

APPENDIX D-19
NH₃-N by Flow Injection Analysis: AP-0059

1.0 PURPOSE

This procedure provides a method for the determination of ammonia in drinking and surface waters.

2.0 SCOPE

2.1 This method covers the determination of ammonia in drinking and surface waters.

2.2 The method is based on reactions that are specific for the ammonium ion.

2.3 The applicable range is 0.1 to 20.0 mg N/L as NH₃.

3.0 SUMMARY

This method is based on the Berthelot reaction. Ammonia reacts with alkaline phenol, then with sodium hypochlorite to form indophenol blue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm, and is directly proportional to the original ammonia concentration in the sample.

4.0 REFERENCES

4.1 U.S. Environmental Protection Agency, *Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020, Revised March 1983, "Nitrogen, Ammonia, Method 350.1 (Colorimetric, Automated Phenate)."

4.2 U.S. Environmental Protection Agency, 40 CFR Part 36 Table 1B, footnote 6, 1994.

4.3 Lachat Instruments, *QuickChem Automated Ion Analyzer Methods Manual*, QuickChem Method 10-107-06-1-A, "Determination Of Ammonia By Flow Injection Analysis, Colorimetry."

4.4 Lachat Instruments, *QuickChem 8000 Automated Ion Analyzer Omnion FIA Software Installation and Tutorial Manual*.

5.0 RESPONSIBILITIES

5.1 It is the responsibility of the laboratory manager to ensure that this procedure is followed.

5.2 It is the responsibility of the team leader to review the results of the procedure.

5.3 It is the responsibility of the Analysts to follow this procedure, evaluate data, and to report any abnormal results or unusual occurrences to the team leader.

6.0 REQUIREMENTS

6.1 Prerequisites

6.1.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to ensure a representative sample and allow for quality control analysis (at least 100 mL).

6.1.2 Samples may be preserved by addition of a maximum of 2 mL of concentrated H₂SO₄ per liter (preferred - 1 mL of 1N H₂SO₄ per 100 mL) and stored at 4°C. Acid preserved samples have a holding time of 28 days.

6.2 Limitations and Actions

6.2.1 If the analyte concentration is above the analytical range of the calibration curve, the sample must be diluted to bring the analyte concentration within range.

6.2.2 Interferences

6.2.2.1 Calcium and magnesium ions may precipitate if present in sufficient concentration. Tartrate or EDTA is added to the sample in-line in order to prevent this problem.

6.2.2.2 Color, turbidity and certain organic species may interfere. Turbidity can be removed by filtration through a 0.45 um pore diameter membrane filter prior to analysis. Sample color may be corrected for by running the samples through the

manifold without color formation (omit Sodium Phenolate, reagent 1). The ammonium concentration is determined by subtracting the value obtained without color formation from the value obtained with color formation.

6.3 Apparatus/Equipment

6.3.1 Balance – analytical, capable of accurately weighing to the nearest 0.0001 g.

6.3.2 Glassware – Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3.3 Flow injection analysis equipment (Lachat model 8000) designed to deliver and react samples and reagents in the required order and ratios.

6.3.3.1 Autosampler

6.3.3.2 Multichannel proportioning pump

6.3.3.3 Reaction unit or manifold

6.3.3.4 Colorimetric detector

6.3.3.5 Data system

6.3.4 Special Apparatus

6.3.4.1 Heating Unit

6.3.5 Syringe filters - Titan nylon 25-mm syringe filters - 0.45 micron. SRI Catalog number 44525-NN or equivalent.

6.3.6 Syringes - 10 cc syringe with Luer Lok, B-D Part 309604 or equivalent. (Smaller volumes are acceptable)

6.4 Reagents and Standards

6.4.1 Preparation of Reagents -

Use deionized water (10 megohm) for all solutions.

Degassing with helium: To prevent bubble formation, degas all solutions except the standards, Sodium Phenolate (Reagent 1) and Sodium Hypochlorite (Reagent 2) with helium. Bubble helium through a degassing tube (Lachat Part 50100) through the solution for at least one minute.

Refrigerate all solutions and standards.

6.4.1.1 **Reagent 1. Sodium Phenolate**

CAUTION: Wear gloves. Phenol causes severe burns and is rapidly absorbed in the body through the skin.

By Volume: In a 1 L volumetric flask, dissolve **88 mL of 88% liquefied phenol** or **83 g crystalline phenol** (C₆H₅OH) in approximately **600 mL water**. While stirring, slowly add **32 g sodium hydroxide** (NaOH). Cool, dilute to the mark, and mix. Do not degas this reagent.

