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) 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.

iv) 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 used, its configuration and the standard curve that is used.

b) Summary of Method
i) The method is based on the Berthelot reaction. 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 as a catalyst.

ii) After a defined reaction period, either through continuous flow or by timing, the color is measured spectrophotometrically at a wavelength of 630-660 nm.

iii) The method measures both ammonia (NH₃) and ammonium ions (NH₄⁺) and results are reported as mg NH₄-N/L. Figure 1 shows how the percentage of each species is dependent on pH.

c) Interferences
i) Color development is pH dependent and 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 or 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 performed by using dual-beam background correction at a different wavelength, a faster time of flight with flow injection, or 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 not 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 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. In this SOP the chemicals needed for the reactions are listed, but not the 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 as a buffer solution to ensure that all samples and standards are analyzed at the same pH. The EPA allows the use of several different buffers depending on interferences. The three common buffers are sodium potassium tartrate (KNaC₄H₇O₆), sodium potassium tartrate + sodium citrate (Na₃C₆H₅O₇·2H₂O), and disodium EDTA.

(2) Alkaline Phenol solution: Liquid or crystalline phenol is combined with sodium hydroxide in a 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: Sodium nitroferricyanide (Na₂Fe(CN)₅NO·2H₂O) is dissolved in reagent water. Nitroprusside is the catalyst for color formation. 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 check standard must be purchased or made from a second source.

(1) Anhydrous ammonium chloride (NH₄Cl): Primary standard-grade NH₄Cl is dried overnight over sulfuric acid and then dissolved in ammonia-free reagent water. This
solution is stable for up to 6 months when refrigerated at ≤ 6°C.

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

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

iv) Artificial Sea Water (ASW): see Chapter 6, Section 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 must 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 not been freshly collected and are frozen, thaw the samples to room temperature.

(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 decade, 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 ≤ MQL or reporting limit.

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

c. LCS/QCS (if the QCS is a CRM, the ICV standard may be omitted);

d. Reagent/method blank;

e. Ten to twenty CBP samples;

f. One matrix spike sample and one duplicate sample;

g. One continuing calibration verification standard (CCV) per decade; and a

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

i. Steps (4) e through (4) h are repeated until all samples are analyzed (or QC samples indicate that the system is out of control and recalibration is necessary).

j. Method blank/LRB

k. CCV standard(s) and/or?

l. LCS/QCS.

(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) Calculate ammonia concentrations from the linear regression obtained from the standard curve in which the concentrations of the standards are entered as the independent variable (x-axis) and the corresponding response is the dependent variable (y-axis).

(2) Results should be reported in units of mg NH₄-N/L.
h) Quality Control

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

ii) 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.

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

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

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

vi) Reference materials: The laboratory must analyze a standard reference material or other second-source performance check with each run.
### Summary of acceptance limits and corrective actions for Ammonia QC samples and parameters

<table>
<thead>
<tr>
<th>INDICATOR</th>
<th>ACCEPTANCE/ ACTION LIMITS</th>
<th>ACTION</th>
<th>FREQUENCY (BATCH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation Coefficient (r)</td>
<td>( r \geq 0.995 )</td>
<td>If &lt; 0.995, evaluate data points of the calibration curve. If any data point is outside established limits, reject as outlier.</td>
<td>1 per batch if acceptable.</td>
</tr>
<tr>
<td>ICV</td>
<td>( \pm 10% )</td>
<td>Recalibrate if outside acceptance limits.</td>
<td>Beginning of run following standard curve.</td>
</tr>
<tr>
<td>QCS</td>
<td>( \pm 10% ) (EPA 1993)</td>
<td>If QCS value is outside ( \pm 10% ) of the QCS concentration, reject the run, correct the problem and rerun samples.</td>
<td>Beginning of run following the ICV.</td>
</tr>
<tr>
<td>CCV</td>
<td>( \pm 10% )</td>
<td>If outside 10%, correct the problem. Rerun all samples following the last in-control CCV.</td>
<td>After every 10-20 samples and at end of batch</td>
</tr>
<tr>
<td>Method Blank/Laboratory Reagent Blank (LRB)</td>
<td>( \leq \text{Method Quantitation Limit} )</td>
<td>If the LRB exceeds the quantitation limit, results are suspect. Rerun the LRB. If the concentration still exceeds the quantitation limit, reject or qualify the data, or raise the quantitation limit.</td>
<td>Following ICV, after every 10-20 samples and at the end of the run.</td>
</tr>
<tr>
<td>Method Quantitation Limit (MQL) check standard.</td>
<td>Within +3s of average MQL check standard output ??? + 30% ???</td>
<td>When the value is outside the predetermined limit and the ICV is acceptable, reanalyze the MQL standard. If the reanalysis is unacceptable, increase the concentration and reanalyze. If this higher concentration meets the acceptance criteria, raise the reporting limit for the batch.</td>
<td>Beginning of run following the LRB</td>
</tr>
<tr>
<td>Laboratory Matrix Spike Sample</td>
<td>( \pm 20% )</td>
<td>If the recovery of any analyte falls outside the designated acceptance limits and the QCS is in control, the recovery problem is judged matrix induced. Repeat the LFM and if the sample results are again outside the acceptable recovery range, the sample should be reported with a “matrix induced bias” qualifier.</td>
<td>After every 10-20 samples</td>
</tr>
<tr>
<td>Laboratory Duplicate Sample</td>
<td>( \pm 20% )</td>
<td>If the RPD fails to meet the acceptance limits, the samples should be reanalyzed. If the RPD again fails to meet the acceptance limits, the sample must be reported with a qualifier identifying the sample analysis result as not having acceptable RPD for duplicate analysis.</td>
<td>After every 10-20 samples.</td>
</tr>
</tbody>
</table>
i) References


(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).


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 (NH₄⁺) increase. As pH levels increase above 9.5, the ammonia (NH₃) increases.