

4500-NH₃ NITROGEN (AMMONIA)*

4500-NH₃ A. Introduction

1. Selection of Method

The two major factors that influence selection of the method to determine ammonia are concentration and presence of interferences. In general, direct manual determination of low concentrations of ammonia is confined to drinking waters, clean surface or groundwater, and good-quality nitrified wastewater effluent. In other instances, and where interferences are present or greater precision is necessary, a preliminary distillation step (B) is required.

A titrimetric method (C), an ammonia-selective electrode method (D), an ammonia-selective electrode method using

known addition (E), a phenate method (F), and two automated versions of the phenate method (G and H) are presented. Methods D, E, F, G, and H may be used either with or without sample distillation. The data presented in Tables 4500-NH₃:I and III should be helpful in selecting the appropriate method of analysis.

Nesslerization has been dropped as a standard method, although it has been considered a classic water quality measurement for more than a century. The use of mercury in this test warrants its deletion because of the disposal problems.

The distillation and titration procedure is used especially for NH₃-N concentrations greater than 5 mg/L. Use boric acid as the absorbent following distillation if the distillate is to be titrated.

The ammonia-selective electrode method is applicable over the range from 0.03 to 1400 mg NH₃-N/L.

The manual phenate method is applicable to both fresh water and seawater and is linear to 0.6 mg NH₃-N/L. Distill into

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Joint Task Group: 20th Edition (4500-NH₃:H)—Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

sulfuric acid (H₂SO₄) absorbent for the phenate method when interferences are present.

The automated phenate method is applicable over the range of 0.02 to 2.0 mg NH₃-N/L.

2. Interferences

Glycine, urea, glutamic acid, cyanates, and acetamide hydrolyze very slowly in solution on standing but, of these, only urea and cyanates will hydrolyze on distillation at pH of 9.5. Hydrolysis amounts to about 7% at this pH for urea and about 5% for cyanates. Volatile alkaline compounds such as hydrazine and amines will influence titrimetric results. Residual chlorine reacts with ammonia; remove by sample pretreatment. If a sample is likely to contain residual chlorine, immediately upon collection, treat with dechlorinating agent as in 4500-NH₃.B.3d.

3. Storage of Samples

Most reliable results are obtained on fresh samples. If samples are to be analyzed within 24 h of collection, refrigerate unacidi-

fied at 4°C. For preservation for up to 28 d, freeze at -20°C unacidified, or preserve samples by acidifying to pH <2 and storing at 4°C. If acid preservation is used, neutralize samples with NaOH or KOH immediately before making the determination. CAUTION: Although acidification is suitable for certain types of samples, it produces interferences when exchangeable ammonium is present in unfiltered solids.

4. Bibliography

- THAYER, G.W. 1970. Comparison of two storage methods for the analysis of nitrogen and phosphorus fractions in estuarine water. *Chesapeake Sci.* 11:155.
- SALLEY, B.A., J.G. BRADSHAW & B.J. NELSON. 1986. Results of Comparative Studies of Preservation Techniques for Nutrient Analysis on Water Samples. Virginia Institute of Marine Science, Gloucester Point.

4500-NH₃ B. Preliminary Distillation Step

1. General Discussion

The sample is buffered at pH 9.5 with a borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds. It is distilled into a solution of boric acid when titration is to be used or into H₂SO₄ when the phenate method is used. The ammonia in the distillate can be determined either colorimetrically by the phenate method or titrimetrically with standard H₂SO₄ and a mixed indicator or a pH meter. The choice between the colorimetric and the acidimetric methods depends on the concentration of ammonia. Ammonia in the distillate also can be determined by the ammonia-selective electrode method, using 0.04N H₂SO₄ to trap the ammonia.

2. Apparatus

a. Distillation apparatus: Arrange a borosilicate glass flask of 800- to 2000-mL capacity attached to a vertical condenser so that the outlet tip may be submerged below the surface of the receiving acid solution. Use an all-borosilicate-glass apparatus or one with condensing units constructed of block tin or aluminum tubes.

b. pH meter.

3. Reagents

a. Ammonia-free water: Prepare by ion-exchange or distillation methods:

1) Ion exchange—Prepare ammonia-free water by passing distilled water through an ion-exchange column containing a strongly acidic cation-exchange resin mixed with a strongly basic anion-exchange resin. Select resins that will remove organic compounds that interfere with the ammonia determination.

Some anion-exchange resins tend to release ammonia. If this occurs, prepare ammonia-free water with a strongly acidic cation-exchange resin. Regenerate the column according to the manufacturer's instructions. Check ammonia-free water for the possibility of a high blank value.

2) Distillation—Eliminate traces of ammonia in distilled water by adding 0.1 mL conc H₂SO₄ to 1 L distilled water and redistilling. Alternatively, treat distilled water with sufficient bromine or chlorine water to produce a free halogen residual of 2 to 5 mg/L and redistill after standing at least 1 h. Discard the first 100 mL distillate. Check redistilled water for the possibility of a high blank.

It is very difficult to store ammonia-free water in the laboratory without contamination from gaseous ammonia. However, if storage is necessary, store in a tightly stoppered glass container to which is added about 10 g ion-exchange resin (preferably a strongly acidic cation-exchange resin)/L ammonia-free water. For use, let resin settle and decant ammonia-free water. If a high blank value is produced, replace the resin or prepare fresh ammonia-free water.

