Method 349.0

Determination of Ammonia in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis

Jia-Zhong Zhang, Cooperative Institute for Marine and Atmospheric Studies
Rosenstiel School of Marine and Atmospheric Science/AOML, NOAA
University of Miami, Miami, FL 33149

Peter B. Ortner, Charles J. Fischer, and Lloyd D. Moore, Jr., National Oceanic and Atmospheric Administration, Atlantic Oceanographic and Meteorological Laboratory, Ocean Chemistry Division, Miami, FL 33149

Project Officer

Elizabeth J. Arar

Version 1.0
September 1997

National Exposure Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

Method 349.0

Determination of Ammonia in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis

1.0 Scope and Application

1.1 This method provides a procedure for the determination of ammonia in estuarine and coastal waters. The method is based upon the indophenol reaction,[1-5] here adapted to automated gas-segmented continuous flow analysis.

The term ammonia as used in this method denotes total concentration of ammonia, including both chemical forms, NH_3 and NH_4^+. Because ionization of NH_4^+ has a pK value of about 9.3, NH_4^+ is the dominant chemical form in natural waters. At pH of 8.2 and 25°C only 8.1% is present as NH_3, the form that can be toxic to fish and other aquatic organisms.

The concentration of ammonia in estuarine and coastal water shows considerable temporal and spatial variability. It rarely exceeds 0.005 mg N/L in oxygenated, unpolluted estuarine and coastal water, but in anoxic water, the amount of ammonia can be as high as 0.28 mg N/L.[6]

Although other forms of nitrogen contribute to primary productivity and nutrient cycling in marine and estuarine waters, ammonia is particularly important. Because ammonia represents the most reduced form of inorganic nitrogen available, it is preferentially assimilated by phytoplankton. Whereas nitrate is the source of nitrogen, it must first be reduced to ammonia before it can be assimilated and incorporated into amino acids and other compounds. Ammonia is released during the decomposition of organic nitrogen compounds by proteolytic bacteria, but also excreted directly by invertebrates along with urea and peptides.[7] In regions of coastal upwelling, ammonia released by zooplankton can play a significant role in supplying the nitrogen that supports phytoplankton production.[8]

Analyte	Chemical Abstracts Service Registry Numbers (CASRN)
Ammonia	7664-41-7

1.2 A statistically determined method detection limit (MDL)[9] of 0.3 µg N/L has been determined by one laboratory from seawaters of four different salinities. The method is linear to 4.0 mg N/L using a Flow Solution System (Alpkem, Wilsonville, Oregon).

1.3 Approximately 60 samples per hour can be analyzed.

1.4 This method should be used by analysts both experienced in the use of automated gas segmented continuous flow colorimetric analyses, and also familiar with matrix interferences and the procedures used in their correction. A minimum of 6-months experience under the close supervision of a qualified analyst is recommended.

2.0 Summary of Method

2.1 The automated gas segmented continuous flow colorimetric method is used for the analysis of ammonia concentration. Ammonia in solution reacts with alkaline phenol and NaDTT (Sect. 7.2.5) at 60°C to form indophenol blue in the presence of sodium nitroferricyanide as a catalyst. The absorbance of indophenol blue at 640 nm is linearly proportional to the concentration of ammonia in the sample. A small systematic negative error caused by differences in the refractive index of seawater and reagent water, and a positive error caused by the matrix effect on the color formation, may be corrected for during data processing.

3.0 Definitions

3.1 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solution containing analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.2 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed

exactly like a sample, and its purpose is to determine whether method performance is within acceptable control limits, and whether the laboratory is capable of making accurate and precise measurements. This is a standard prepared in reagent water that is analyzed as a sample.

3.3 Laboratory Fortified Sample Matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.4 Laboratory Reagent Blank (LRB) -- An aliquot of reagent water that is treated exactly as a sample including exposure to all labware, equipment, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or apparatus.

3.5 Linear Dynamic Range (LDR) -- The absolute quantity or concentration range over which the instrument response to an analyte is linear.

3.6 Method Detection Limit (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.[9]

3.7 Reagent Water (RW) -- Type 1 reagent grade water equal to or exceeding the standards established by the American Society for Testing and Materials (ASTM). Reverse osmosis systems or distilling units followed by Super-Q Plus Water System that produce water with 18 megohm resistance are examples of acceptable water sources. To avoid contamination of ammonia from the air, the reagent water should be stored in a sealed or a collapsible container and used the day of preparation.

3.8 Refractive Index (RI) -- The ratio of the velocity of light in a vacuum to that in a given medium. The relative refractive index is the ratio of the velocity of light in two different media, such as estuarine or sea water versus reagent water. The correction for this difference is referred to as refractive index correction in this method.

3.9 Stock Standard Solution (SSS) -- A concentrated solution of method analyte prepared in the laboratory using assayed reference compounds or purchased from a reputable commercial source.

3.10 Primary Dilution Standard Solution (PDS) -- A solution prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.11 Quality Control Sample (QCS) -- A solution of method analyte of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.12 Synchronization Peak Solution -- A synchronization peak is required by most data acquisition programs to initialize the peak finding parameters. The first cup in every run must always be identified as a SYNC sample. The SYNC sample is usually a high concentration standard, but can be any sample that generates a peak at least 25% of full scale.

3.13 Color SYNC Peak Solution -- A colored solution used to produce a synchronization peak in the refractive index measurement in which no color reagent is pumped through system.

3.14 Sensitivity Drift -- The change in absorbance for a given concentration of analyte due to instrumental or chemical drift during the course of measurement.

3.15 Matrix Effect -- The change of absorbance in different matrices due to the effect of ionic strength and composition on the kinetics of color forming reactions.

