AMMONIUM NITROGEN, LOW LEVEL
Manual Salicylate Method

Background: This method was adapted from the Lab Manual of the Central Analytical Laboratory at Natural Resources Research Institute, University of Minnesota-Duluth (Ameel, J., E. Ruzycki and R.P. Axler. 1998. Analytical chemistry and quality assurance procedures for natural water samples. 6th edition. Central Analytical Laboratory, NRRI Tech. Rep. NRRI/TR-98/03 revised annually). These methods are based on American Public Health Association (APHA) and US EPA compilations of water quality methods and the Lab and its Methods Manual is certified biannually by the Minnesota Department of Health. For ease of use by teachers using the *Water on the Web* curriculum, some simplifications have been added since class data will likely be used only for instructional purposes. It is also assumed that water chemistry test kits such as those produced by Hach Chemical Company, and others, will be used by many instructors. These will include detailed instructions in the kit. However, where appropriate, the *WOW* lecture module slides will include troubleshooting tips and techniques suggestions.

Safety: It is assumed that instructors are proficient in the handling of hazardous chemicals. Dangerous concentrated acids and toxic and carcinogenic chemicals may be used in some methods.

Sampling: Stream and lake surface samples can be collected by dipping the sample bottle by hand, using an extension pole or lowering a sampler from a bridge, dock or boat. Be sure to stand downstream from the actual sampling site. Deep-water samples can be collected using a Van Dorn or Kemmerer sampler.

Sample containers should be rinsed with 10% HCl, soaked for 48 hrs in technical lab-grade deionized water (DIW) then rinsed 3 times with ultrapure (MQW or milliQ deionized/filtered water) prior to use. In the classroom, if high quality de-ionized water is not available, buy a few gallons from a local supermarket. De-ionized is preferred over distilled, if available.

Samples must be filtered through a 0.45 um (micron) membrane filter ASAP. Samples are preserved either by addition of ultrapure concentrated H_2SO_4 to a pH < 2, or frozen. Holding time is 28 days if preserved.   If lab analyses will be performed within about 48 hours, the filtered samples can be refrigerated.

General: This method is used for the determination of low levels of ammonia in fresh water with the concentrations of ~5 ppb to 500 ppb. Higher levels are determined by quantitative dilution with DIW. The method uses the blue indophenol reaction between ammonia, salicylate and hypochlorite at high pH.   Appropriate QA/QC (Quality Assurance and Quality Control) should be followed.

Equipment

1.   12 mL glass test tubes
2.   Spectrophotometer*
3.   Vortex stirrer **
4.   Water bath set at 37°C ***

5.   10 mL, 1mL, and 0.1mL pipettes

4 cm or greater cell preferred; 1 cm cell will suffice but with increased level of detection
*** if test tube vortexer is unavailable, use tight fitting plastic caps or parafilm to seal the top and then invert and shake vigorously for ~5 seconds – but do it the same for every sample and standard.*
**** An insulated ice chest will work as a water bath but pre-incubate the test tube rack in the bath at an initial temperature of ~ 40°C before adding the reagents to allow for cooling.*

Reagents

1.   Sodium Salicylate solution, 40%: Dissolve 40 g sodium salicylate in ~ 50 mL MQW in a 100 mL **[*C]** volumetric flask. Fill to the mark with MQW (make fresh daily).
2.   NaOH (0.1N), Dissolve 2.0 g of solid NaOH **[*C]** in 500 mL MQW. Make fresh weekly. Store in a plastic bottle.
3.   Hypochlorite solution, 1.93% in 0.1 N NaOH, i.e., Standard Clorox has 5.25% sodium hypochlorite so mix 37 mL of bleach with 63 mL 0.1N of NaOH. Make fresh daily.
4.   Potassium ferrocyanide solution **[*T]**. Dissolve 2.0 g $K_4Fe(CN)_6$ •$3H_2O$ and 10 g
        $Na_3Citrate$ •$2H_2O$ in ~ 50 mL 0.1N NaOH. Fill to 100 mL with 0.1N NaOH. Stable for
        weeks if stored cool and dark.

Hazards

*T = toxicity hazard. This is a type of chemical hazard but is flagged separately since the chemical may be extremely toxic to biological systems if ingested or inhaled. Special handling procedures are listed in the Material Safety Data Sheet (MSDS).

*C = chemical hazard. The reactivity of the chemical or chemicals with each other or tissue involved through contact may create a reaction that is potentially injurious through the creation of heat, explosion, toxic gases and/or destruction of exposed tissue.

Standards: Run an individual calibration curve for each set of samples covering the expected ammonium-N range. Prepare a 25 mg/L NH_4-N stock using NH_4Cl. Dry NH_4Cl for about 2 hours at 105°C. Dissolve 0.0955 g of NH_4Cl in about 500 mL MQW. Dilute to 1000 mL with MQW. The following is the range of 0-500 ppb NH_4-N.

Standard range.			
ppb N	mL of Stock	ppb N	mL of Stock
0	0	100	0. 400
10	0.040	250	1.0
25	0.100	500	2.0
50	0.200		
Final volume of 100mL for each standard.			

Procedure

1.   Fill sample tubes with 7 mL of blank, standard or sample
2.   Add 1 mL of salicylate reagent. (Vortex**)

3.   Add 1 mL of potassium ferrocyanide solution. (Vortex**)
4.   Add 0.4 mL of hypochlorite solution. (Vortex**)
5.   Cover tubes with para-film and place in water bath*** at 37°C for 45 minutes.
6.   Remove and allow to cool for 15 minutes, vortex and measure absorbance at 660 nm with a spectrophotometer using a 4cm or 1 cm cell. Read If absorbances are > 1.0 check the linearity of the standard curve and use a smaller cuvette size if available. For educational purposes a cuvette cell size of 1 cm should suffice.

7.   Re-read standards and blank at the end of the run

8.   After run, rinse all glassware in DIW and fill with 0.1 N HCl for storage.

Calculations:

- Enter initial and final standard absorbances into a regression using either a spreadsheet or calculator. The regression coefficient r^2 (a measure of linearity) should be > 0.99 (lower values may be instructional if the class is asked to explain why the fit is poor). It is important to ask students to plot the calibration curves in order to look for outliers.

- Calculate ug NH_4-N/L (ppbN) by entering sample absorbances into the regression. Depending on light path, the N range for this method is from < 5 to ~ 500 ugN/L. If sample is < 5 ugN/L, report as below detection. If > ~500 ugN/L, dilute and re-run (be sure to check the standard curve before re-running samples; a smooth but non-linear curve may suffice for instructional purposes).

QA/QC:

- Use correct cell size depending on sample range
- Verify wavelength setting before reading absorbances
- Use Quality Control Check Standards and internal spikes for estimating true accuracy, and replicates for estimating precision as required
- Refer to WOW Module 13 (Unit 4) for detailed Quality Assurance and Quality Control information

References:

Bower, C.E. and T. Holm-Hansen, 1980. A salicylate-hypochlorite method determining ammonia in sea water. Can. J. Fish. Aquat. Sci. 37:794-798.

U.S. Environmental Protection Agency. Method 351.2. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020. Revised March 1983, August 1993.

Verdouw, H., C.J.A. van Echteld and E.M.J. Dekkers, 1978. Water Research 12:399-402.