Use ammonia-free distilled water for preparing all reagents, rinsing, and sample dilution.

b. Borate buffer solution: Add 88 mL 0.1N NaOH solution to 500 mL approximately 0.025M sodium tetraborate (Na₂B₄O₇) solution (9.5 g Na₂B₄O₇ · 10 H₂O/L) and dilute to 1 L.

c. Sodium hydroxide, 6N.

d. Dechlorinating reagent: Dissolve 3.5 g sodium thiosulfate (Na₂S₂O₃ · 5H₂O) in water and dilute to 1 L. Prepare fresh weekly. Use 1 mL reagent to remove 1 mg/L residual chlorine in 500-mL sample.

e. Neutralization agent.

1) Sodium hydroxide, NaOH, 1N.

2) Sulfuric acid, H_2SO_4 , 1*N*.

f. Absorbent solution, plain boric acid: Dissolve 20 g H_3BO_3 in water and dilute to 1 L.

g. Indicating boric acid solution: See Section 4500-NH₃.C.3a and b.

h. Sulfuric acid, 0.04*N*: Dilute 1.0 mL conc H_2SO_4 to 1 L.

4. Procedure

a. Preparation of equipment: Add 500 mL water and 20 mL borate buffer, adjust pH to 9.5 with 6*N* NaOH solution, and add to a distillation flask. Add a few glass beads or boiling chips and use this mixture to steam out the distillation apparatus until distillate shows no traces of ammonia.

b. Sample preparation: Use 500 mL dechlorinated sample or a known portion diluted to 500 mL with water. When NH₃-N concentration is less than 100 $\mu\text{g/L}$, use a sample volume of 1000 mL. Remove residual chlorine by adding, at the time of collection, dechlorinating agent equivalent to the chlorine residual. If necessary, neutralize to approximately pH 7 with dilute acid or base, using a pH meter.

Add 25 mL borate buffer solution and adjust to pH 9.5 with 6*N* NaOH using a pH meter.

c. Distillation: To minimize contamination, leave distillation apparatus assembled after steaming out and until just before starting sample distillation. Disconnect steaming-out flask and

immediately transfer sample flask to distillation apparatus. Distill at a rate of 6 to 10 mL/min with the tip of the delivery tube below the surface of acid receiving solution. Collect distillate in a 500-mL erlenmeyer flask containing 50 mL indicating boric acid solution for titrimetric method. Distill ammonia into 50 mL 0.04*N* H_2SO_4 for the ammonia-selective electrode method and for the phenate method. Collect at least 200 mL distillate. Lower distillation receiver so that the end of the delivery tube is free of contact with the liquid and continue distillation during the last minute or two to cleanse condenser and delivery tube. Dilute to 500 mL with water.

When the phenate method is used for determining NH₃-N, neutralize distillate with 1*N* NaOH solution.

d. Ammonia determination: Determine ammonia by the titrimetric method (C), the ammonia-selective electrode methods (D and E), or the phenate methods (F and G).

5. Bibliography

- NICHOLS, M.S. & M.E. FOOTE. 1931. Distillation of free ammonia from buffered solutions. *Ind. Eng. Chem., Anal. Ed.* 3:311.
- GRIFFIN, A.E. & N.S. CHAMBERLIN. 1941. Relation of ammonia nitrogen to breakpoint chlorination. *Amer. J. Pub. Health* 31:803.
- PALIN, A.T. 1950. Symposium on the sterilization of water. Chemical aspects of chlorination. *J. Inst. Water Eng.* 4:565.
- TARAS, M.J. 1953. Effect of free residual chlorination of nitrogen compounds in water. *J. Amer. Water Works Assoc.* 45:47.

4500-NH₃ C. Titrimetric Method

1. General Discussion

The titrimetric method is used only on samples that have been carried through preliminary distillation (see Section 4500-NH₃.B). The following table is useful in selecting sample volume for the distillation and titration method.

Ammonia Nitrogen in Sample mg/L	Sample Volume mL
5-10	250
10-20	100
20-50	50.0
50-100	25.0

2. Apparatus

Distillation apparatus: See Section 4500-NH₃.B.2a and b.

3. Reagents

Use ammonia-free water in making all reagents and dilutions.

a. Mixed indicator solution: Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol. Combine solutions. Prepare monthly.

b. Indicating boric acid solution: Dissolve 20 g H_3BO_3 in water, add 10 mL mixed indicator solution, and dilute to 1 L. Prepare monthly.

c. Standard sulfuric acid titrant, 0.02*N*: Prepare and standardize as directed in Alkalinity, Section 2320B.3c. For greatest accuracy, standardize titrant against an amount of Na_2CO_3 that has been incorporated in the indicating boric acid solution to reproduce the actual conditions of sample titration; 1.00 mL = $14 \times \text{normality} \times 1000 \mu\text{g N}$. (For 0.02*N*, 1.00 mL = 280 $\mu\text{g N}$.)

4. Procedure

a. Proceed as described in Section 4500-NH₃.B using indicating boric acid solution as absorbent for the distillate.

b. Sludge or sediment samples: Rapidly weigh to within $\pm 1\%$ an amount of wet sample, equivalent to approximately 1 g dry weight, in a weighing bottle or crucible. Wash sample into a 500-mL kjeldahl flask with water and dilute to 250 mL. Proceed as in ¶ 4a but add a piece of paraffin wax to distillation flask and collect only 100 mL distillate.

c. Titrate ammonia in distillate with standard 0.02*N* H_2SO_4 titrant until indicator turns a pale lavender.

d. Blank: Carry a blank through all steps of the procedure and apply the necessary correction to the results.