4.0 Interferences

4.1 Hydrogen sulfide at concentrations greater than 2 mg S/L can negatively interfere with ammonia analysis. Hydrogen sulfide in samples should be removed by acidification with sulfuric acid to a pH of about 3, then stripping with gaseous nitrogen.

4.2 The addition of sodium citrate and EDTA complexing reagent eliminates the precipitation of calcium and magnesium hydroxides when calcium and

magnesium in seawater samples mix with high pH (about 13) reagent solution.[4]

4.3 Sample turbidity is eliminated by filtration or centrifugation after sample collection.

4.4 As noted in Section 2.1 refractive index and salt error interferences occur when sampler wash solution and calibration standards are not matched with samples in salinity, but are correctable. For low concentration samples (< 20 µg N/L), low nutrient seawater (LNSW) with salinity matched to samples, sampler wash solutions and calibration standards is recommended to eliminate matrix interferences.

5.0 Safety

5.1 Water samples collected from the estuarine and coastal environment are rarely hazardous. However, the individual who collects samples should use proper technique.

5.2 Good laboratory technique should be used when preparing reagents. Laboratory personnel should obtain material safety data sheets (MSDS) for al chemicals used in this method. A lab coat, safety goggles, and gloves should be worn when handling the concentrated acid.

5.3 Chloroform is used as a preservative in this method. Use in a properly ventilated area, such as a fume hood.

6.0 Equipment and Supplies

6.1 Gas Segmented Continuous Flow Autoanalyzer Consisting of:

6.1.1 Automatic sampler.

6.1.2 Analytical cartridge with reaction coils and heater.

6.1.3 Proportioning pump.

6.1.4 Spectrophotometer equipped with a tungsten lamp (380-800 nm) or photometer with a 640 nm interference filter (maximum 2 nm bandwidth).

6.1.5 Strip chart recorder or computer based data acquisition system.

6.1.6 Nitrogen gas (high-purity grade, 99.99%).

6.2 Glassware and Supplies

6.2.1 Gaseous ammonia concentration in the laboratory air should be minimal to avoid sample or reagent contamination. Remove any NH_4OH solution stored in the laboratory. Smoking should be strictly forbidden. An air filtration unit might also be used to obtain ammonia-free lab air.

6.2.2 All labware used in the analysis must be free of residual ammonia to avoid sample or reagent contamination. Soaking with laboratory grade detergent, rinsing with tap water, followed by rinsing with 10% HCl (v/v) and then thoroughly rinsing with reagent water was found to be sufficient when working at moderate and high concentration of ammonia. Ammonia is known for its high surface reactivity.[10] When working at low levels of ammonia (< 20 µg N/L), further cleaning of labware is mandatory. Plastic bottles and glass volumetric flasks should be cleaned in an ultrasonic bath with reagent water for 60 minutes. Bottles and sample tubes made of glass can be easily cleaned by boiling in reagent water. Repeat the cleaning process with fresh reagent water prior to use if necessary.

6.2.3 Automatic pipetters with disposable pipet tips capable of delivering volumes ranging from 100 µL to 1000 µL and 1 mL to 10 mL.

6.2.4 Analytical balance, with accuracy to 0.1 mg, for preparing standards.

6.2.5 60-mL glass or high density polyethylene sample bottles, glass volumetric flasks and glass sample tubes.

6.2.6 Drying oven.

6.2.7 Desiccator.

6.2.8 Membrane filters with 0.45 µm nominal pore size. Plastic syringes with syringe filters.

6.2.9 Centrifuge.

6.2.10 Ultrasonic water bath cleaner.

7.0 Reagents and Standards

Note: All reagents must be of analytical reagent grade.

7.1 Stock Reagent Solutions

7.1.1 Complexing Reagent - Dissolve 140 g of sodium citrate dihydrate($Na_3C_6H_5O_7.2H_2O$, FW 294.11), 5 g of sodium hydroxide (NaOH, FW 40) and 10 g of disodium EDTA ($Na_2C_{10}H_{14}O_8N_2.2H_2O$, FW 372.24), in approximately 800 mL of reagent water, mix and dilute to 1 L with reagent water. The pH of this solution is approximately 13. This solution is stable for 2 months.

7.1.2 Stock Ammonium Sulfate Solution (100 mg N/L) - Quantitatively transfer 0.4721 g of pre-dried (105°C for 2 hours) ammonium sulfate ($(NH_4)_2SO_4$, FW 132.15) to a 1000 mL glass volumetric flask containing approximately 800 mL of reagent water and dissolve the salt. Add a few drops of chloroform as a preservative. Dilute the solution to the mark with reagent water. Store in a glass bottle in the refrigerator at 4°C. It is stable for 2 months.[11]

7.1.3 Low Nutrient Sea Water (LNSW) - Obtain natural low nutrient seawater from surface water of the Gulf Stream or Sargasso Sea (salinity 36 ‰, < 7 µg N/L) and filter it through 0.3 micron pore size glass fiber filters. If this is not available, commercial low nutrient sea water (< 7 µg N/L) with salinity of 35 ‰ (Ocean Scientific International, Wormley, U.K.) can be substituted. **NOTE:** Don't use artificial seawater in this method.

7.2 Working Reagents

7.2.1 Brij-35 Start-up Solution - Add 2 mL of Brij-35 surfactant (ICI Americas, Inc.) to 1000 mL reagent water and mix gently.

Note: Brij-35 is a trade name for polyoxyethylene(23) lauryl ether ($C_{12}H_{25}(OCH_2CH_2)_{23}OH$, FW=1199.57, CASRN 9002-92-0).

7.2.2 Working Complexing Reagent - Add 1 mL Brij-35 to 200 mL of stock complexing reagent, mix gently. Prepare this solution daily. This volume of solution is sufficient for an 8-hour run.

