O·I·Analytical **Methodology**

ISO 9001 SGS

Ammonia Nitrogen, USEPA by Flow Injection Analysis (FIA)

(Cartridge Part #A002053)

1.0 Scope and Application

1.1 This method is used for the determination of ammonia in drinking water, surface water, and domestic and industrial wastes according to USEPA Method 350.1 (Reference 15.4).

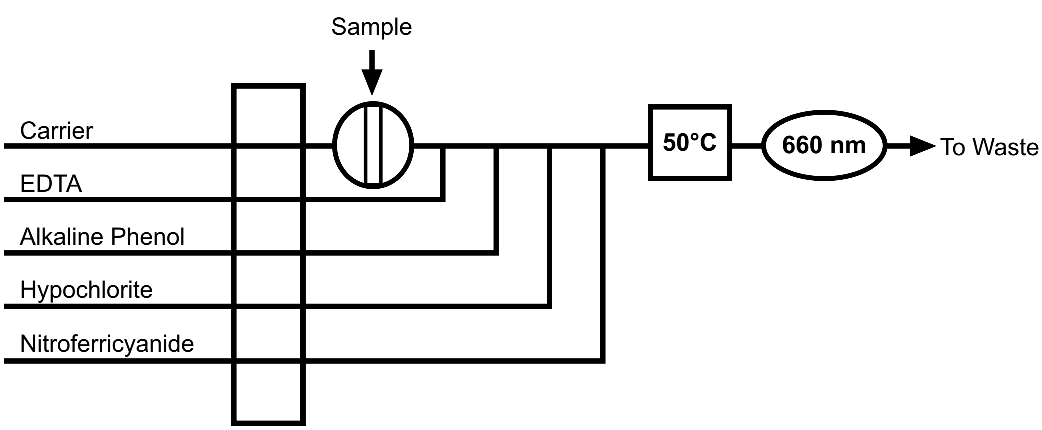
1.2 The Method Detection Limit (MDL) of this method is 0.002 mg/L ammonia as nitrogen (N). The applicable range of the method is 0.01–25 mg/L ammonia as nitrogen. The range may be extended to analyze higher concentrations by sample dilution.

2.0 Summary of Method

2.1 Ammonia reacts with alkaline phenol and hypochlorite to form indophenol blue in an amount that is proportional to the ammonia concentration. The blue color is intensified with sodium nitroferricyanide, and the absorbance is measured at 660 nm (References 15.4, 15.5, and 15.7).

2.2 The quality of the analysis is assured through reproducible calibration and testing of the Flow Injection Analysis (FIA) system.

2.3 A general flow diagram of the FIA system is shown below (see Section 17.0 for a detailed flow diagram).



3.0 Definitions

Definitions for terms used in this method are provided in Section 16.0, “Glossary of Definitions and Purposes.”

4.0 Interferences

4.1 Eliminate precipitation of calcium and magnesium hydroxides by adding EDTA.

4.2 Filter turbid samples prior to analysis.

4.3 Samples with background absorbance at the analytical wavelength may interfere (References 15.4 and 15.7).

5.0 Safety

5.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been fully established. Each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level.

5.2 For reference purposes, a file of Material Safety Data Sheets (MSDS) for each chemical used in this method should be available to all personnel involved in this chemical analysis. The preparation of a formal safety plan is also advisable.

5.3 The following chemicals used in this method may be highly toxic or hazardous and should be handled with extreme caution at all times. Consult the appropriate MSDS before handling.

5.3.1 Ammonium Sulfate, $(NH_4)_2SO_4$ (FW 132.14)

5.3.2 Chloroform, $CHCl_3$ (FW 120.39)

5.3.3 Ethylenediaminetetraacetic Acid, Disodium Salt Dihydrate (EDTA), $C_{10}H_{16}N_2Na_2O_8 \cdot 2H_2O$ (FW 372.24)

5.3.4 Kleenflow™ Acidic (OI Analytical Part #A001251)

5.3.5 Kleenflow Basic (OI Analytical Part # A001252)

5.3.6 Phenol, solid or liquefied, 88%, C_6H_5OH (FW 94.11)

5.3.7 Sodium Hydroxide, NaOH (FW 40.00)

5.3.8 Sodium Hypochlorite Solution, 5.25% available chlorine (household bleach), NaOCl (FW 74.44)

5.3.9 Sodium Nitroferricyanide Dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$ (FW 297.95)

5.4 Unknown samples may be potentially hazardous and should be handled with extreme caution at all times.

5.5 Proper personal protective equipment (PPE) should be used when handling or working in the presence of chemicals.

5.6 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.