5. Calculation

a. Liquid samples:

$$\text{mg NH}_3\text{-N/L} = \frac{(A - B) \times 280}{\text{mL sample}}$$

b. Sludge or sediment samples:

$$\text{mg NH}_3\text{-N/kg} = \frac{(A - B) \times 280}{\text{g dry wt sample}}$$

where:

A = volume of H₂SO₄ titrated for sample, mL, and

B = volume of H₂SO₄ titrated for blank, mL.

6. Precision and Bias

Three synthetic samples containing ammonia and other constituents dissolved in distilled water were distilled and analyzed by titration.

Sample 1 contained 200 μg NH₃-N/L, 10 mg Cl⁻/L, 1.0 mg NO₃⁻-N/L, 1.5 mg organic N/L, 10.0 mg PO₄³⁻/L, and 5.0 mg silica/L. The relative standard deviation and relative error for the 21 participating laboratories were 69.8% and 20%, respectively.

Sample 2 contained 800 μg NH₃-N/L, 200 mg Cl⁻/L, 1.0 mg NO₃⁻-N/L, 0.8 mg organic N/L, 5.0 mg PO₄³⁻/L, and 15.0 mg silica/L. The relative standard deviation and relative error for the 20 participating laboratories were 28.6% and 5%, respectively.

Sample 3 contained 1500 μg NH₃-N/L, 400 mg Cl⁻/L, 1.0 mg NO₃⁻-N/L, 0.2 mg organic N/L, 0.5 mg PO₄³⁻/L, and 30.0 mg silica/L. The relative standard deviation and relative error for the 21 participating laboratories were 21.6%, and 2.6%, respectively.

7. Bibliography

- MEEKER, E.W. & E.C. WAGNER. 1933. Titration of ammonia in the presence of boric acid. *Ind. Eng. Chem., Anal. Ed.* 5:396.
- WAGNER, E.C. 1940. Titration of ammonia in the presence of boric acid. *Ind. Eng. Chem., Anal. Ed.* 12:711.

4500-NH₃ D. Ammonia-Selective Electrode Method

1. General Discussion

a. Principle: The ammonia-selective electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an electrode internal solution of ammonium chloride. Dissolved ammonia (NH_{3(aq)} and NH₄⁺) is converted to NH_{3(aq)} by raising pH to above 11 with a strong base. NH_{3(aq)} diffuses through the membrane and changes the internal solution pH that is sensed by a pH electrode. The fixed level of chloride in the internal solution is sensed by a chloride ion-selective electrode that serves as the reference electrode. Potentiometric measurements are made with a pH meter having an expanded millivolt scale or with a specific ion meter.

b. Scope and application: This method is applicable to the measurement of 0.03 to 1400 mg NH₃-N/L in potable and surface waters and domestic and industrial wastes. High concentrations of dissolved ions affect the measurement, but color and turbidity do not. Sample distillation is unnecessary. Use standard solutions and samples that have the same temperature and contain about the same total level of dissolved species. The ammonia-selective electrode responds slowly below 1 mg NH₃-N/L; hence, use longer times of electrode immersion (2 to 3 min) to obtain stable readings.

c. Interference: Amines are a positive interference. This may be enhanced by acidification. Mercury and silver interfere by complexing with ammonia, unless the NaOH/EDTA solution (3c) is used.

d. Sample preservation: Refrigerate at 4°C for samples to be analyzed within 24 h. Preserve samples high in organic and nitrogenous matter, and any other samples for longer storage, by lowering pH to 2 or less with conc H₂SO₄.

2. Apparatus

a. Electrometer: A pH meter with expanded millivolt scale capable of 0.1 mV resolution between -700 mV and +700 mV or a specific ion meter.

*b. Ammonia-selective electrode.**

c. Magnetic stirrer, thermally insulated, with TFE-coated stirring bar.

3. Reagents

a. Ammonia-free water: See Section 4500-NH₃.B.3a. Use for making all reagents.

b. Sodium hydroxide, 10N.

c. NaOH/EDTA solution, 10N: Dissolve 400 g NaOH in 800 mL water. Add 45.2 g ethylenediaminetetraacetic acid, tetrasodium salt, tetrahydrate (Na₄EDTA · 4 H₂O) and stir to dissolve. Cool and dilute to 1000 mL.

d. Stock ammonium chloride solution: Dissolve 3.819 g anhydrous NH₄Cl (dried at 100°C) in water, and dilute to 1000 mL; 1.00 mL = 1.00 mg N = 1.22 mg NH₃.

e. Standard ammonium chloride solutions: See ¶ 4a below.

