Ammonium (NH_4^+) Protocol

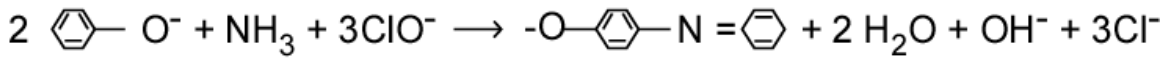
(Modified from Strickland and Parsons)

The following method is specific for ammonia. The technique is a modification of the phenol-hypochlorite method published by Solarzano, L. (1969).

Introduction

Nitrogen, in the form of NH_3 (ammonia gas) or NH_4+ (ammonium ion), is the first breakdown product of N-containing organic matter. A sensitive colorimetric test for ammonia nitrogen in seawater was not developed until 1969. Prior to that time samples had to be painstakingly distilled before analysis. The method presented here is suitable for both seawater and freshwater although for highest precision at low concentrations some modifications may be made for the analysis of freshwater.

The method employs the so-called 'indophenol-blue reaction between ammonia, phenol and hypochlorite in an alkaline medium. Indophenol is an intensely colored amino-derivative of phenylquinone-monoamine:



The resulting blue color is similar to that of the indophenol dyes and its intensity is proportional to the ammonium concentration. The indophenol colorometric reaction was modified by the introduction of the catalyst nitroprusside which accentuates the blue color at room temperature.

NOTE: Due to the ubiquity of ammonia gas, test tubes are easily contaminated during long-term storage. Test tubes should be pre-reacted (see next page) one or two days before the lab and allowed to dry.

Solutions:

1. **Phenol solution**:
   In a fume hood, dissolve 10 g of crystalline analytical reagent grade phenol in 100 mls of 95% v/v ethyl alcohol (or 22 mls 90% Phenol in 200 ml 95% ETOH). This solution is stable for several months.

2. **Sodium Nitroprusside Solution**:
   Re-crystallize sodium nitroprusside: dissolve 5g of sodium nitroprusside in 10 ml of DI water. (This can take several hours). All the nitroprusside may not dissolve. Use a stir bar to help dissolve crystals. Add some DI if necessary to help dissolve. Add 25 mls of 95% ethyl alcohol. Freeze overnight to reform crystals and then filter (use cold ETOH to wash crystals) and dry the crystals in a dessicator. Re-crystallized sodium nitroprusside will give lower reagent blanks. Dissolve 1.0 g of *re-crystallized* sodium nitroprusside in 200 ml of DI water. Store in an amber bottle. The solution is stable for at least one month.

3. **Alkaline Reagent**:

Dissolved 100 g of sodium citrate and 5 g of sodium hydroxide (analytical reagent grade) in 500 ml of DI water. This solution is stable indefinitely. Use caution when handling NaOH.

4. **Standard Ammonia Solution** (10,000 μM stock):
   Dry 5 grams of NH_4Cl in an oven set at 60° C for 24 hours. Once this is cool, add 0.5349 g of dried NH_4Cl to a 1 L volumetric flask and fill to the line with DI. Add 1ml chloroform to preserve. This solution is 0.01 M NH_4Cl (10,000 uM NH_4Cl). This solution is stable for many months if well stoppered.

Each Day before samples are run:

Make up **Oxidizing Solution**:
Mix 100 ml of the Alkaline Reagent with 25 ml of Sodium Hypochlorite Solution (chlorine bleach). Keep solution stoppered while not in use. Prepare fresh every day.

Make up a set of standards using the 10,000 μM NH_4Cl stock solution. Include a DI blank with your standard curve. We often use the concentrations of 0, 0.5 μM, 1 μM, 5 μM, 10 μM, 50 μM, and 100 μM for our measurements. The analysis is not linear beyond 100 μM.

For ammonia analysis, only use test tubes that have been pre-reacted with the reagents. To pre-react new tubes: rinse tubes three times with DI water. (Once tubes have been pre-reacted, they can be used over and over with only DI rinses after use; rinse 3X) Pipette 3 ml of DI water into each test tube. IN THE HOOD: Add reagents: 0.12 ml of phenol, 0.12 ml of sodium nitroprusside solution, and 0.3 ml of oxidizing solution to each test tube, using the vortex to mix after each reagent is added. Allow pre-reacting tubes to stand at room temperature for 1 hour. Invert tubes (make sure you are wearing gloves) to allow reagents to contact all surfaces of the tubes. Pour waste from tubes into NH_3 waste container in the hood. Fill tubes once with DI water and empty into the NH_3 waste container in the hood. Rinse tubes twice more with DI water (empty tubes in the sink).

Allow tubes to dry completely, and cover with parafilm to avoid contamination by atmospheric NH_3.

Turn on the spectrophotometer and allow warming up for 20min. Set the wavelength to 640nm. *Refer to "Ammonium or phosphate on the Shimadzu 1601 Spectrophotometer".*

The three reagents: **Phenol Solution**, **Sodium Nitroprusside Solution**, and **Oxidizing Solution** may be dispensed from re-pipettor bottles. Make sure that the re-pipettors are set to dispense the correct volume of reagent for the volume of sample that you will be analyzing.

Dilute samples if necessary to bring them within range of your standard curve.

IN THE HOOD:
To **3 ml** of sample add the following reagents (using a repipettor makes this go quickly). Mix the test tube using the vortex mixer after *each* reagent is added.

- **0.12 ml** of Phenol Solution,
- **0.12 ml** of Sodium Nitroprusside Solution,

- **0.3 ml** of Oxidizing Solution.

Allow the samples to stand at a temperature between 20° C and 27° C for at least 1 hour in the dark. Color will continue to develop over several hours, so standards and unknowns should be measured in approximately the same time frame. Keep tubes covered with parafilm to lessen the contamination by atmospheric ammonia.

Read the absorption at 640 nm.

Dump waste into clearly marked container labeled: NH_4+ WASTE: PHENOL, ETHANOL, SODIUM-NITROPRUSSIDE, SODIUM-CITRATE, NAOH

CLEAN UP!!!

References:

Solarzano, L. (1969). Determination of ammonium in natural waters by phenol hypochlorite method. *Limnol. Oceanogr.*, 14:799-800.

Strickland,J.D.H. and T.R.Parsons *A practical handbook of Seawater Analysis* 1972 Ottawa, Fisheries Research Board of Canada 2nd. Ed.