By weight: To a tared 1 L container, add **888 g water**. Add **94.2 g of 88 liquefied phenol** or **83 g crystalline phenol** (C₆H₅OH). While stirring, slowly add **32 g sodium hydroxide** (NaOH). Cool and invert to mix. Do not degas this reagent.

6.4.1.2 **Reagent 2. Sodium Hypochlorite**

By Volume: In a **500 mL** volumetric flask, dilute **250 mL Regular Clorox bleach** [5.25% sodium hypochlorite (NaOCl), The Clorox Company, Oakland, CA] to mark with **water**. Invert to mix.

By weight: To a tared **500 mL** container, add **250 g Regular Clorox bleach** [5.25% sodium hypochlorite (NaOCl), The Clorox Company, Oakland, CA] and **250 g water**. Invert to mix.

6.4.1.3 **Reagent 3. Buffer**

By Volume: In a **1 L** volumetric flask, dissolve **50.0 g disodium ethylenediamine tetraacetate dihydrate** (Na₂EDTA • 2H₂O) and **5.5 g sodium hydroxide** (NaOH) in about **900 mL water**. Dilute to the mark and invert or stir to mix.

By weight: To a tared **1 L** container, add **50.0 g disodium ethylenediamine tetraacetate dihydrate** (Na₂EDTA • 2H₂O) and **5.5 g sodium hydroxide** (NaOH). Add **968 g water**. Invert or stir to mix.

6.4.1.4 **Reagent 4. Sodium Nitroprusside**

By Volume: In a **1 L** volumetric flask, dissolve **3.50 g sodium nitroprusside** (Sodium Nitroferricyanide [Na₂Fe(CN)₅NO • 2H₂O]) dilute to the mark with **water**. Stir or shake to mix.

By weight: To a tared **1 L** flask, dissolve **3.50 g sodium nitroprusside** (Sodium Nitroferricyanide [Na₂Fe(CN)₅NO • 2H₂O]) and **1000 g water**. Stir or shake to mix.

6.4.2 Preparation of Standards

Note: Following are standards preparations for running 3 channels simultaneously for PO₄-P, NH₃-N and NO₂-N + NO₃-N. Also included is the preparation of a NO₂-N standard which is used to assess the cadmium reduction column's efficiency.

6.4.2.1 **Standard 1. Stock Orthophosphate Standard - 1000 mg P/L as PO₄**

Dry **primary standard grade anhydrous potassium phosphate monobasic** (KH₂PO₄) for one hour at 105°C. In a 1 L volumetric flask dissolve **4.396 g primary standard grade anhydrous potassium phosphate monobasic** (KH₂PO₄) in about **800 mL water**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.2 **Standard 2. Stock Ammonia Standard - 1000 mg N/L as NH₃**

Dry **ammonium chloride** (NH₄Cl) for two hours at 105°C. In a 1 L volumetric flask dissolve **3.819 g ammonium chloride** (NH₄Cl) in about **800 mL water**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.3 **Standard 3. Stock Nitrate Standard - 1000 mg N/L as NO₃⁻**

In a 1 L volumetric flask dissolve **7.220 g potassium nitrate** (KNO₃) in about **600 mL water**. Add **2 mL chloroform**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.4 **Standard 4. Stock Nitrite Standard - 1000 mg N/L as NO₂⁻**

In a 1 L volumetric flask dissolve **4.93 g sodium nitrate** (NaNO₂) in about **800 mL water**. Add **2 mL chloroform**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.5 **Standard 5. Working Standard - 50 mg/L PO₄-P, NH₃-N and NO₃-N**

In a 1 L volumetric flask add about 600 mL water. Pipette 50 mL from each of the **Stock Orthophosphate Standard** (standard 1), the **Stock Ammonia Standard** (standard 2), and the **Stock Nitrate Standard** (standard 3). Dilute to mark with water and mix.

6.4.2.6 **Standard 6. Working Nitrite Standard - 20 mg N/L as NO₂⁻**

In a 1 L volumetric flask add about 700 mL water. Pipette 20 mL **Stock Nitrate Standard** (standard 4). Dilute to mark with water and mix.

6.4.2.7 **Standard 7. Working Quality Control Standard - 32.61 mg P/L as PO₄³⁻, 31.06 mg N/L as NH₄, and 27.11 mg N/L as NO₃⁻.**

In a 500 mL volumetric flask add about 300 mL water. Pipette 50 mL of the E M Science 1000 mg/L **Phosphate Standard Solution** (326.1 mg P/L), 20 mL of the E M Science 1000 mg/L **Ammonia Standard Solution** (776.5 mg N/L), and 60 mL of the E M Science 1000 mg/L **Nitrate Standard Solution** (225.9 mg N/L). Dilute to mark with water and mix.