7.2.3 Sodium Nitroferricyanide Solution - Dissolve 0.25 g of sodium nitroferricyanide ($Na_2Fe(CN)_5NO.2H_2O$, FW 297.97) in 400 mL of reagent water, dilute to 500 mL with reagent water. Store in an amber bottle at room temperature.

7.2.4 Phenol Solution - Dissolve 1.8 g of solid phenol (C_6H_5OH, FW 94.11) and 1.5 g of sodium hydroxide (NaOH, FW 40) in 100 mL of reagent water. Prepare this solution fresh daily.

7.2.5 NaDTT Solution - Dissolve 0.5 g of sodium hydroxide (NaOH, FW 40) and 0.2 g dichloroisocyanuric acid sodium salt (NaDTT, $NaC_3Cl_2N_3O_3$, FW 219.95) in 100 mL of reagent water. Prepare this solution fresh daily.

7.2.6 Colored SYNC Peak Solution - Add 50 µL of blue food coloring solution to 1000 mL reagent water and mix thoroughly. Further dilute this solution to obtain a peak of between 25 to 100 percent full scale according to the AUFS setting used for refractive index measurement.

7.2.7 Primary Dilution Standard Solution - Prepare a primary dilution standard solution (5 mg N/L) by diluting 5.0 mL of stock standard solution to 100 mL with reagent water. Prepare this solution daily.

Note: This solution should be prepared to give an intermediate concentration appropriate for further dilution in preparing the calibration solutions. Therefore, the concentration of a primary dilution standard solution must be adjusted according to the desired concentration range of calibration solutions.

7.2.8 Calibration Standards - Prepare a series of calibration standards (CAL) by diluting suitable volumes of a primary dilution standard solution (Section 7.2.7) to 100 mL with reagent water or low nutrient seawater. Prepare these standards daily. The concentration range of calibration standards should bracket the expected concentrations of samples and not span more than two orders of magnitude. At least five calibration standards with equal increments in concentration should be used to construct the calibration curve.

When working with samples of a narrow range of salinities (± 2 ‰) or samples containing low ammonia concentration (< 20 µg N/L), it is recommended that the CAL solutions be prepared in Low Nutrient Seawater (Section 7.1.4) diluted to the salinity of samples, and the Sampler Wash Solution also be Low Nutrient Seawater (Section 7.1.4) diluted to the same salinity. **NOTE:** *If this procedure is employed, it is not necessary to perform the matrix effect and refractive index corrections outlined in Sections 12.2 and 12.3.*

When analyzing samples of moderate and high ammonia concentration (> 20 µg N/L) with varying salinities, calibration standard solutions and sampler wash solutions can be prepared in reagent water. The corrections for matrix effect and refractive index should be subsequently applied (Sections 12.2 and 12.3).

7.2.9 Saline Ammonia Standards - If CAL solutions are not prepared to match sample salinity, then saline ammonia standards must be prepared in a series of salinities in order to quantify the matrix effect (the change in the colorimetric response of ammonia due to the change in the composition of the solution). The following dilution of Primary Dilution Standard Solution (Section 7.2.7) and LNSW with reagent water to 100 mL in volumetric flasks, are suggested.

Salinity (‰)	Volume of LNSW(mL)	Volume of PDS(mL)	Conc. mg N/L
0	0	2	.10
9	25	2	.10
18	50	2	.10
27	75	2	.10
35	98	2	.10

8.0 Sample Collection, Preservation and Storage

8.1 Sample Collection - Samples collected for nutrient analyses from estuarine and coastal waters are normally collected using one of two methods: hydrocast or submersible pump systems.

8.1.1 A hydrocast uses a series of sampling bottles (Niskin, Go-Flo or equivalent) attached at fixed intervals to a hydro wire. These bottles are sent through the water column open and are closed either electronically or via a mechanical messenger when the bottles have reached the desired depth.

8.1.2 In a submersible pump system, a weighted hose is sent to the desired depth in the water column and water is pumped from that depth to the deck of the ship for sample processing.

8.1.3 For collecting surface samples, an acid - cleaned plastic bucket or a large plastic bottle can be used as convenient samplers. Wash the sampler three times with sample water before collecting samples.

8.1.4 Turbid samples must be filtered through a 0.45 µm membrane filter as soon as possible after collection. Wash the filter with reagent water before use. Pass at least 100 mL of sample through the filter and discard before taking the final sample. Care must be taken to avoid the contamination of ammonia especially handling low concentrations of ammonia (< 20 µg N/L) samples.[10] An alternative technique to remove particulate is centrifugation.

8.1.5 60-mL glass or high density polyethylene bottles are used for sample storage. Sample bottles should be rinsed 3 times with about 20 mL of sample, shaking with the cap in place after each rinse. Pour the rinse water into the cap to dissolve and rinse away salt crusts trapped in the threads of the cap. Finally, fill the sample bottle about 3/4 full, and screw the cap on firmly.

8.2 Sample Preservation - After collection and filtration or centrifugation, samples should be analyzed as soon as possible. If samples will be analyzed within 3 hours then keep refrigerated in tightly sealed, glass or high density polyethylene bottles in the dark at 4°C until the analysis can be performed.

8.3 Sample Storage - At low concentrations of ammonia (< 20 µg N/L), no preservation technique is satisfactory. *Samples must be analyzed within 3 hours of collection*. At moderate and high concentrations of ammonia (> 20 µg N/L) samples can be preserved by the addition of 2 mL of chloroform per liter of sample and refrigerated in the dark at 4°C. Samples can be stored in either glass or high density polyethylene bottles. A maximum holding time for preserved estuarine and coastal water samples with moderate to high concentrations of ammonia is two weeks.[12]

9.0 Quality Control

9.1 Each laboratory using this method is required to implement a formal quality control (QC) program. The minimum requirements of this program consists of an initial demonstration of performance, continued analysis of Laboratory Reagent Blanks (LRB), laboratory duplicates and Laboratory Fortified Blanks (LFB) with each set of samples as a continuing check on performance.

