Kjeldahl methods (4500-N_{org} B and C) determine nitrogen in the trinegative state. They fail to account for nitrogen in the form of azide, azine, azo, hydrazone, nitrate, nitrite, nitrile, nitro, nitroso, oxime, and semi-carbazone. *Kjeldahl nitrogen* is the sum of organic nitrogen and ammonia nitrogen.

The major factor that influences the selection of a macro- or semimicro-Kjeldahl method to determine organic nitrogen is its concentration. The macro-Kjeldahl method applies to samples containing either low or high concentrations of organic nitrogen but requires a relatively large sample volume for low concentrations. In the semimicro-Kjeldahl method, which applies to samples containing high concentrations of organic nitrogen, choose a sample volume that contains organic plus ammonia nitrogen in the range of 0.2 to 2 mg.

The block digestion method (4500-N_{org} D) is a micro method with an automated analysis step capable of measuring organic nitrogen as low as 0.1 mg/L when blanks are carefully controlled.

2. Storage of Samples

The most reliable results are obtained on fresh samples. If an immediate analysis is not possible, preserve samples for Kjeldahl digestion by acidifying to pH 1.5 to 2.0 with concentrated H_2SO_4 and storing at 4 °C. Do not use $HgCl_2$ because it interferes with ammonia removal.

3. Interferences

a. Nitrate: During Kjeldahl digestion, nitrate in excess of 10 mg/L can oxidize a portion of the ammonia released from the digested organic nitrogen, producing N_2O and resulting in a negative interference. When sufficient organic matter in a low state of oxidation is present, nitrate can be reduced to ammonia, resulting in a positive interference. The conditions under which significant interferences occur are not well defined and there is no proven way to eliminate the interference with the Kjeldahl methods described herein.

b. Inorganic salts and solids: The acid and salt content of the Kjeldahl digestion reagent is intended to produce a digestion temperature of about 380 °C. If the sample contains a very large quantity of salt or inorganic solids that dissolve during digestion, the temperature may rise above 400 °C, at which point pyrolytic loss of nitrogen begins to occur. To prevent an excessive digestion temperature, add more H_2SO_4 to maintain the acid–salt balance. Not all salts cause precisely the same temperature rise, but adding 1 mL H_2SO_4 per gram of salt in the sample gives reasonable results. Add the extra acid and the digestion reagent to both sample and reagent blank. Too much acid lowers the digestion temperature below 380 °C and results in incomplete digestion and recovery. If necessary, add sodium hydroxide-sodium thiosulfate before the final distillation step to neutralize the excess acid.

Large amounts of salt or solids also may cause bumping during distillation. If this occurs, add more dilution water after digestion.

c. Organic matter: During Kjeldahl digestion, H_2SO_4 oxidizes organic matter to CO_2 and H_2O. If a large amount of organic matter is present, a large amount of acid will be consumed, the ratio of salt to acid will increase, and the digestion temperature will increase. If enough organic matter is present, the temperature will rise above 400 °C, resulting in pyrolytic loss of nitrogen.