4. Procedure

a. Preparation of standards: Prepare a series of standard solutions covering the concentrations of 1000, 100, 10, 1, and 0.1

* Orion Model 95-10, EIL Model 8002-2, Beckman Model 39565, or equivalent.

mg NH₃-N/L by making decimal dilutions of stock NH₄Cl solution with water.

b. Electrometer calibration: Place 100 mL of each standard solution in a 150-mL beaker. Immerse electrode in standard of lowest concentration and mix with a magnetic stirrer. Limit stirring speed to minimize possible loss of ammonia from the solution. Maintain the same stirring rate and a temperature of about 25°C throughout calibration and testing procedures. Add a sufficient volume of 10*N* NaOH solution (1 mL usually is sufficient) to raise pH above 11. If the presence of silver or mercury is possible, use NaOH/EDTA solution in place of NaOH solution. If it is necessary to add more than 1 mL of either NaOH or NaOH/EDTA solution, note volume used, because it is required for subsequent calculations. Keep electrode in solution until a stable millivolt reading is obtained. Do not add NaOH solution before immersing electrode, because ammonia may be lost from a basic solution. Repeat procedure with remaining standards, proceeding from lowest to highest concentration. Wait until the reading has stabilized (at least 2 to 3 min) before recording millivolts for standards and samples containing ≤ 1 mg NH₃-N/L.

c. Preparation of standard curve: Using semilogarithmic graph paper, plot ammonia concentration in milligrams NH₃-N per liter on the log axis vs. potential in millivolts on the linear axis starting with the lowest concentration at the bottom of the scale. If the electrode is functioning properly a tenfold change of NH₃-N concentration produces a potential change of about 59 mV.

d. Calibration of specific ion meter: Refer to manufacturer's instructions and proceed as in ¶s 4a and b.

e. Measurement of samples: Dilute if necessary to bring NH₃-N concentration to within calibration curve range. Place 100 mL sample in 150-mL beaker and follow procedure in ¶ 4b above. Record volume of 10*N* NaOH added. Read NH₃-N concentration from standard curve.

5. Calculation

$$\text{mg NH}_3\text{-N/L} = A \times B \times \left[\frac{100 + D}{100 + C} \right]$$

where:

A = dilution factor,

B = concentration of NH₃-N/L, mg/L, from calibration curve,

C = volume of 10*N* NaOH added to calibration standards, mL, and

D = volume of 10*N* NaOH added to sample, mL.

4500-NH₃:I. PRECISION AND BIAS OF AMMONIA-SELECTIVE ELECTRODE

Level mg/L	Matrix	Mean Recovery %	Precision	
			Overall % RSD	Single Operator % RSD
0.04	Distilled water	200	125	25
	Effluent water	100	75	0
0.10	Distilled water	180	50	10
	Effluent water	470	610	10
0.80	Distilled water	105	14	5
	Effluent water	105	38	7.5
20	Distilled water	95	10	5
	Effluent water	95	15	10
100	Distilled water	98	5	2
	Effluent water	97	—	—
750	Distilled water	97	10.4	1.6
	Effluent water	99	14.1	1.3

Source: AMERICAN SOCIETY FOR TESTING AND MATERIALS. Method 1426-79. American Soc. Testing & Materials, Philadelphia, Pa.

6. Precision and Bias

For the ammonia-selective electrode in a single laboratory using surface water samples at concentrations of 1.00, 0.77, 0.19, and 0.13 mg NH₃-N/L, standard deviations were ±0.038, ±0.017, ±0.007, and ±0.003, respectively. In a single laboratory using surface water samples at concentrations of 0.10 and 0.13 mg NH₃-N/L, recoveries were 96% and 91%, respectively. The results of an interlaboratory study involving 12 laboratories using the ammonia-selective electrode on distilled water and effluents are summarized in Table 4500-NH₃:I.

7. Bibliography

- BANWART, W.L., J.M. BREMNER & M.A. TABATABAI. 1972. Determination of ammonium in soil extracts and water samples by an ammonia electrode. *Comm. Soil Sci. Plant Anal.* 3:449.
- MIDGLEY, C. & K. TERRANCE. 1972. The determination of ammonia in condensed steam and boiler feed-water with a potentiometric ammonia probe. *Analyst* 97:626.
- BOOTH, R.L. & R.F. THOMAS. 1973. Selective electrode determination of ammonia in water and wastes. *Environ. Sci. Technol.* 7:523.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020, National Environmental Research Center, Cincinnati, Ohio.
- AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1979. Method 1426-79. American Soc. Testing & Materials, Philadelphia, Pa.

4500-NH₃ E. Ammonia-Selective Electrode Method Using Known Addition

1. General Discussion

a. Principle: When a linear relationship exists between concentration and response, known addition is convenient for mea-

suring occasional samples because no calibration is needed. Because an accurate measurement requires that the concentration at least double as a result of the addition, sample concentration must be known within a factor of three. Total concentration of

ammonia can be measured in the absence of complexing agents down to 0.8 mg NH₃-N/L or in the presence of a large excess (50 to 100 times) of complexing agent. Known addition is a convenient check on the results of direct measurement.

b. See Section 4500-NH₃.D.1 for further discussion.

2. Apparatus

Use apparatus specified in Section 4500-NH₃.D.2.

3. Reagents

Use reagents specified in Section 4500-NH₃.D.3.

Add standard ammonium chloride solution approximately 10 times as concentrated as samples being measured.

4. Procedure

a. Dilute 1000 mg/L stock solution to make a standard solution about 10 times as concentrated as the sample concentrate.

b. Add 1 mL 10N NaOH to each 100 mL sample and immediately immerse electrode. When checking a direct measurement, leave electrode in 100 mL of sample solution. Use magnetic stirring throughout. Measure mV reading and record as E₁.

c. Pipet 10 mL of standard solution into sample. Thoroughly stir and immediately record new mV reading as E₂.

5. Calculation

a. $\Delta E = E_1 - E_2$.

b. From Table 4500-NH₃:II find the concentration ratio, Q, corresponding to change in potential, ΔE. To determine original total sample concentration, multiply Q by the concentration of the added standard:

$$C_o = Q C_s$$

where:

- C_o = total sample concentration, mg/L,
- Q = reading from known-addition table, and
- C_s = concentration of added standard, mg/L.

c. To check a direct measurement, compare results of the two methods. If they agree within ±4%, the measurements probably are good. If the known-addition result is much larger than the direct measurement, the sample may contain complexing agents.