9.2 Initial Demonstration of Performance (Mandatory)

9.2.1 The initial demonstration of performance is used to characterize instrument performance by determining the MDL and LDR and laboratory performance by analyzing quality control samples prior to analysis of samples using this method.

9.2.2 A method detection limit (MDL) should be established for the method analyte, using a low level seawater sample containing, or fortified at, approximately 5 times the estimated detection limit. To determine MDL values, analyze at least seven replicate aliquots of water which have been processed through the entire analytical method. Perform all calculations defined in the method and report concentration in appropriate units. Calculate the MDL as follows:

$$MDL = (t)(S)$$

where, S = the standard deviation of the replicate analyses

t = Student's t value for n-1 degrees of freedom at the 99% confidence limit; t = 3.143 for six degrees of freedom.

MDLs should be determined every 6 months or whenever a significant change in background or instrument response occurs or a new matrix is encountered.

9.2.3 The LDR should be determined by analyzing a minimum of eight calibration standards ranging from 0.002 to 2.00 mg N/L across all sensitivity settings (Absorbance Units Full Scale output range setting) of the detector. Standards and sampler wash solutions should be prepared in low nutrient seawater with salinities similar to that of samples to avoid the necessity to correct for salt error, or refractive index. Normalize responses by multiplying the response by the Absorbance Units Full Scale output range setting. Perform the linear regression of normalized response vs. concentration and obtain the constants m and b, where m is the slope and b is the y-intercept. Incrementally analyze standards of higher concentration until the measured absorbance response, R, of a standard no longer yields a calculated concentration C_C, that is within 100 ± 10% of known concentration, C, where $C_C = (R-b)/m$. That concentration defines the upper limit of the LDR for the instrument. Should samples be encountered that have a concentration that is ≥ 90% of the upper limit of LDR, then these samples must be diluted and reanalyzed.

9.3 Assessing Laboratory Performance (Mandatory)

9.3.1 Laboratory Reagent Blank (LRB) - A laboratory should analyze at least one LRB with each set of samples. LRB data are used to assess contamination from the laboratory environment. Should an analyte value in the LRB exceed the MDL, then laboratory or reagent contamination should be suspected. When the LRB value constitutes 10% or more of the analyte concentration determined for a sample, duplicates of the sample must be prepared and analyzed again after the source of contamination has been corrected and acceptable LRB values have been obtained.

9.3.2 Laboratory Fortified Blank (LFB) - A laboratory should analyze at least one LFB with each set of samples. The LFB must be at a concentration within the daily calibration range. The LFB data are used to calculate accuracy as percent recovery. If the recovery of the analyte falls outside the required control limits of 90 -110%, the source of the problem should be identified and resolved before continuing the analyses.

9.3.3 The laboratory must use LFB data to assess laboratory performance against the required control limits of 90 -110%. When sufficient internal performance data become available (usually a minimum of 20 to 30 analyses), optional control limits can be developed from the percent mean recovery (x) and standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{Upper Control Limit} = x + 3S$$
$$\text{Lower Control Limit} = x - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each 5 to 10 new recovery measurements, new control limits can be calculated using only the most recent 20 to 30 data points. Also the standard deviation (S) data should be used to establish an ongoing precision statement for the level of concentrations included in the LFB. These data must be kept on file and available for review.

9.4 Assessing Analyte Recovery - Laboratory Fortified Sample Matrix (LFM)

9.4.1 A laboratory should add a known amount of analyte to a minimum of 5% of the total number of samples or one LFM per sample set, whichever is greater. The analyte added should be 2-4 times the ambient concentration and should be at least four times greater than the MDL.

9.4.2 Calculate percent recovery of analyte, corrected for background concentration measured in a separate unfortified sample. These values should be compared with the values obtained from the LFBs. Percent recoveries may be calculated using the following equation:

$$R = \frac{(C_S - C)}{S} \times 100$$

where, R = percent recovery

C_S = measured fortified sample addition in mg N/L

C = sample background concentration (mg N/L)

S = concentration in mg N/L added to the environmental sample.

9.4.3 If the recovery of the analyte falls outside the required control limits of 90-110%, but the laboratory performance for that analyte is within the control limits, the fortified sample should be prepared again and analyzed. If the result is the same after reanalysis, the recovery problem encountered with the fortified sample is judged to be matrix related and the sample data should be flagged accordingly.

10.0 Calibration and Standardization

10.1 At least five calibration standards should be prepared fresh daily for system calibration.

10.2 A calibration curve should be constructed for each sample set by analyzing a series of calibration standard solutions. A sample set should contain no more than 60 samples. For a large number of samples make several sample sets with individual calibration curves.

10.3 Analyze the calibration standards, in duplicate, before the actual samples.

10.4 The calibration curve containing five data points or more that bracket the conentrations of samples should have a correlation coefficient, r, of 0.995 or better and the range should not be greater than two orders of magnitude.

10.5 Use a high CAL solution followed by two blank cups to quantify system carryover. The difference in peak heights between two blank cups is due to the carryover from the high CAL solution. The carryover coefficient, k, is calculated as follows:

$$k = \frac{P_{b1} - P_{b2}}{P_{high}}$$

where, P_{high} = the peak height of the high ammonia standard

P_{b1} = the peak height of the first blank sample

P_{b2} = the peak height of the second blank sample

The carryover coefficient, k, should be measured in seven replicates to obtain a statistically significant number. The carryover coefficient should be remeasured with any change in manifold plumbing or upon replacement of pump tubes.

