METHOD 9210

1. COMMENT: Several commenters (59, 61, 64) thought that, in Method 9210, there is a possibility for loss of analyte when samples are treated for interferences through coprecipitation.

   RESPONSE: The Agency disagrees ~~wiht~~ with this comment. No loss of analyte was noticed in any of the studies using this method. If at some later time information is presented to the Agency detailing and identifying the loss of analyte due to the coprecipitation process, we will consider it at that time. No changes have been made to the method based on this comment.

2. COMMENT: Several commenters (59, 61, 64) found the narrow working range of Method 9210 to 2 to 100 mg/L to be within the 90 to 110% recoveries.

   RESPONSE: ~~**JB, I don't understand these comments. I looked back at the comments and this was compiled correctly.**~~ The Agency is unsure of the meaning or intent of this comment. If the working range of this method is too narrow for the applications of your laboratory, alternative methods are available. No changes have been made to the method based on this comment.

3. COMMENT: Several commenters (59, 61, 64) stated that the time of ISE in Method 9210 should be equal for all samples to ensure consistency in measurement.

   RESPONSE: The Agency disagrees with this comment. The times in all of the ISE methods are dependent on the conditions necessary to successfully analyze each analyte. If the instrument manufacturer of laboratory has documented various times, then the flexibility of the method allows for these deviations but no change to the method is warranted.

METHOD 9210

POTENTIOMETRIC DETERMINATION OF NITRATE
IN AQUEOUS SAMPLES WITH ION-SELECTIVE ELECTRODE

1.0 SCOPE AND APPLICATION

1.1 This method can be used for measuring total solubilized nitrate in drinking waters, natural surface waters, groundwaters, domestic and industrial wastewaters, and in soil extracts (ASTM methods D4646-87, D5233-92 or D3987-85).

> NOTE: This method is for the analysis of simple nitrate ion rather than total nitrate, as analysis using the ion-selective electrode is not preceded by a distillation step.

1.2 The method detection limit is 2.0 mg/L. Nitrate concentrations from 0.2 to 1,000 mg/L may be measured. However, using a linear calibration, results less than 2 mg/L may be biased up to approximately 420 percent high; results greater than 400 mg/L may be biased up to approximately 50 percent low.

1.3 ISEs must be used carefully, and results must be interpreted cautiously, since an ISE may be affected by numerous analytical interferences which may either increase or decrease the apparent analyte concentration, or which may damage the ISE. Effects of most interferences can be minimized or eliminated by adding appropriate chemical reagents to the sample. Obtaining the most accurate results, therefore, requires some knowledge of the sample composition.

> NOTE: ISE manufacturers usually include a list of interferences in the instruction manual accompanying an ISE, along with recommended methods for minimizing or eliminating effects of these interferences.

2.0 SUMMARY OF METHOD

2.1 Total solubilized nitrate is determined potentiometrically using a nitrate ion-selective electrode (ISE) in conjunction with a double-junction reference electrode and a pH meter with an expanded millivolt scale or an ISE meter capable of being calibrated directly in terms of nitrate concentration.

2.2 Standards and samples are mixed 50:1 with an ionic strength adjustment solution (ISA). Calibration is performed by analyzing a series of standards and plotting mV vs. nitrate-nitrogen concentration on semilog paper or by calibrating the ion meter directly in terms of nitrate concentration.

3.0 INTERFERENCES

3.1 The nitrate electrode responds to numerous interfering anions. Most of the interferants, however, can be rendered harmless by adding suitable reagents. Cyanide, bisulfide, bicarbonate, carbonate, and phosphate are removed by adjusting the solution pH to 4 with boric acid. Chloride, bromide, and iodide are removed by adding silver sulfate solution. Nitrite is also an interferant, as shown in Table 1; nitrite is removed by adding sulfamic acid. The amounts of silver sulfate and sulfamic acid needed will vary based on the concentrations of interferants. As a general guide, 1 mL of silver sulfate will eliminate chloride interference in a 50 mL sample containing 35 mg/L Cl^-; 1 mL of sulfamic acid solution will eliminate nitrite interference in a 50 mL sample containing 95 mg/L NO_2^-.

3.2 Temperature changes affect electrode potentials. Using an ISE calibrated at 22°C, a 20.0 mg/L nitrate solution was measured as 20.6 mg/L at 22 °C and 12.9 mg/L at 32°C (see Ref. 4). Therefore, standards and samples must be equilibrated at the same temperature (± 1°C).

3.3 The user should be aware of the potential of intereferences from colloidal substances and that, if necessary, the samples may be filtered.