6. Precision and Bias

In 38 water samples analyzed by both the phenate and the known-addition ammonia-selective electrode method, the electrode method yielded a mean recovery of 102% of the values obtained by the phenate method when the NH₃-N concentrations varied between 0.30 and 0.78 mg/L. In 57 wastewater samples similarly compared, the electrode method yielded a mean recovery of 108% of the values obtained by the phenate method using distillation when the NH₃-N concentrations varied between 10.2 and 34.7 mg N/L. In 20 instances in which two to four replicates

TABLE 4500-NH₃:II.* VALUES OF Q VS. ΔE (59 MV SLOPE) FOR 10% VOLUME CHANGE

ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q
5.0	0.297	9.0	0.178	16.0	0.0952	24.0	0.0556	32.0	0.0354
5.1	0.293	9.1	0.176	16.2	0.0938	24.2	0.0549	32.2	0.0351
5.2	0.288	9.2	0.174	16.4	0.0924	24.4	0.0543	32.4	0.0347
5.3	0.284	9.3	0.173	16.6	0.0910	24.6	0.0536	32.6	0.0343
5.4	0.280	9.4	0.171	16.8	0.0897	24.8	0.0530	32.8	0.0340
5.5	0.276	9.5	0.169	17.0	0.0884	25.0	0.0523	33.0	0.0335
5.6	0.272	9.6	0.167	17.2	0.0871	25.2	0.0517	33.2	0.0333
5.7	0.268	9.7	0.165	17.4	0.0858	25.4	0.0511	33.4	0.0329
5.8	0.264	9.8	0.164	17.6	0.0846	25.6	0.0505	33.6	0.0326
5.9	0.260	9.9	0.162	17.8	0.0834	25.8	0.0499	33.8	0.0323
6.0	0.257	10.0	0.160	18.0	0.0822	26.0	0.0494	34.0	0.0319
6.1	0.253	10.2	0.157	18.2	0.0811	26.2	0.0488	34.2	0.0316
6.2	0.250	10.4	0.154	18.4	0.0799	26.4	0.0482	34.4	0.0313
6.3	0.247	10.6	0.151	18.6	0.0788	26.6	0.0477	34.6	0.0310
6.4	0.243	10.8	0.148	18.8	0.0777	26.8	0.0471	34.8	0.0307
6.5	0.240	11.0	0.145	19.0	0.0767	27.0	0.0466	35.0	0.0304
6.6	0.237	11.2	0.143	19.2	0.0756	27.2	0.0461	36.0	0.0289
6.7	0.234	11.4	0.140	19.4	0.0746	27.4	0.0456	37.0	0.0275
6.8	0.231	11.6	0.137	19.6	0.0736	27.6	0.0450	38.0	0.0261
6.9	0.228	11.8	0.135	19.8	0.0726	27.8	0.0445	39.0	0.0249
7.0	0.225	12.0	0.133	20.0	0.0716	28.0	0.0440	40.0	0.0237
7.1	0.222	12.2	0.130	20.2	0.0707	28.2	0.0435	41.0	0.0226
7.2	0.219	12.4	0.128	20.4	0.0698	28.4	0.0431	42.0	0.0216
7.3	0.217	12.6	0.126	20.6	0.0689	28.6	0.0426	43.0	0.0206
7.4	0.214	12.8	0.123	20.8	0.0680	28.8	0.0421	44.0	0.0196
7.5	0.212	13.0	0.121	21.0	0.0671	29.0	0.0417	45.0	0.0187
7.6	0.209	13.2	0.119	21.2	0.0662	29.2	0.0412	46.0	0.0179
7.7	0.207	13.4	0.117	21.4	0.0654	29.4	0.0408	47.0	0.0171
7.8	0.204	13.6	0.115	21.6	0.0645	29.6	0.0403	48.0	0.0163
7.9	0.202	13.8	0.113	21.8	0.0637	29.8	0.0399	49.0	0.0156
8.0	0.199	14.0	0.112	22.0	0.0629	30.0	0.0394	50.0	0.0149
8.1	0.197	14.2	0.110	22.2	0.0621	30.2	0.0390	51.0	0.0143
8.2	0.195	14.4	0.108	22.4	0.0613	30.4	0.0386	52.0	0.0137
8.3	0.193	14.6	0.106	22.6	0.0606	30.6	0.0382	53.0	0.0131
8.4	0.190	14.8	0.105	22.8	0.0598	30.8	0.0378	54.0	0.0125
8.5	0.188	15.0	0.103	23.0	0.0591	31.0	0.0374	55.0	0.0120
8.6	0.186	15.2	0.1013	23.2	0.0584	31.2	0.0370	56.0	0.0115
8.7	0.184	15.4	0.0997	23.4	0.0576	31.4	0.0366	57.0	0.0110
8.8	0.182	15.6	0.0982	23.6	0.0569	31.6	0.0362	58.0	0.0105
8.9	0.180	15.8	0.0967	23.8	0.0563	31.8	0.0358	59.0	0.0101

* Orion Research Inc. Instruction Manual, Ammonia Electrode, Model 95-12, Boston, MA. 02129.

of these samples were analyzed, the mean standard deviation was 1.32 mg N/L. In three measurements at a sewer outfall, distillation did not change statistically the value obtained by the electrode method. In 12 studies using standards in the 2.5- to 30-mg N/L range, average recovery by the phenate method was 97% and by the electrode method 101%.