The carryover correction (CO) of a given peak, i, is proportional to the peak height of the preceding sample, P_{i-1}.

$$CO = (k)x(P_{i-1})$$

To correct a given peak height reading, P_i, subtract the carryover correction.[13,14]

$$P_{i,c} = P_i - CO$$

where $P_{i,c}$ is corrected peak height. The correction for carryover should be applied to all the peak heights throughout a run. The carryover coefficient should be less than 5% in this method.

10.6 Place a high standard solution at the end of each sample run to check for sensitivity drift. Apply sensitivity drift correction to all the samples. The sensitivity drift during a run should be less than 5%.

Note: Sensitivity drift correction is available in most data acquisition software supplied with autoanalyzers. It is assumed that the sensitivity drift is linear with time. An interpolated drift correction factor is calculated for each sample according to the sample position during a run. Multiply the sample peak height by the corresponding sensitivity drift correction factor to obtain the corrected peak height for each sample.

11.0 Procedure

11.1 If samples are stored in a refrigerator, remove samples and equilibrate to room temperature prior to analysis.

11.2 Turn on the continuous flow analyzer and data acquisition components and warm up at least 30 minutes.

11.3 Set up cartridge and pump tubes as shown in Figure 1.

11.4 Set spectrophotometer wavelength to 640 nm, and turn on lamp.

11.5 Set the Absorbance Unit Full Scale (AUFS) range on the spectrophotometer at an appropriate setting according to the highest concentration of ammonia in the samples. The highest setting appropriate for this method is 0.2 AUFS for 6 mg N/L.

11.6 Prepare all reagents and standards.

11.7 Choose an appropriate wash solution for sampler wash. For analysis of samples with a narrow range of salinities (± 2 ‰) or for samples containing low ammonia concentrations (< 20 µg N/L), it is recommended that the CAL solutions be prepared in Low Nutrient Seawater (Section 7.1.4) diluted to the salinity of samples, and that the Sampler Wash Solution also be Low Nutrient Seawater diluted to the same salinity. For samples with varying salinities and higher ammonia concentrations (> 20 µg N/L), it is suggested that the reagent water used for the sampler wash solution and for preparing calibration standards and procedures in Section 12.2 and 12.3 be employed.

11.8 Begin pumping the Brij-35 start-up solution (Section 7.2.1) through the system and obtain a steady baseline. Place the reagents on-line. The reagent baseline will be higher than the start-up solution baseline. After the reagent baseline has stabilized, reset the baseline.

Note: To minimize the noise in the reagent baseline, clean the flow system by sequentially pumping the sample line with reagent water, 1 N HCl solution, reagent water, 1 N NaOH solution for few minutes each at tahe end of the daily analysis. Make sure to rinse the system well with reagent water after pumping NaOH solution to prevent precipitation of $Mg(OH)_2$ when seawater is introduced into the system. Keep the reagents and samples free of particulate. Filter the reagents and samples if necessary.

If the baseline drifts upward, pinch the waste line for a few seconds to increase back pressure. If absorbance drops down rapidly when back pressure increases, this indicates that there are air bubbles trapped in the flow cell. Attach a syringe at the waste outlet of the flowcell. Air bubbles in the flowcell can often be eliminated by simply attaching a syringe for a few minutes or, if not, dislodged by pumping the syringe piston. Alternatively, flushing the flowcell with alcohhol was found to be effective in removing air bubbles from the flowcell.

11.9 The sampling rate is approximately 60 samples per hour with 30 seconds of sample time and 30 seconds of wash time.

11.10 Use cleaned sample cups or tubes (follow the procedures outlined in Section 6.2.2). Place CAL solutions and saline standards (optional) in sampler. Complete filling the sampler tray with samples, laboratory reagent blanks, laboratory fortified blanks, laboratory fortified sample matrices, and QC samples. Place a blank after every ten samples.

11.11 Commence analysis.

12.0 Data Analysis and Calculations

12.1 Concentrations of ammonia in samples are calculated from the linear regression, obtained from the standard curve in which the concentrations of the

calibration standards are entered as the independent variable, and their corresponding peak heights are the dependent variable.

12.2 Refractive Index Correction for Estuarine and Coastal Samples

12.2.1 If reagent water is used as the wash solution, the operator has to quantify the refractive index correction due to the difference in salinity between sample and wash solution. The following procedures are used to measure the relationship between the sample salinity and refractive index on a particular detector.

12.2.2 First, analyze a set of ammonia standards in reagent water with color reagent using reagent water as the wash and obtain a linear regression of peak height versus concentration.

12.2.3 Second, replace reagent water wash solution with Low Nutrient Seawater wash solution.

Note: In ammonia analysis absorbance of the reagent water is higher than that of the LNSW. When using reagent water as a wash solution, the change in refractive index causes the absorbance of seawater to become negative. To measure the absorbance due to refractive index change in different salinity samples, Low Nutrient Seawater must be used as the wash solution to bring the baseline down.

12.2.4 Third, replace the phenol solution (Section 7.2.4) and NaDTT solution (Section 7.2.5) with reagent water. All other reagents remain the same. Replace the synchronization sample with the colored SYNC peak solution (Section 7.2.6).

12.2.5 Prepare a series of different salinity samples by diluting the LNSW. Commence analysis and obtain peak heights for different salinity samples. The peak heights for the refractive index correction must be obtained at the same AUFS range setting and on the same spectrophotometer as the corresponding standards (Section 12.2.2).

12.2.6 Using LNSW as the wash water, a maximum absorbance will be observed for reagent water. No change in refractive index will be observed in the seawater sample. Assuming the absolute absorbance for reagent water (relative to the seawater baseline) is equal to the absorbance for seawater (relative to reagent water baseline), subtract the absorbances of samples of various salinities from that of reagent water. The results are the apparent absorbance due to the change in refractive index between samples of various salinities relative to the reagent water baseline.