Note: 1000 mg/L standards by other reputable laboratory vendors may be substituted.

6.4.2.8 **Calibration Standards**

Standards are diluted to **500 mL** with **water**.

	Calibration Standards Concentration mg/L	Prepared From	
		Concentration mg/L	Aliquot mL
1	20.00	50	200
2	10.00	50	100
3	4.00	50	40
4	2.50	50	25
5	1.00	10	50
6	0.10	1	50
7	0.02	0.10	100
8	0.00	Water	0

For standards for samples that have 1 mL of 1 N H₂SO₄ added per 100 mL, add **5 mL** of **1N H₂SO₄** to each standard after building to volume.

Note: If other acid concentrations are used to preserve samples, match for standards.

6.4.2.9 **Cadmium Reduction Column Efficiency Check Standard - 2.00 mg N/L as NO₂⁻**

In a **500 mL** volumetric flask add about **300 mL water**. Pipette **50 mL** of the **Working Nitrite Standard** (standard 6). Dilute to mark with **water**, add **5 mL** of **1N H₂SO₄** and mix.

6.4.2.10 **Laboratory Control Standard - 1.63 mg P/L as PO₄, 1.55 mg N/L as NH₃, and 1.36 mg N/L as NO₃⁻.**

In a **1 L** volumetric flask add about **700 mL water**. Pipette **50 mL** of the **Working Quality Control Standard** (standard 7). Dilute to mark with **water**, add **10 mL** of **1N H₂SO₄** and mix.

6.5 Quality Control Sample Requirements

Begin and end each run by measuring a laboratory control standard, a midpoint calibration standard run as a sample, and a reagent blank. When the run is long enough, every twentieth sample should be followed by the above three QC check samples. Recovery should be 90 to 110% of the expected value.

7.0 PROCEDURE

7.1 Procedure Instructions

7.1.1 The instrument is calibrated each day of use and may be calibrated with each sample tray.

7.1.2 Prepare reagents and standards as described in section 6.4.

7.1.3 Set up manifold as shown in section 9.2.

7.1.4 Enter data system parameters as in section 9.1.

7.1.5 Pump deionized water through all reagent lines and check for leaks and smooth flow. Allow 15 minutes for heating unit to warm up to 60°C. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

7.1.6 Pour samples and standards into vials. If samples have particulate matter, filter them into the sample vial with a syringe and nylon syringe filter. Load standard and sample trays.

7.1.7 Place samples and standards in the autosampler. Enter the information required by the data system, such as standard concentration, and sample identification.

7.1.8 Calibrate the instrument by injecting the standards. The data system will then associate the concentration with the instrument responses for each standard.

7.1.9 If samples require color correction, inject the samples with color development, then inject the samples with water replacing the color reagent (reagent 1).

- 7.1.10 At end of run, remove all transmission lines from reagents and place them in water. Pump for about five minutes.
- 7.1.11 To prevent baseline drifts, peaks that are too wide, or other problems with NH₃-N precision, clean the NH₃-N manifold by placing the manifold reagent lines in 1M hydrochloric acid (1 volume concentrated HCl added to 11 volumes of water). Pump for about 5 minutes.
- 7.1.12 Remove all reagent lines from the hydrochloric acid and place them in water. Pump until the HCl is thoroughly washed out (about 5 minutes).
- 7.1.13 Remove the transmission lines from the water and pump all lines dry.
- 7.2 Calculations and Recording Data
- 7.2.1 Calibration is done by injecting standards. The data system will then automatically prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation provided by the software.
- 7.2.2 Create a custom report. (Lachat Instruments, *QuickChem 8000 Automated Ion Analyzer Omnion FIA Software Installation and Tutorial Manual*, page 43, "Task 11 - Creating a Custom Report")
- 7.2.3 Report only those values that fall between the lowest and highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.
- 7.2.4 Samples that require color correction: From the value obtained with color developer added, subtract the value obtained without color developer. When a large number of samples are analyzed, use a spreadsheet to calculate the color correction.
- 7.2.5 Report results in mg NH₃-N/L.

8.0 SAFETY

8.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Use routine laboratory protective clothing (lab coat, gloves, and eye protection) when handling these reagents. Thoroughly wash any skin that comes into contact with any of these chemicals. Avoid creating or inhaling dust or fumes from solid chemicals.

