SECTION D.2
AMMONIA NITROGEN

CEDR Method Code: NH4F L01

a) Scope and Application

i) This method describes the determination of low-level ammonia nitrogen concentrations in filtered samples taken from fresh and estuarine surface waters.

ii) The reaction chemistry described may be used with auto-analyzer instruments with segmented flow, flow injection or discrete mixing apparatus. The analytical range is determined by the instrument, its configuration and the standard curve that is constructed.

iii) Typical MDLs and reporting limits are 0.005 and 0.01 mg/L, respectively.

b) Summary of Method

i) The procedure is based on EPA Method 350.1, without the sample distillation requirement. The chemistry is based on the Berthelot reaction, where alkaline phenol and sodium hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration present. The blue color formed is intensified by the use of sodium nitroprusside and measured spectrophotometrically at a wavelength of 630-660 nm.

ii) The method measures both ammonia (NH_3) and ammonium ions (NH_4^+) and results are reported as mg NH_4-N/L. Figure 1 shows how the percentage of each species is dependent on pH.

iii) Tidal monitoring program samples are filtered in the field through 0.7 µm glass fiber filters. Nontidal monitoring program samples are filtered in the field through a 0.45 µm capsule filters, with some exceptions.

c) Interferences

i) Color development is pH dependent − it is recommended that samples be in the pH range of 4 to 10 so that the buffer can adjust for variances in sample pH.

ii) Turbidity can bias the results through the absorption and scattering of light. A second filtration may be necessary to remove this effect.

iii) Refractive Index interferences should be corrected for when analyzing estuarine/coastal samples. This can be achieved by using dual-beam background correction at a different wavelength, increasing the flow rate, or by matching the salinity of the calibration standards and rinse/blank water to the salinity of the samples.

iv) High concentrations of calcium and magnesium can cause a precipitate to form and result in spikes in absorption spectra. The use of a buffer containing sodium citrate, tartrate or EDTA can mitigate the calcium interference. For water samples very high in magnesium, such as seawater, the use of a sodium citrate buffer is recommended.

d) Apparatus and Materials

i) Continuous-flow automated analytical system equipped with an auto sampler, manifold, proportioning pump, tubing heater, colorimeter, photomultiplier, detector (λ = 630-660 nm), and a computer-based data system. Flow injection and discrete spectrophotometric instrumentation are considered equivalent to continuous-flow systems when using the same reaction chemistry. Changing the buffer to mitigate interferences is not considered a reaction chemistry change.

ii) Nitrogen-free glassware: All glassware used in the determination must be low in residual ammonia to avoid sample/reagent contamination. Washing glassware with 10-50% HCl and thoroughly rinsing with reagent water has been found to be effective. Some laboratories use nitrogen-free detergents instead of, or before acid rinsing. The glassware cleaning procedure will be considered sufficient if all quality control samples are within the expected ranges.

e) Reagents and Standards

i) Stock reagent solutions: The prescribed recipe for these reagents is generally instrument dependent and may change according to the concentration of the samples being analyzed. The chemicals needed for reagents and standards are listed, but not in specific amounts. For continuous flow analyzers, a surfactant such as Brij™ may be added to one or more reagents.

(1) Buffer solution: This reagent is used to ensure that all samples and standards are analyzed at the same pH. The U.S. EPA allows the use of several different buffers depending on interferences. Three common buffers are: sodium potassium tartrate ($KNaC_4H_4O_6$), sodium potassium tartrate + sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), and disodium EDTA.

(2) Alkaline Phenol solution: Liquid or crystalline phenol is combined with sodium hydroxide under a ventilation hood. The use of crystalline phenol is preferred since a preservative such as oxalic acid is added to liquid phenol by the manufacturer. Prepare this reagent weekly in an amber glass bottle and store in a refrigerator at ≤ 6°C.

(3) Sodium Nitroprusside: Nitroprusside is a catalyst for color formation. Sodium nitroferricyanide ($Na_2Fe(CN)5NO \cdot 2H_2O$) is dissolved in reagent water. Its shelf life is 6 months when protected from atmospheric contamination.

(4) Sodium hypochlorite solution: Dissolve a proper portion, according to method, of a hypochlorite solution with approximately 5% free chlorine into reagent water. Prepare this reagent weekly and refrigerate when not in use.

ii) Calibration Standards: Laboratories may purchase or prepare stock and working standards. The calibration verification standard must be purchased or made from a second source, and be traceable to a national standard.