12.2.7 For each sample of varying salinity, calculate the apparent ammonia concentration due to refractive index from its peak height corrected to reagent water baseline (Section 12.2.5) and the regression equation of ammonia standards obtained with color reagent being pumped through the system (Section 12.2.2). Salinity is entered as the independent variable and the apparent ammonia concentration due to refractive index is entered as the dependent variable. The resulting regression allows the operator to calculate apparent ammonia concentration due to refractive index when the sample salinity is known. Thus, the operator would not be required to obtain refractive index peak heights for all samples.

12.2.8 The magnitude of refractive index correction can be minimized by using a low refractive index flowcell. An example of a typical result using a low refractive index flowcell follows:

Salinity ($‰_o$)	Apparent ammonia conc. due to refractive index (µg N/L)
0.0	0.00
4.5	0.18
9.1	0.45
13.9	0.66
17.9	0.86
27.6	1.30
36.2	1.63

Note: You must calculate the refractive index correction for your particular detector. The refractive index must be redetermined whenever a significant change in the design of the flowcell or a new matrix is encountered.

12.2.9 An example of a typical equation is:

Apparent ammonia (µg N/L) = 0.0134 + 0.0457S

where S is sample salinity in parts per thousand. The apparent ammonia concentration due to refractive index so obtained should then be added to samples of

corresponding salinity when reagent water was used as the wash solution for samples analysis.

If a low refractive index flowcell is used and ammonia concentration is greater than 200 µg N/L, the correction for refractive index becomes negligible.

12.3 Correction for Matrix Effect in Estuarine and Coastal Samples

12.3.1 When calculating concentrations of samples of varying salinities from standards and wash solution prepared in reagent water, it is necessary to first correct for refractive index errors, then correct for the change in color development due to the differences in composition between samples and standards (matrix effect). Even where the refractive index correction may be small, the correction for matrix effect can be appreciable.

12.3.2 Plot the salinity of the saline standards (Section 7.2.9) as the independent variable, and the apparent concentration of ammonia (mg N/L) from the peak height (corrected for refractive index) calculated from the regression of standards in reagent water, as the dependent variable for all saline standards. The resulting regression equation allows the operator to correct the concentrations of samples of known salinity for the color enhancement due to matrix effect. An example of a typical result follows:

Salinity (‰)	Peak height of 0.140 mg N/L	Uncorrected NH_3 conc. calculated from standards in reagent water (mg N/L)
0	2420	0.1400
4.5	2856	0.1649
9.1	2852	0.1649
13.9	2823	0.1635
17.9	2887	0.1673
27.6	2861	0.1663
36.2	2801	0.1633

12.3.3 Using the reagent described in Section 7.0, as shown above, matrix effect becomes a single factor independent of sample salinity. An example of a typical equation to correct for matrix effect is:

Corrected concentration (mg N/L)
= Uncorrected concentration /1.17(mg N/L)

12.3.4 Results of sample analyses should be reported in mg N/L or in µg N/L.

mg N/L = ppm (parts per million)
µg N/L = ppb (part per billion)

13.0 Method Performance

13.1 Single Laboratory Validation

13.1.1 Method Detection Limit- A method detection limit (MDL) of 0.3 µg N/L has been determined by one laboratory from spiked LNSW of three different salinities as follows:

Salinity (‰)	$[NH_3]$ (µg N/L)	SD (µg N/L)	Recovery (%)	MDL (µg N/L)
36.2	0.7	0.0252	95.4	0.0792
36.2	0.7	0.0784	100.8	0.2463
36.2	1.4	0.0826	104.7	0.2595
36.2	1.4	0.0966	105.6	0.3035
17.9	0.7	0.0322	106.5	0.1012
17.9	0.7	0.0182	92.2	0.0572
17.9	1.4	0.0938	109.1	0.2947
17.9	1.4	0.0882	100	0.2771
4.5	0.7	0.0672	95.1	0.2111
4.5	1.4	0.1008	94.1	0.3167
4.5	1.4	0.126	106.7	0.3959
0.0	0.7	0.077	98.2	0.2419
0.0	0.7	0.0784	100.8	0.2463
0.0	1.4	0.0854	101.9	0.2683

13.1.2 Single Analyst Precision - A single laboratory analyzed three samples collected from the Miami River and Biscayne Bay, Florida. Seven replicates of each sample were processed and analyzed with salinity ranging from 4.8 to 35.0. The results were as follows:

Sample	Salinity (‰)	Concentration (µg N/L)	RSD (%)
1	35.5	6.3	7.19
2	20.0	72.1	1.57
3	4.8	517.6	0.64

13.1.3 Laboratory Fortified Sample Matrix - Laboratory fortified sample matrices were processed in three different salinities ranging from 4.8 to 35.0 and ambient ammonia concentrations from 0.0 to 72.1 µg N/L. Seven replicates of each sample were analyzed and the results were as follows:

Salinity (‰)	Concentration ambient (µg N/L)	Concentration fortified (µg N/L)	RSD (%)	Recovery (%)
35.5	6.3	70	5.01	98.3
20.0	72.1	140	1.71	98.3
4.8	0.0	280	1.81	98.1

13.1.4 Linear Dynamic Range - A linear dynamic range (LDR) of 4.0 mg N/L has been determined by one laboratory from spiked LNSW using a Flow Solution System (Alpkem, Wilsonville, Oregon).

13.1.5 Sample Preservation Study - Natural samples have been preserved by freezing, acidification and addition of chloroform and phenol as preservatives to the samples stored in glass and high density polyethylene bottles. Table 1 summarized the results of preservation study.