4500-NH₃ F. Phenate Method

1. General Discussion

a. Principle: An intensely blue compound, indophenol, is formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by sodium nitroprusside.

b. Interferences: Complexing magnesium and calcium with citrate eliminates interference produced by precipitation of these ions at high pH. There is no interference from other trivalent forms of nitrogen. Remove interfering turbidity by distillation or filtration. If hydrogen sulfide is present, remove by acidifying samples to pH 3 with dilute HCl and aerating vigorously until sulfide odor no longer can be detected.

2. Apparatus

Spectrophotometer for use at 640 nm with a light path of 1 cm or greater.

3. Reagents

a. Phenol solution: Mix 11.1 mL liquified phenol ($\geq 89\%$) with 95% v/v ethyl alcohol to a final volume of 100 mL. Prepare weekly. CAUTION: *Wear gloves and eye protection when handling phenol; use good ventilation to minimize all personnel exposure to this toxic volatile substance.*

b. Sodium nitroprusside, 0.5% w/v: Dissolve 0.5 g sodium nitroprusside in 100 mL deionized water. Store in amber bottle for up to 1 month.

c. Alkaline citrate: Dissolve 200 g trisodium citrate and 10 g sodium hydroxide in deionized water. Dilute to 1000 mL.

d. Sodium hypochlorite, commercial solution, about 5%. This solution slowly decomposes once the seal on the bottle cap is broken. Replace about every 2 months.

e. Oxidizing solution: Mix 100 mL alkaline citrate solution with 25 mL sodium hypochlorite. Prepare fresh daily.

f. Stock ammonium solution: See Section 4500-NH₃.D.3d.

g. Standard ammonium solution: Use stock ammonium solution and water to prepare a calibration curve in a range appropriate for the concentrations of the samples.

4. Procedure

To a 25-mL sample in a 50-mL erlenmeyer flask, add, with thorough mixing after each addition, 1 mL phenol solution, 1 mL sodium nitroprusside solution, and 2.5 mL oxidizing solution. Cover samples with plastic wrap or paraffin wrapper film. Let color develop at room temperature (22 to 27°C) in subdued light for at least 1 h. Color is stable for 24 h. Measure absorbance at

TABLE 4500-NH₃:III. PRECISION DATA FOR MANUAL PHENATE METHOD BASED ON TRIPPLICATE ANALYSES OF AMMONIUM SULFATE

Lab/ Analyst	NH ₃ -N Concentration mg/L	Optical Density	Relative Standard Deviation %
1/1	0.1	0.129	1.55
1/2	0.1	0.114	9.66
2/1	0.1	0.100	10.2
2/2	0.1	0.122	2.36
3/1	0.1	0.112	3.61
3/2	0.1	0.107	1.94
1/1	0.3	0.393	0.39
1/2	0.3	0.364	0.32
2/1	0.3	0.372	2.64
2/2	0.3	0.339	0.90
3/1	0.3	0.370	0.31
3/2	0.3	0.373	0.46
1/1	0.5	0.637	0.77
1/2	0.5	0.630	0.56
2/1	0.5	0.624	1.65
2/2	0.5	0.618	0.86
3/1	0.5	0.561	0.27
3/2	0.5	0.569	0.91

640 nm. Prepare a blank and at least two other standards by diluting stock ammonia solution into the sample concentration range. Treat standards the same as samples.

5. Calculations

Prepare a standard curve by plotting absorbance readings of standards against ammonia concentrations of standards. Compute sample concentration by comparing sample absorbance with the standard curve.

6. Precision and Bias

For the manual phenate method, reagent water solutions of ammonium sulfate were prepared and analyzed by two analysts in each of three laboratories. Results are summarized in Table 4500-NH₃:III.

7. Bibliography

- SOLORZANO, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol. Oceanogr.* 14:799.
- PARSONS, T.R., Y. MAITA & C.M. LALLI. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Elmsford, N.Y.

4500-NH₃ G. Automated Phenate Method

1. General Discussion

a. Principle: Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.

b. Interferences: Seawater contains calcium and magnesium ions in sufficient concentrations to cause precipitation during analysis. Adding EDTA and sodium potassium tartrate reduces the problem. Eliminate any marked variation in acidity or alkalinity among samples because intensity of measured color is pH-dependent. Likewise, insure that pH of wash water and standard ammonia solutions approximates that of sample. For example, if sample has been preserved with 0.8 mL conc H₂SO₄/L, include 0.8 mL conc H₂SO₄/L in wash water and standards. Remove interfering turbidity by filtration. Color in the samples that absorbs in the photometric range used for analysis interferes.

c. Application: Ammonia nitrogen can be determined in potable, surface, and saline waters as well as domestic and industrial wastewaters over a range of 0.02 to 2.0 mg/L when photometric measurement is made at 630 to 660 nm in a 10- to 50-mm tubular flow cell at rates of up to 60 samples/h. Determine higher concentrations by diluting the sample.

2. Apparatus

Automated analytical equipment: An example of the required continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-NH₃:1.