6.0 Apparatus, Equipment, and Supplies

6.1 Flow Injection Analysis (FIA) System (OI Analytical Flow Solution® 3000) consisting of the following:

6.1.1 120-Place Autosampler

6.1.2 Expanded Range (ER) Photometric Detector with 5-mm path length flowcell and 660-nm optical filter

6.1.4 Data Acquisition System (PC or Notebook PC) with WinFLOW™ software

6.1.5 Ammonia Nitrogen, USEPA Cartridge (Part #A002053)

6.2 Sampling equipment—Sample bottle, amber glass, with polytetrafluoroethylene (PTFE)-lined cap. Clean by washing with detergent and water, rinsing with two aliquots of reagent water, and drying by baking at 110°–150°C for a minimum of one hour.

6.3 Standard laboratory equipment including volumetric flasks, pipettes, syringes, etc. should all be cleaned, rinsed, and dried per bottle cleaning procedure in Section 6.2.

7.0 Reagents and Calibrants

7.1 Raw Materials

7.1.1 Ammonium Sulfate, $(NH_4)_2SO_4$ (FW 132.14)

7.1.2 Brij®-35, 30% w/v (OI Analytical Part #A21-0110-33)

7.1.3 Chloroform, $CHCl_3$ (FW 120.39)

7.1.4 Deionized Water (ASTM Type I or II)

7.1.5 Ethylenediaminetetraacetic Acid, Disodium Salt Dihydrate (EDTA), $C_{10}H_{16}N_2Na_2O_8•2H_2O$ (FW 372.24)

7.1.6 Kleenflow Acidic (OI Analytical Part #A001251)

7.1.7 Kleenflow Basic (OI Analytical Part # A001252)

7.1.8 Phenol, solid or liquefied, 88%, C_6H_5OH (FW 94.11)

7.1.9 Sodium Hydroxide, NaOH (FW 40.00)

7.1.10 Sodium Hypochlorite Solution, 5.25% available chlorine (household bleach), NaOCl (FW 74.44)

7.1.11 Sodium Nitroferricyanide Dihydrate, $Na_2Fe(CN)_5NO \bullet 2H_2O$ (FW 297.95)

7.2 Reagent Preparation

Note: For best results, filter and degas all reagents prior to use.

7.2.1 Reagent Water

7.2.1.1 Degassed and deionized reagent water can be prepared in one of the following manners:

7.2.1.1.1 Place distilled/deionized water under a strong vacuum for 15–20 minutes. Magnetic stirring or sonification will aid in the degassing process.

7.2.1.1.2 Purge distilled/deionized water with a stream of nitrogen gas (or other inert gas) through a glass frit for approximately 5 minutes.

7.2.1.1.3 Boil distilled/deionized water in an Erlenmeyer flask for 15–20 minutes. Remove the flask from the heat source, cover it with an inverted beaker, and allow it to cool to room temperature.

7.2.1.2 After preparing the degassed reagent water, store the reagent water in a tightly sealed container to protect it from reabsorption of atmospheric gases. For best results, store degassed reagent water under a slight vacuum when not in use.

7.2.2 Start-up Solution (500 mL)

7.2.2.1 Add 1 mL of Brij-35 to approximately 400 mL of reagent water (Section 7.2.1) in a 500-mL volumetric flask.

7.2.2.2 Dilute to 500 mL with reagent water and mix gently.

7.2.3 Stock Complexing Reagent, 0.5% EDTA (1 L)

7.2.3.1 Dissolve 5 g of ethylenediaminetetraacetic acid, disodium salt dihydrate and 1 g of sodium hydroxide in approximately 800 mL of reagent water in a 1-L volumetric flask.

7.2.3.2 Dilute to 1,000 mL with reagent water and mix well.

7.2.4 Working Complexing Reagent (250 mL)

7.2.4.1 Add 250 µL of Brij-35 to 250 mL of stock complexing reagent (Section 7.2.3) and mix gently.

Note: This volume of solution will be sufficient for an 8 hour run. Prepare this solution fresh daily.