There is no significant difference in recovery of ammonia from samples stored in glass and high density polyethylene bottles, suggesting either glass or high density polyethylene bottles can be used for storage of ammonia samples.

For low concentration of ammonia samples (< 20 µg N/L, sample 1 in table 1), no preservation technique is satisfactory. Samples must be analyzed within 3 hours of collection.

Freezing cannot preserve ammonia in samples for more than one week. Acidified samples must be neutralized with NaOH solution prior to analysis. Addition of NaOH to acidified samples induces the precipitation of $Mg(OH)_2$ and $Ca(OH)_2$. Centrifuging the samples cannot completely eliminate the interference, therefore, acidification is not suitable preservation technique. Addition of phenol increases the absorbance of samples. Phenol is not recommended as a suitable preservative although samples preserved with phenol were stable as those preserved by chloroform.[12]

For moderate and high concentrations of ammonia (> 20 µg N/L) samples, it is suggested samples be preserved by the addition of 2 mL of chloroform per liter of sample and refrigerated in the dark at 4°C. A maximum holding time for preserved estuarine and coastal water samples with moderate to high concentrations of ammonia is two weeks.[10]

13.2 Multi-Laboratory Validation

Multi-laboratory data is unavailable at this time.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society, Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202) 872-4477.

15.0 Waste Management

15.1 The U.S. Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

For further information on waste management consult *The Waste Management Manual for Laboratory Personnel*, available from the American Chemical Society at the address listed in Section 14.2.

16.0 References

1. Solorzano, L. 1969. Determination of ammonia in natural waters by the phenylhypochlorite method. Limnol. Oceanogr., 14:799-801.

2. Head, P.C., 1971. An automated phenolhypochlorite method for the determination of ammonia in sea water. Deep-Sea Research, 18:531-532.

3. Slawyk, G., and MacIsaac, J.J., 1972. Comparison of two automated ammonia methods in a region of coastal upwelling. Deep-Sea Research, 19:521-524.

4. Hansen, H.P. and Grasshoff , K. 1983, Automated Chemical Analysis, In *Methods of Seawater Analysis* (Grasshoff, K., M. Ehrhardt and K. Kremling, Eds) Weinheim, Verlag Chemie, Germany. pp363-365.

5. Mautoura, R.F.C. and E.M.S. Woodward, 1983. Optimization of the indophenol blue method for the automated determination of ammonia in estuarine waters. Estuarine, Coastal and Shelf Science, 17:219-224.

6. Zhang J-Z, and F. J. Millero 1993. The chemistry of anoxic waters in the Cariaco Trench. Deep-Sea Res., 40:1023-1041.

7. Raymont, J.E.G. 1980. Plankton and productivity in the oceans. Pergamon Press, Oxford, England.

8. Smith, S.L. and T.E. Whitledge. 1977. The role of zooplankton in the regeneration of nitrogen in a coastal upwelling off northwest Africa. Deep-Sea Res. 24: 49-56.

9. Code of Federal Regulations 40, Ch. 1, Pt. 136 Appendix B. Definition and Procedure for the Determination of Method Detection Limit. Revision 1.11.

10. Eaton, A.D. and V. Grant, 1979. Sorption of ammonium by glass frits and filters: implications for analyses of blakish and freshwater. Limnol. Oceanogr. 24:397-399.

11. Aminot A. and R. Kerouel, 1996. Stability and preservation of primary calibration solutions of nutrients. Mar. Chem. 52:173-181.

12. Degobbis, D. 1973. On the storage of seawater samples for ammonia determination. Limnol. Oceanogr., 18:146-150.

13. Angelova, S, and H.W.Holy. 1983. Optimal speed as a function of system performance for continuous flow analyzers. Analytica Chimica Acta, 145:51-58.

14. Zhang, J.-Z. 1997. Distinction and quantification of carry-over and sample interaction in gas segmented continuous flow analysis. Journal of Automatic Chemistry, 19(6):205-212.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