4.0 APPARATUS AND MATERIALS

4.1 pH/mV meter capable of reading to 0.1 mV or an ISE meter.

4.2 Nitrate ISE (Orion 9307 or equivalent) and double-junction reference electrode (Orion 9002 or equivalent).

4.3 Thermally isolated magnetic stirrer, Teflon®-coated stir bar, and stopwatch.

4.4 Volumetric flask, 100 mL.

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Reagent water. All references to water in this method refer to reagent water, as defined in Chapter One.

5.3 ISA adjustor solution (2M, $(NH_4)_2SO_4$): Dissolve 26.4 g of ammonium sulfate in reagent water to make 100 mL of solution.

5.4 Boric acid (1M, H_3BO_3): Dissolve 6.2 g of boric acid in reagent water to make 100 mL of preservative solution (for numerous anions and bacteria).

5.5 Silver sulfate (0.05 M, Ag_2SO_4) to remove interferences noted in Step 3.1. A saturated silver sulfate solution contains approximately 5.5 g/L of solubilized silver.

5.6 Sulfamic acid (0.1 M, $HOSO_2NH_2$) to remove nitrite from sample, as noted in Step 3.1.

5.7 Nitrate calibration stock solution (1,000 mg/L, NO_3^-): Dissolve 0.1631 g of potassium nitrate (dried two hours at 110°C and stored in a desiccator) in reagent water, add 1.00 mL of preservative solution, and dilute to 100 mL in a volumetric flask. Store in a clean bottle.

5.8 Nitrate calibration standards: Prepare a series of calibration standards by diluting the 1,000 mg/L nitrate standard. A suitable series is given in the table below.

mL of 1,000 mg/L NO_3^- Solution	Concentration when Diluted to 50.0 mL (mg/L NO_3^--N)
0.0500	1.00
0.150	3.00
0.500	10.0
1.50	30.0
5.00	100.0

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 In most environmental samples, nitrate is not affected by complexation, precipitation, inorganic oxidation-reduction reactions, and protonation. In the presence of a reducing agent (*e.g.*, organic matter), however, bacteria will utilize nitrate as an oxidant, causing a slow decrease in the nitrate concentration. This potential interference can be obviated by using a preservative. Therefore, samples must be preserved by adding 1 mL of 1M boric acid solution per 100 mL of sample.

6.3 Samples should be stored at 4° C and must be analyzed within three (3) days of collection.

7.0 PROCEDURE

7.1 Calibration

7.1.1 When using a nitrate ISE and a separate double-junction reference electrode, ensure that reference electrode inner and outer chambers are filled with solutions recommended by the manufacturer. Equilibrate the electrodes for at least one hour in a 100 mg/L nitrate standard before use.

7.1.2 Calibrate the nitrate ISE using standards that narrowly bracket the expected sample concentration. If the sample concentration is unknown, calibrate with 3.00 mg/L and 30.0 mg/L nitrate standards. Add 50.0 mL of standard, 0.50 mL of preservative solution, and 1.00 mL of ISA to a 100-mL beaker. Add a Teflon®-coated magnetic stir bar, place the beaker on a magnetic stir plate, and stir at slow speed (no visible vortex). Immerse the electrode tips to just above the rotating stir bar. If using an ISE meter, calibrate the meter in terms of nitrate concentration following the manufacturer's instructions. If using a pH/mV meter, record the meter reading (mV) as soon as the reading is stable, but in no case should the time exceed five minutes after immersing the electrode tips. Prepare a calibration curve by plotting measured potential (mV) as a function of the logarithm of nitrate concentration. The slope must be 54-60 mV per decade of nitrate concentration. If the slope is not acceptable, the ISE may not be working properly. For corrective action, consult the ISE operating manual.

7.2 Allow samples and standards to equilibrate to room temperature.

7.3 Prior to and between analyses, rinse the electrodes thoroughly with reagent water and gently shake off excess water. Low-level measurements are faster if the electrode tips are first immersed five minutes in reagent water.

7.4 Add 50.0 mL of sample and 1.00 mL of ISA to a 100-mL beaker. Add a Teflon®-coated magnetic stir bar. Place the beaker on a magnetic stir plate and stir at a slow speed (no visible vortex). Immerse the electrode tips to just above the rotating stir bar. Record the meter reading (mV or concentration) as soon as the reading is stable, but in no case should the time exceed five minutes after immersing the electrode tips. If reading mV, determine nitrate-nitrogen concentration from the calibration curve.

7.5 When analyses have been completed, rinse the electrodes thoroughly and store them in a 100 mg/L nitrate standard solution. If the electrodes will not be used more than one day, drain the reference electrode internal filling solutions, rinse with reagent water, and store dry.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One for specific quality control procedures.