7.2.5 Stock Sodium Hydroxide Solution, 10 N (1 L)

7.2.5.1 While stirring, carefully add 400 g of sodium hydroxide to approximately 500 mL of reagent water in a 1-L volumetric flask.

7.2.5.2 Allow the solution to cool to room temperature. Dilute to 1,000 mL with reagent water and mix well.

Warning: Mixing sodium hydroxide with water produces a great of amount of heat. Take appropriate precautions.

Note: Store in a polyethylene container at room temperature. If stored properly, this solution is stable for 4–6 weeks.

7.2.6 Alkaline Phenol (1 L)

7.2.6.1 While stirring, carefully add 80 mL of stock sodium hydroxide solution (Section 7.2.5) to approximately 700 mL of reagent water in a 1-L volumetric flask.

7.2.6.2 Cool the solution in an ice bath.

7.2.6.3 While stirring, carefully add 83 g of phenol (or 94 mL of liquified phenol) in small aliquots, cooling the solution after each addition.

7.2.6.4 Dilute to 1,000 mL with reagent water and mix well.

Warning: Mixing sodium hydroxide or phenol with water produces a great of amount of heat. Take appropriate precautions.

Note: Store in an amber polyethylene container at 4°C. If stored properly, this solution is stable for 4–6 weeks.

7.2.7 Working Hypochlorite Solution (100 mL)

7.2.7.1 Add 50 mL of sodium hypochlorite (5.25% available chlorine) to approximately 30 mL of reagent water in a 100-mL volumetric flask.

7.2.7.2 Dilute to 100 mL with reagent water and mix well.

Note: Prepare this solution fresh daily.

7.2.8 Sodium Nitroferricyanide Solution (1 L)

7.2.8.1 Dissolve 0.5 g of sodium nitroferricyanide dihydrate in approximately 800 mL of reagent water in a 1-L volumetric flask.

7.2.8.2 Dilute to 1,000 mL with reagent water and mix well.

Note: Store in an amber polyethylene bottle at 4°C. If stored properly, this solution is stable for 4–6 weeks.

7.2.9 Carrier, Sample Blank, and Sampler Wash Solution—Reagent Water

7.3 Calibrant Preparation

7.3.1 Stock Calibrant 1,000 mg/L Ammonia Nitrogen (1 L)

7.3.1.1 Dissolve 4.717 g of ammonium sulfate (dried at 100°C) in approximately 800 mL of reagent water in a 1-L volumetric flask.

7.3.1.2 Dilute to 1,000 mL with reagent water and mix well.

7.3.1.3 Preserve the solution with 100 µL of chloroform.

Note: Store in a amber bottle at 4°C. If stored properly, this solution is stable for 4–6 weeks.

7.3.2 Intermediate Calibrant 100 mg/L Ammonia Nitrogen (100 mL)

7.3.2.1 Use a volumetric pipet to add 10 mL of stock calibrant (Section 7.3.1) to approximately 80 mL of reagent water in a 100-mL volumetric flask.

7.3.2.2 Dilute to 100 mL with reagent water and mix well.

Note: Prepare this solution fresh daily.

7.3.3 Working Calibrants (100 mL)

7.3.3.1 Add the designated volumes of stock calibrant (see Equation 1) to the required number of 100-mL volumetric flasks that each contain approximately 80 mL of reagent water.

7.3.3.2 Dilute each solution to the mark with reagent water and mix well.

Note: Prepare working calibrants fresh daily.

EQUATION 1

$$C_1V_1 = C_2V_2$$

Where:
C_1 = *Concentration (in mg/L) of stock solution (or calibrant)*
V_1 = *Volume (in L) of stock solution (or calibrant) to be used*
C_2 = *Desired concentration (in mg/L) of working calibrant to be prepared*
V_2 = *Final volume (in L) of working calibrant to be prepared*

By solving this equation for the volume of stock solution to be used (V_1), the following equation is obtained:

$$V_1 = \frac{C_2V_2}{C_1}$$

Since the desired concentration (C_2), the final volume (V_2), and the concentration of the stock solution (C_1) are all known for any given calibrant concentration in a defined volume, the volume of stock solution to be used (V_1) is easily calculated.

7.3.3.3 Calibrants covering the entire range of this analysis can be prepared from the following tables.

Note: These tables assume use of a 100-µL sample loop.