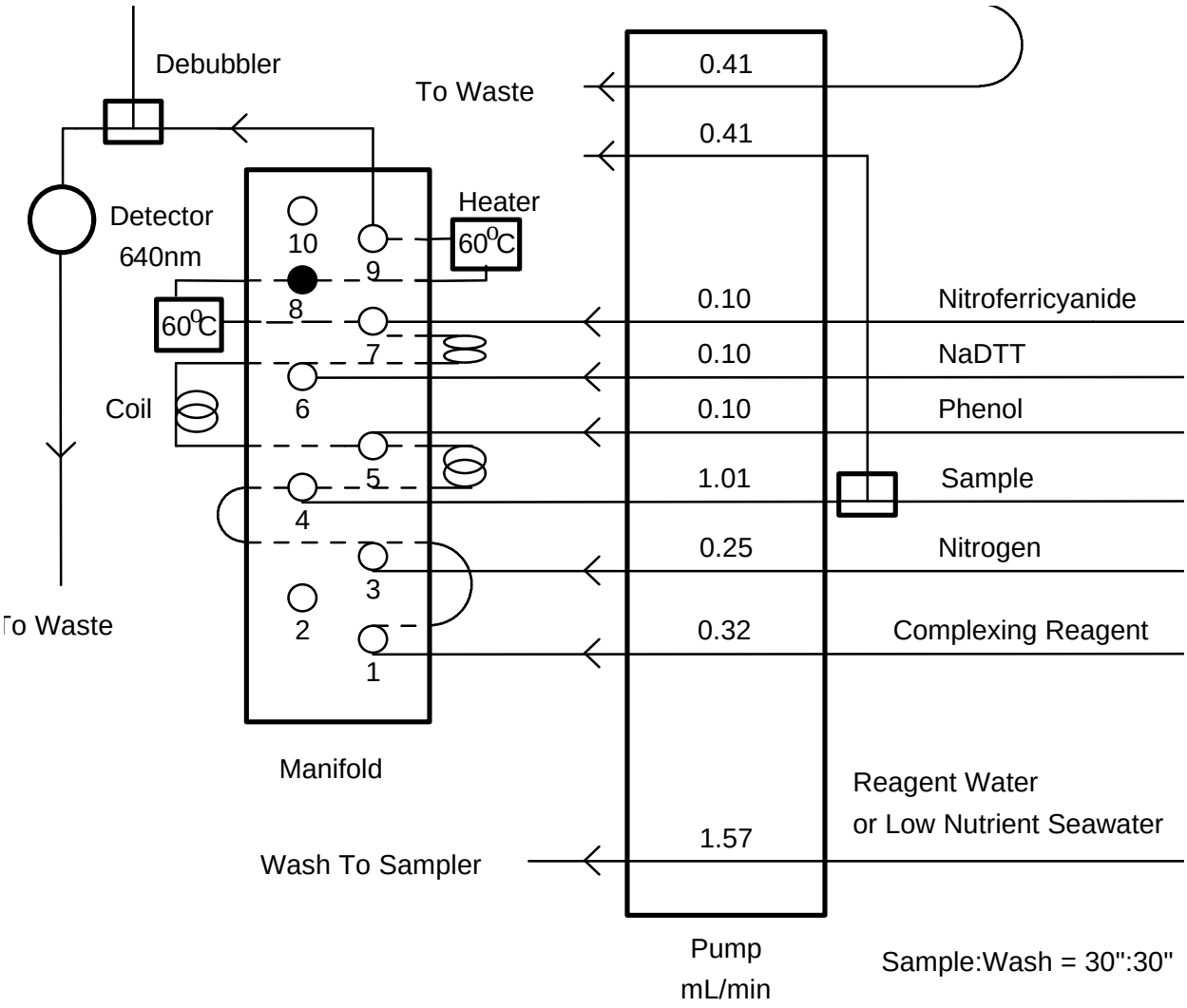

Figure 1. Manifold Configuration for Ammonia Analysis.

Table 1 . Percentage Recovery[A] of Ammonia From Natural Water Samples Preserved by Freezing, Acidification, Addition of Chloroform and Phenol.

sample[B]	method[C]	bottle[D]	time(day)				
			0	7	14	21	28
1	none	HDPE	100	349	345	18	91
	freezing	glass	100	100	0	0	0
		HDPE	100	102	0	0	0
	H_2SO_4 [E]	glass	200	564	285	73	55
		HDPE	200	113	64	45	36
	$CHCl_3$	glass	193	135	29	47	36
		HDPE	193	193	18	44	36
	phenol [F]	glass	153	36	44	0	0
		HDPE	153	36	0	0	0
1+	freezing	glass	100	101	82	77	102
		HDPE	100	97	76	61	81
	H_2SO_4 [E]	glass	95	105	69	54	37
		HDPE	95	91	91	88	116
	$CHCl_3$	glass	96	105	85	78	89
		HDPE	96	102	85	78	92
	phenol [F]	glass	130	133	110	148	123
		HDPE	130	128	102	103	118
2	none	HDPE	100	32	0	0	0
	freezing	glass	100	109	93	77	88
		HDPE	100	107	82	67	91
	H_2SO_4 [E]	glass	252	162	66	62	50
		HDPE	252	193	45	41	27
	$CHCl_3$	glass	99	114	83	75	96
		HDPE	99	98	80	70	83
	phenol [F]	glass	108	107	88	74	93
		HDPE	108	101	83	74	86
2+	freezing	glass	99	108	109	111	106
		HDPE	99	106	95	78	91
	H_2SO_4 [E]	glass	100	107	51	86	88
		HDPE	100	102	39	98	107
	$CHCl_3$	glass	99	106	116	94	105
		HDPE	99	107	98	95	103
	phenol [F]	glass	117	121	106	105	116
		HDPE	117	124	107	106	117
3	none	HDPE	100	104	14	1	0
	freezing	glass	100	-	116	64	106
		HDPE	100	108	105	65	75
	H_2SO_4 [E]	glass	101	106	44	74	61
		HDPE	101	108	111	106	109
	$CHCl_3$	glass	100	96	98	96	94
		HDPE	100	93	97	95	95
	phenol [F]	glass	112	106	107	112	125
		HDPE	112	112	108	110	112

[A] Recovery is calculated based on the ammonia concentration in non-preserved sample at day 0. Samples with recoveries higher than 100% are subject to interference either from precipitation or phenol.

[B] For salinity and concentration of ammonia in samples 1, 2, 3 see Section 13.1.2.

Sample 1+ and 2+ are the fortified samples 1 and 2 at ammonia concentrations 76.3 and 202.1 µg N/L, respectively.

C Methods of preservation:
None: stored the samples in high density polyethylene carboys at room temperature without any preservative added.
Freezing: Frozen and stored at -20°C.
H_2SO_4 : Acidified to pH 1.8 with H_2SO_4, and stored at 4°C. Neutralized the acid with NaOH solution before analysis.
$CHCl_3$: Added 2 mL chloroform per 1000 mL sample, and stored at 4°C.
Phenol: Added 8 g phenol per 1000 mL sample, and stored at 4°C.

C Glass and high density polyethylene bottles were compared to determine the effect of sample bottle type on the preservation.

E Adding NaOH to neutralize acidified samples induced the precipitation of $Mg(OH)_2$ and $Ca(OH)_2$. Centrifuging the samples can not completely eliminate the interference, therefore, acidification is not suitable preservation technique.

F Although samples preserved with phenol were stable as those preserved by chloroform, an absorbance increase was observed, therefore, this is not recommended as a suitable preservation technique.