3. Reagents

a. Ammonia-free distilled water: See Section 4500-NH₃.B.3a. Use for preparing all reagents and dilutions.

b. Sulfuric acid, H₂SO₄, 5N, air scrubber solution: Carefully add 139 mL conc H₂SO₄ to approximately 500 mL water, cool to room temperature, and dilute to 1 L.

c. Sodium phenate solution: In a 1-L erlenmeyer flask, dissolve 93 mL liquid (≥89%) phenol in 500 mL water. In small increments and with agitation, cautiously add 32 g NaOH. Cool flask under running water and dilute to 1 L. CAUTION: *Minimize exposure of personnel to this compound by wearing gloves and eye protection, and using proper ventilation.*

d. Sodium hypochlorite solution: Dilute 250 mL bleach solution containing 5.25% NaOCl to 500 mL with water.

e. EDTA reagent: Dissolve 50 g disodium ethylenediamine tetraacetate and approximately six pellets NaOH in 1 L water. For salt-water samples where EDTA reagent does not prevent precipitation of cations, use sodium potassium tartrate solution prepared as follows:

Sodium potassium tartrate solution: To 900 mL water add 100 g NaKC₄H₄O₆ · 4H₂O, two pellets NaOH, and a few boiling chips, and boil gently for 45 min. Cover, cool, and dilute to 1 L. Adjust pH to 5.2 ± 0.05 with H₂SO₄. Let settle overnight in a cool place and filter to remove precipitate. Add 0.5 mL polyoxyethylene 23 lauryl ether* solution and store in stoppered bottle.

f. Sodium nitroprusside solution: Dissolve 0.5 g Na₂(NO)Fe(CN)₅ · 2H₂O in 1 L water.

g. Ammonia standard solutions: See Sections 4500-NH₃.D.3d and e. Use standard ammonia solution and water to prepare the calibration curve in the appropriate ammonia concentration range. To analyze saline waters use substitute ocean water of the following composition to prepare calibration standards:

Constituent	Concentration g/L
NaCl	24.53
MgCl ₂	5.20
CaCl ₂	1.16
KCl	0.70
SrCl ₂	0.03
Na ₂ SO ₄	4.09
NaHCO ₃	0.20
KBr	0.10
H ₃ BO ₃	0.03
NaF	0.003

Subtract blank background response of substitute seawater from standards before preparing standard curve.

4. Procedure

a. Eliminate marked variation in acidity or alkalinity among samples. Adjust pH of wash water and standard ammonia solutions to approximately that of sample.

b. Set up manifold and complete system as shown in Figure 4500-NH₃:1.

c. Obtain a stable base line with all reagents, feeding wash water through sample line.

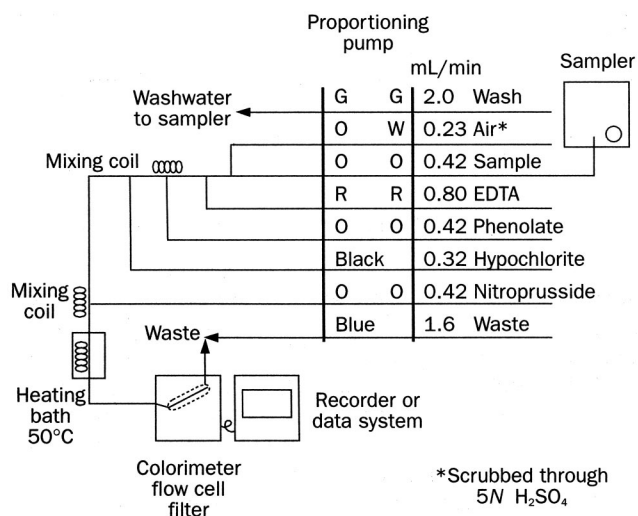


Figure 4500-NH₃:1. Ammonia manifold.

* Brij-35, available from ICI Americas, Wilmington, DE.

d. Typically, use a 60/h, 6:1 cam with a common wash.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against $\text{NH}_3\text{-N}$ concentrations in standards. Compute sample $\text{NH}_3\text{-N}$ concentration by comparing sample response with standard curve.

6. Precision and Bias

For an automated phenate system in a single laboratory using surface water samples at concentrations of 1.41, 0.77, 0.59, and 0.43 mg $\text{NH}_3\text{-N/L}$, the standard deviation was ± 0.005 mg/L, and

at concentrations of 0.16 and 1.44 mg $\text{NH}_3\text{-N/L}$, recoveries were 107 and 99%, respectively.

7. Bibliography

- HILLER, A. & D. VAN SLYKE. 1933. Determination of ammonia in blood. *J. Biol. Chem.* 102:499.
- FIORÉ, J. & J.E. O'BRIEN. 1962. Ammonia determination by automatic analysis. *Wastes Eng.* 33:352.
- AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1966. Manual on Industrial Water and Industrial Waste Water, 2nd ed. American Soc. Testing & Materials, Philadelphia, Pa.
- O'CONNOR, B., R. DOBBS, B. VILLIERS & R. DEAN. 1967. Laboratory distillation of municipal waste effluents. *J. Water Pollut. Control Fed.* 39:25.

4500-NH₃ H. Flow Injection Analysis

1. General Discussion

a. *Principle:* A water sample containing ammonia or ammonium cation is injected into an FIA carrier stream to which a complexing buffer, alkaline phenol, and hypochlorite are added. This reaction, the Berthelot reaction, produces the blue indophenol dye. The blue color is intensified by the addition of nitro-ferricyanide. The resulting peak's absorbance is measured at 630 nm. The peak area is proportional to the concentration of ammonia in the original sample.