(1) Anhydrous ammonium chloride (NH_4Cl): Primary standard-grade NH_4Cl is dried to remove moisture and cooled in a desiccator. Weigh desired amount on an analytical balance and dissolve in ammonia-free reagent water. This solution is stable for up to

6 months when refrigerated at ≤ 6°C.

(2) Prepare a series of working standards each analysis by diluting suitable volumes of stock solutions with reagent or ASW water. Prepare working standards daily that bracket the expected concentration of the samples.

iii) Reagent water, ammonia-free: see Chapter 6, Section C.4.2

iv) Artificial Sea Water (ASW): see Chapter 6, Section C.4.3.

(1) ASW may be used instead of reagent water to match the salinity of the standards to the salinity of the samples being analyzed. If precipitation occurs, eliminate the magnesium sulfate in the ASW.

(2) When analyzing samples of varying salinities, it may be necessary to prepare standards in a series of salinities to quantify the "salt error", i.e., the shift in the colorimetric response of ammonia due to the change in the ionic strength of the solution. Salinity matching is unnecessary if using a flow injection analyzer or if background correction is built into the instrument.

f) Sample Handling

i) Samples should be analyzed as quickly as possible. If the samples are to be analyzed within 48 hours of collection, keep refrigerated at ≤ 6°C.

ii) If samples will not be analyzed within 48 hours of collection, freeze and store them at -20°C or less for a maximum of 28 days.

g) Procedure

i) Calibration: Set up calibration standards to establish a curve that brackets the expected concentration of samples. See Section 6.C.5 for additional calibration requirements.

ii) Sample analysis

(1) If samples have been frozen, allow to thaw.

(2) Allow the instrument to warm up sufficiently to obtain a steady instrument state, ready to collect data. Use a sampling rate which ensures reliable results.

(3) Analytical sequence: The samples and associated QC samples and standards should be run according to the following sequence.

a. Three or more calibration standards per order of magnitude, within the linear range of the instrument;

i. One calibration standard with zero analyte concentration to estimate the y-intercept.
ii. The lowest standard must have a concentration ≤ PQL or ≤ reporting limit.

b. Initial calibration verification (ICV) standard, traceable to a national standard;

c. Reagent/Method blank;

d. Twenty CBP samples;

e. One matrix spike sample and one duplicate sample;

f. Method blank or laboratory reagent blank (LRB).

g. One continuing calibration verification standard (CCV) per order of magnitude.

h. Repeat steps (3) d through (3) g until all samples are analyzed (or QC samples indicate that the system is out of control and recalibration is necessary).

(4) If a low concentration sample peak follows a high concentration sample peak, a certain amount of carryover can be expected in continuous flow instruments. If the low concentration peak is not clearly defined, it is recommended to reanalyze that sample at the end of the sample run.

iii) Calculations

(1) Prepare a calibration curve by plotting instrument response against standard concentrations. Compute sample concentration by comparing sample response with the standard curve. Multiply concentration by the appropriate dilution factor.

(2) Results should be reported in units of mg NH_4-N/L.

h) Quality Control

i) This method should be used by analysts experienced in the use of automated colorimetric analyses, matrix interferences and procedures for their correction. Analyst training and/or a demonstration of capability should be documented.

ii) Method detection limits (MDL): Method detection limits should be established using the procedures in Chapter 6, Section C.8.

iii) Calibration

(1) Linear calibration range: Calibration standards should bracket the range of CBP samples.

(2) Correlation coefficient (r): The correlation coefficient must be 0.995 or better for the calibration curve to be used.

iv) Method blank: see Chapter 6, Section C.6.1.

v) Matrix spike sample: see Chapter 6, Section C.6.4.

vi) Laboratory duplicate: see Chapter 6, Section C.6.3.

vii) Certified reference material: The laboratory must analyze an ammonia certified reference material (CRM) for verify the accuracy of the initial calibration. Alternatively, a material from a second-source or lot that is traceable to a national standard may be used.