9.0 NOTES

9.1 Data System Parameters

Method Filename: PANHANOW.MET
 Method Description: Ortho P (a) = 4.0 to 0.02 mg P/L
 NH₃-N (a) = 20.0 to 0.1 mg N/L
 NO₂-N/NO₃-N (a) = 20.0 to 0.2 mg N/L

Analyte Data:

Analyte Name: Ammonia (NH₃)-N
 Concentration Units: mg NH₃-N/L
 Chemistry: Direct
 Inject to Peak Start (s): 28.0
 Peak Base Width (s): 21.000
 % Width Tolerance: 100.000
 Threshold: 8000.000
 Autodilution Trigger: Off
 QuickChem Method: 10-107-06-1-A

Calibration Data:

Levels: (mg NH ₃ -N/L)	1: 20.000	2: 10.000	3: 4.000
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	5: 1.000	6: 0.100	8: 0.000
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Calibration Rep Handling:	Average
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Calibration Fit Type:	1 st Order Poly
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Force through Zero:	No
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Weighing Method:	None
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Concentration Scaling:	None
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Sampler Timing:

Method Cycle Period:	70.0
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Min. Probe in Wash Period:	9.0
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Probe in Sample Period:	30.0
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Valve Timing:

Method Cycle Period:	70.0
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Sample Reaches 1 st Valve:	18.0
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Valve:	On
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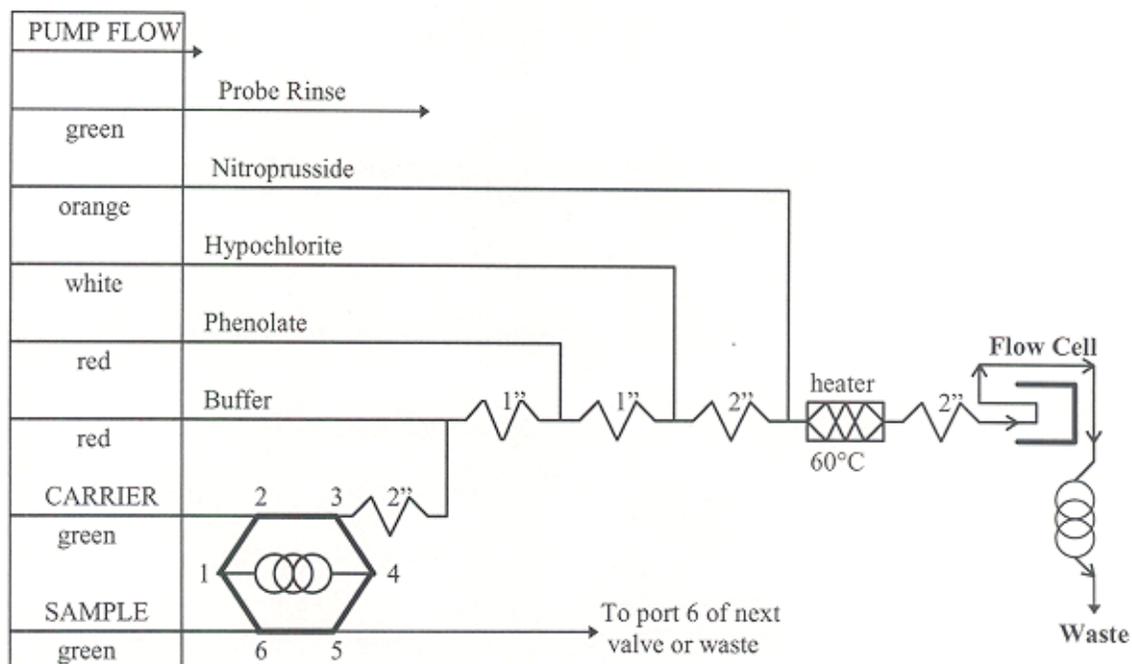
Load Time:	0.0
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Load period	25.0
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Inject Period:	45.0
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Sample Loop:	13 cm x 0.5 mm i.d.
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9.2 Ammonia Manifold Diagram



Sample Loop = 13 cm x 0.5 mm i.d.

Interference Filter = 630 nm

Carrier is DI Water

All manifold tubing is **0.8 mm (0.32 in) i.d.** Lachat Part No. 50028. This is **5.2 uL/cm**. The sample loop uses **0.5 mm (0.022") i.d.** tubing.

1 is **70 cm** of tubing on a **4.5 cm** coil support.

Apparatus: The  includes 650 cm of tubing wrapped around the heater block at the specified temperature.

10.0 ATTACHMENTS AND APPENDICES

None

End of Procedure