Also see Section 4500-NH₃-F and Section 4130, Flow Injection Analysis (FIA).

b. *Interferences:* Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Also see Section 4500-NH₃-A. Some interferences are removed by distillation; see Section 4500-NH₃-B.

2. Apparatus

Flow injection analysis equipment consisting of:

- FIA injection valve* with sample loop or equivalent.
- Multichannel proportioning pump.*
- FIA manifold* (Figure 4500-NH₃:2) with tubing heater and flow cell. In Figure 4500-NH₃:2, relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*
- Absorbance detector*, 630 nm, 10-nm bandpass.
- Injection valve control and data acquisition system.*

* Teflon or equivalent.

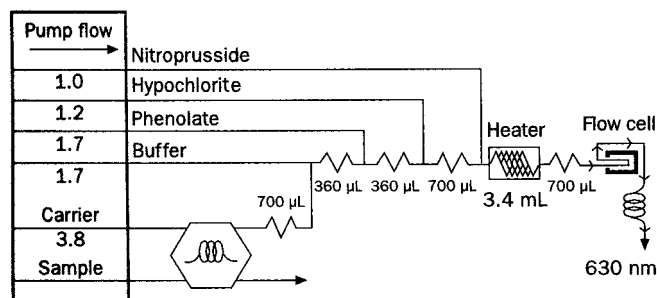


Figure 4500-NH₃:2. FIA ammonia manifold.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. To prevent bubble formation, degas carrier and buffer with helium. Use He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. *Buffer:* To a 1-L tared container add 50.0 g disodium ethylenediamine tetraacetate and 5.5 g sodium hydroxide, NaOH. Add 968 mL water. Mix with a magnetic stirrer until dissolved.

b. *Phenolate:* CAUTION: Wear gloves. Phenol causes severe burns and is rapidly absorbed into the body through the skin. To a tared 1-L container, add 888 g water. Add 94.2 g 88% liquefied phenol or 83 g crystalline phenol, C₆H₅OH. While stirring, slowly add 32 g NaOH. Cool and invert to mix thoroughly. Do not degas.

c. *Hypochlorite:* To a tared 500-mL container add 250 g 5.25% sodium hypochlorite, NaOCl bleach solution† and 250 g water. Stir or shake to mix.

d. *Nitroprusside*: To a tared 1-L container add 3.50 g sodium nitroprusside (sodium nitroferrocyanide), Na₂Fe(CN)₅NO · 2H₂O, and 1000 g water. Invert to mix.

e. *Stock ammonia standard*, 1000 mg N/L: In a 1-L volumetric flask dissolve 3.819 g ammonium chloride, NH₄Cl, that has been dried for 2 h at 110°C, in about 800 mL water. Dilute to mark and invert to mix.

f. *Standard ammonia solutions*: Prepare ammonia standards in desired concentration range, using the stock standard (¶ 3e), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-NH₃:2 and follow method supplied by manufacturer or laboratory standard operating procedure for this method. Follow quality control procedures described in Section 4020.

5. Calculations

Prepare standard curves by plotting the absorbance of standards processed through the manifold versus ammonia concentration. The calibration curve is linear.

6. Precision and Bias

a. *Recovery and relative standard deviation*: The results of single-laboratory studies with various matrices are given in Table 4500-NH₃:IV.

b. *MDL*: A 650-μL sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 0.020-mg N/L standard. These gave a mean of 0.0204 mg N/L, a standard deviation of 0.0007 mg N/L, and an MDL of 0.002 mg N/L.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and Procedure for the Determination of Method Detection Limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

TABLE 4500-NH₃:IV. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg NH ₃ -N/L	Recovery %	Relative Standard Deviation %	
Wastewater treatment plant influent	Reference sample*	—	104	—	
	Blank†	0.4	100	—	
		0.8	102	—	
	Site A‡§	0	—	—	0.5
		0.4	108	—	—
		0.8	105	—	—
	Site B‡§	0	—	—	0.5
		0.4	105	—	—
		0.8	105	—	—
	Site C‡§	0	—	—	1.1
		0.4	109	—	—
		0.8	107	—	—
Wastewater treatment plant effluent	Reference sample*	—	106	—	
	Blank†	0.4	105	—	
		0.8	105	—	
	Site A‡	0	—	—	ND
		0.4	90	—	—
		0.8	88	—	—
	Site B‡	0	—	—	<0.1
		0.4	93	—	—
		0.8	94	—	—
	Site C‡	0	—	—	<0.1
		0.4	89	—	—
		0.8	91	—	—
Landfill leachate	Reference sample*	—	106	—	
	Blank†	0.4	105	—	
		0.8	106	—	
	Site A‡#	0	—	—	1.9
		0.4	125	—	—
		0.8	114	—	—
	Site B‡#	0	—	—	0.4
		0.4	96	—	—
		0.8	106	—	—
	Site C‡#	0	—	—	0.2
		0.4	102	—	—
		0.8	107	—	—

ND = not detectable.
 * U.S. EPA QC sample, 1.98 mg N/L.
 † Determined in duplicate.
 ‡ Samples without known additions determined four times; samples with known additions determined in duplicate.
 § Site A and C samples diluted 20-fold, Site B sample diluted 100-fold. Typical relative difference between duplicates 0.2%.
 || Samples not diluted. Typical relative difference between duplicates <1%.
 # Site A and C samples diluted 50-fold; Site B sample diluted 150-fold. Typical relative difference between duplicates 0.3%.