Table 6.D.2. FREQUENCY OF ROUTINE CALIBRATION, BLANK AND QC SAMPLES FOR AMMONIA NITROGEN

Control Sample	Frequency of Application	Acceptance Criteria	Corrective Action
Instrument Calibration	Each analysis day	Using all standards in curve, r ≥ 0.995. Linear at PQL or RL	Repeat full calibration.
Initial Calibration Verification (ICV)– *2nd source or CRM, Traceable to a national standard*	After calibration standards, prior to sample analysis	90-110% recovery of known concentration	Recalibrate and verify prior to analysis.
Method Blank	Beginning and end of preparation batch (≤ 20 samples)	≤ PQL or reporting limit (RL)	Reanalyze another aliquot of blank solution. Investigate possible sources of contamination.
Continuing Calibration Verification (CCV)	At the end of each preparation batch (≤ 20 samples)	90-110% recovery of known concentration	Investigate problem; rerun all samples following the last in-control CCV or ICV.
Matrix Spike Sample	At least 1 per 20 samples	80-120% recovery	Spike another sample aliquot and analyze. If still exceeds control limits, suspect matrix interference and remove interference if possible.
Laboratory Duplicate Sample	At least 1 per 20 samples	20% RPD[1]	Analyze another sample aliquot. Qualify the sample result if still exceeds 20%.

[1] Laboratories may establish less stringent RPD criteria for duplicate samples near the reporting limit.

i) References

(a) U.S. Environmental Protection Agency. 2012. 40 CFR Part 136 – Guidelines Establishing Test Procedures for the Analysis of Pollutants under the Clean Water Act; Analysis and Sampling Procedures. Federal Register/Volume 77 / No. 97/ May 18, 2012/ Rules and Regulations

(b) U.S. EPA 1993. "Methods for the Determination of Inorganic Substances in Environmental Samples,'' NERL–CI, EPA/600/R–93/100, August, 1993. Method 350.1, Rev. 2.0, ammonia (as N) - Automated, spectrophotometric.

(c) Fishman and Friedman. 1985. Methods for the Determination of Inorganic Substances in Water and Fluvial Sediments. TWRI/USGS; Book 5; Chapter A1; Denver, CO. Methods I-2523-85 (dissolved) and I-4523-85 (total).

(d) American Public Health Association. 2012. "Standard Methods for the Examination of Water and Wastewater", Method 4500-NH_3 G-2011, Ammonia Nitrogen by the Automated Phenate Method or 4500-NH_3 H-2011, Flow Injection Analysis.

(e) Solorzano, L. 1969. "Determination of Ammonia in Natural Water by the Phenylhypochlorite Method for the determination of Ammonia in Sea Water". Deep Sea Research, 18:531-532

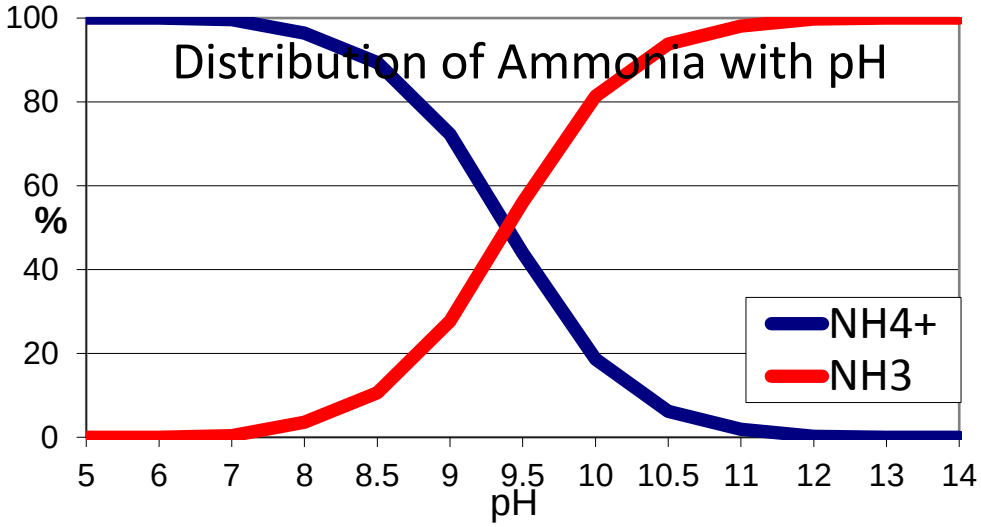

Figure 1. Distribution of Ammonia with pH. At pH 9.25, both ammonia and ammonium ions are present in a 1:1 ratio. As pH levels decrease, the fraction of ammonium ions (NH_4^+) increase. As pH levels increase above 9.5, the ammonia (NH_3) increases.