8.2 Initial Calibration Verification standard (ICV): After performing the calibration step (7.1), verify calibration by analyzing an ICV. The ICV contains a known nitrate concentration at the mid-range of the calibration standards and is from an independent source. ICV recovery must be 90-110 percent. If not, the source of error must be found and corrected. An acceptable ICV must be analyzed prior to sample analysis. The ICV also serves as a laboratory control sample.

8.3 Continuing Calibration Verification standard (CCV): After every 10 samples, and after the final sample, a CCV must be analyzed. The CCV contains a known nitrate concentration at mid-calibration range. CCV recovery must be 90-110 percent. If not, the error source must be found and corrected. If ISE calibration has changed, all samples analyzed since the last acceptable CCV must be re-analyzed.

8.4 Reagent blank: After the ICV and after every CCV, a reagent blank must be analyzed. A reagent blank is a 1 percent solution of preservative solution in reagent water, mixed 50:1 with ISA. The indicated reagent blank concentration must be less than 1 mg/L nitrate. If not, the contamination source must be found and corrected. All samples analyzed since the last acceptable reagent blank must be re-analyzed.

8.5 Matrix spike: Follow the matrix spike protocols presented in Chapter One. The spike concentration must be 10 times the detection limit and the volume added must be negligible (less than or equal to one-thousandth the sample aliquot volume). Spike recovery must be 75-125 percent. If not, samples must be analyzed by the method of standard additions.

9.0 METHOD PERFORMANCE

9.1 In a single-laboratory evaluation, a series of standards with known nitrate concentrations was analyzed with a nitrate ISE. Measurements were obtained over three consecutive days using an Orion 9307 nitrate ISE and an Orion 9002 double-junction reference electrode connected to an Orion 940 ISE meter. A two-point calibration (5.00 and 50.0 mg/L nitrate) was performed prior to analysis. The results are listed in Table 2.

9.2 In the same study, three groundwater samples were spiked with nitrate at four different concentrations and measured with the nitrate ISE. (The groundwater samples initially contained <0.1-2.3 mg/L nitrate.) Each spiked sample was analyzed at each concentration, and the mean recoveries and RSDs are given in Table 3.

9.3 A 50 g portion of soil, which initially contained 0.7 mg/kg nitrate, was spiked with 25.0 mg/kg nitrate to obtain an anion concentration in a single extract volume within the linear range of the ISE. The extract was then analyzed for nitrate using this ISE method, and 89% of the soil spike was recovered.

10.0 REFERENCES

1. Franson, Mary Ann H., Ed. Standard Methods for the Examination of Water and Wastewater, 18th Edition. American Public Health Association, Washington, DC, 1992.

2. Model 93-07 Nitrate Electrode Instruction Manual. Orion Research, Inc., Boston, MA, 1986.

3. Miller, E.L., Waltman, D.W., and Hillman, D.C. Single-Laboratory Evaluation of Fluoride, Chloride, Bromide, Cyanide, and Nitrate Ion-Selective Electrodes for Use in SW-846 Methods. Lockheed Engineering and Sciences Company for Environmental Monitoring Systems Laboratory, U.S. EPA. September 1990. EPA/600/X-90/221.

Table 1. Nitrate ISE Interferences

Analyte Concentration (mg/L)	Interference	Measured Concentration (mg/L)	RSD (%)
25.0	None	26	6.2
25.0	0.01 M H_2SO_4	24.5	5.9
25.0	100 mg/L NO_2^-	46	9.1
25.0	100 mg/L NO_2^- + 500 mg/L $HOSO_2NH_2$	26	6.3

Table 2. Results from a Single-Laboratory Accuracy Evaluation of a Nitrate ISE

Nitrate Concentration (mg/L)	Nitrate Detected (mg/L)	Nitrate Recovery (percent)	Rel. Std. Deviation (percent)
0.100	1.01	1,010	53
0.200	1.04	520	17
0.500	1.23	246	8
1.00	1.71	171	2
2.00	2.45	123	7
5.00	5.0	100	0
10.0	11.0	110	8
20.0	18.9	95	14
50.0	50	100	1
100	96	96	13
200	164	82	3
400	310	77	8
1,000	480	48	17

Table 3. Mean Spike Recoveries of Nitrate in Three Groundwater Samples

Analyte Spike Concentration (mg/L)	Spike Recovery (percent)	Rel. Std. Deviation (percent)
2.00	113	10.7
3.00	106	7.6
5.00	98	1.2
10.0	89	2.7

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