Center for Applied
Isotope Studies
UNIVERSITY OF GEORGIA

#

Protocol for colorimetric analysis of ammonium
From EPA Method 350.1

5. Apparatus

5.1 Technicon AutoAnalyzer Unit (AAI or AAII) consisting of:
    5.1.1 Sampler.
    5.1.2 Manifold (AAI) or Analytical Cartridge (AAII).
    5.1.3 Proportioning pump.
    5.1.4 Heating bath with double delay coil (AAI).
    5.1.5 Colorimeter equipped with 15 mm tubular flow cell and 630-660 nm filters.
    5.1.6 Recorder.
    5.1.7 Digital printer for AAII (optional).

6. Reagents

6.1 Distilled water: Special precaution must be taken to insure that distilled water is free of ammonia. Such water is prepared by passage of distilled water through an ion exchange column comprised of a mixture of both strongly acidic cation and strongly basic anion exchange resins. The regeneration of the ion exchange column should be carried out according to the instruction of the manufacturer.

NOTE 1: All solutions must be made using ammonia-free water.

6.2 Sulfuric acid 5N: Air scrubber solution. Carefully add 139 ml of conc. sulfuric acid to approximately 500 ml of ammonia-free distilled water. Cool to room temperature and dilute to 1 liter with ammonia-free distilled water.

6.3 Sodium phenolate: Using a 1 liter Erlenmeyer flask, dissolve 83 g phenol in 500 ml of distilled water. In small increments, cautiously add with agitation, 32 g of NaOH. Periodically cool flask under water faucet. When cool, dilute to 1 liter with distilled water.

6.4 Sodium hypochlorite solution: Dilute 250 ml of a bleach solution containing 5.25% NaOCl (such as "Clorox") to 500 ml with distilled water. Available chlorine level should approximate 2 to 3%. Since "Clorox" is a proprietary product, its formulation is subject to change. The analyst must remain alert to detecting any variation in this product significant to its use in this procedure. Due to the instability of this product, storage over an extended period should be avoided.

6.5 Disodium ethylenediamine-tetraacetate (EDTA) (5%): Dissolve 50 g of EDTA (disodium salt) and approximately six pellets of NaOH in 1 liter of distilled water.

NOTE 2: On salt water samples where EDTA solution does not prevent precipitation of cations, sodium potassium tartrate solution may be used to advantage. It is prepared as follows:

6.5.1 Sodium potassium tartrate solution: 10% NaKC4H4O6 x 4H2O. To 900 ml of distilled water add 100 g sodium potassium tartrate. Add 2 pellets of NaOH and a few boiling chips, boil gently for 45 minutes. Cover, cool, and dilute to 1 liter with ammonia-free distilled water. Adjust pH to 5.2 +/-.05 with H2SO4. After allowing to settle overnight in a cool place, filter to remove precipitate. Then add 1/2 ml Brij-35 (note 4) (available from Technicon Corporation) solution and store in stoppered bottle.

6.6 Sodium nitroprusside (0.05%): Dissolve 0.5 g of sodium nitroprusside in 1 liter of distilled water.

6.7 Stock solution: Dissolve 3.819 g of anhydrous ammonium chloride, NH4CI, dried at 105 degrees C, in distilled water, and dilute to 1000 ml. 1.0 ml = 1.0 mg NH3-N.

6.8 Standard Solution A: Dilute 10.0 ml of stock solution (6.7) to 1000 ml with distilled water. 1.0 ml = 0.01 mg NH3-N.

6.9 Standard solution B: Dilute 10.0 ml of standard solution A (6.8) to 100.0 ml with distilled water. 1.0 ml = 0.001 mg NH3-N.

6.10 Using standard solutions A and B. prepare the following standards in 100 ml volumetric flasks (prepare fresh daily):

ml Standard Solution/100 ml	NH3-N, mg/l
Solution B	
1.0	0.01
2.0	0.02
5.0	0.05
10.0	0.10
Solution A	
2.0	0.20
5.0	0.50
8.0	0.80
10.0	1.00
15.0	1.50
20.0	2.00

NOTE 3: When saline water samples are arnlyzed, Substitute Ocean Water (SOW) should be used for preparing the above standards used for the calibration curve; otherwise, distilled water is used. If SOW is used, subtract its blank background response from the standards before preparing the standard curve.

Substitute Ocean Water (SOW)

NaCl	24.53 g/l	NaHCO3	0.20 g/l
MgCl2	5.20 g/l	KBr	0.10 g/l
Na2SO4	4.09 g/l	H3BO3	0.03 g/l
CaCl2	1.16 g/l	SrCl2	0.03 g/l
KCl	0.70 g/l	NaF	0.003 g/l

7. Procedure

7.1 Since the intensity of the color used to quantify the concentration is pH dependent, the acid concentration of the wash water and the standard ammonia solutions should approximate that of the samples. For example, if the samples have been preserved with 2 ml conc. H2SO4/liter, the wash water and standards should also contain 2 ml conc. H2SO4/liter.

7.2 For a working range of 0.01 to 2.00 mg NH3-N/l (AAI), set up the manifold as shown in Figure 1. For a working range of .01 to 1.0 mg NH3-N/l (AAII), set up the manifold as shown in Figure 2. Higher concentrations may be accommodated by sample dilution.

7.3 Allow both colorimeter and recorder to warm up for 30 minutes. Obtain a stable baseline with all reagents, feeding distilled water through sample line.

7.4 For the AAI system, sample at a rate of 20/hr, 1:1. For the AAII use a 60/hr 6:1 cam with a common wash.

7.5 Arrange ammonia standards in sampler in order of decreasing concentration of nitrogen. Complete loading of sampler tray with unknown samples.

7.6 Switch sample line from distilled water to sampler and begin analysis.

8. Calculations

8.1 Prepare appropriate standard curve derived from processing ammonia standards through manifold. Compute concentration of samples by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 In a single laboratory (EMSL), using surface water samples at concentrations of 1.41, 0.77, 0.59 and 0.43 mg NH3-N/l, the standard deviation was +/-0.005.

9.2 In a single laboratory (EMSL), using surface water samples at concentrations of 0.16 and 1.44 mg NH3-N/l, recoveries were 107% and 99%, respectively.

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