Final Concentration (mg/L)	Vol. of Inter. Cal. (µL)	Conc. of Inter. Cal. (mg/L)	Final Volume (mL)
0.01	10	100	100
0.05	50	100	100
0.10	100	100	100
0.50	500	100	100
1.0	1,000	100	100

Final Concentration (mg/L)	Vol. of Stock Cal. (µL)	Conc. of Stock Cal. (mg/L)	Final Volume (mL)
5.0	500	1,000	100
10	1,000	1,000	100
20	2,000	1,000	100
25	2,500	1,000	100

8.0 Sample Collection, Preservation, and Storage

8.1 Samples should be collected in plastic or glass bottles that have been thoroughly cleaned and rinsed with reagent water (Section 7.2.1).

8.2 The volume of sample collected should be sufficient to ensure that a representative sample is obtained, replicate analysis is possible, and waste disposal is minimized.

8.3 Preserve and store samples by adding concentrated sulfuric acid to pH < 2 and refrigerating at 4°C. Sample analysis should be performed as soon as possible to eliminate loss of analyte.

8.5 Preserved sample should be adjusted to pH 5–7 with sodium hydroxide prior to analysis.

8.6 Holding time for preserved samples is 28 days from the time of collection (Reference 15.6).

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 15.2). The minimum requirements of this program consist of an initial demonstration of laboratory capability and the periodic analysis of Laboratory Control Samples (LCSs) and Matrix Spike/Matrix Spike Duplicates (MS/MSDs) as a continuing check on performance. Laboratory performance is compared to established performance criteria to determine if the results of the analyses meet the performance characteristics of the method.

9.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable precision and accuracy with this method. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology and to allow the analyst to overcome sample matrix interferences, the analyst is permitted certain options to improve performance or lower the costs of measurements. Alternate determinative techniques, such as the substitution of spectroscopic or other techniques, and changes that degrade method performance are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in this method for the analyte(s) of interest.

9.1.2.1 Each time a modification is made to this method, the analyst is required to repeat the procedure in Section 9.2. If the detection limit of the method will be affected by the change, the laboratory is required to demonstrate that the MDL is lower than one-third the regulatory compliance level or as low as or lower than that listed in Section 1.2. If calibration will be affected by the change, the analyst must recalibrate the instrument per Section 10.4.

9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the information in this subsection, at a minimum.

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.

9.1.2.2.2 A narrative stating the reason(s) for the modification.

9.1.2.2.3 Results from all quality control (QC) tests comparing the modified method to this method including:

a) calibration (Section 10.4)

b) calibration verification (Section 9.5)

c) initial precision and recovery (Section 9.2.2)

d) analysis of blanks (Section 9.4)

e) ongoing precision and recovery (Section 9.6)

f) matrix spike and matrix spike duplicate (Section 9.3)

9.1.2.2.4 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

a) sample numbers and other identifiers

b) analysis dates and times

c) analysis sequence/run chronology

d) sample weight or volume

e) sample volume prior to each cleanup step, if applicable

f) sample volume after each cleanup step, if applicable

g) final sample volume prior to injection

h) injection volume

i) dilution data, differentiating between dilution of a sample or modified sample

j) instrument and operating conditions

k) other operating conditions

l) detector

m) printer tapes, disks, and other recording of raw data

n) quantitation reports, data system outputs, and other data necessary to link raw data to the results reported

9.1.3 Analyses of MS/MSD samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in Section 9.3.

9.1.4 Analyses of laboratory reagent blanks (LRBs) are required to demonstrate freedom from contamination and that the compounds of interest and interfering compounds have not been carried over from a previous analysis. The procedures and criteria for analysis of an LRB are described in Section 9.4.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through the analysis of the LCS that the analytical system is in control. This procedure is described in Section 9.6.

9.1.6 The laboratory should maintain records to define the quality of data that is generated. Development of accuracy statements is described in Sections 9.3.8 and 9.6.3.

9.1.7 Accompanying QC for the determination of ammonia is required per analytical batch. An analytical batch is a set of samples analyzed at the same time to a maximum of 10 samples. Each analytical batch of 10 or fewer samples must be accompanied by a laboratory reagent blank (LRB, Section 9.4), a laboratory control sample (LCS, Section 9.6), and a matrix spike and matrix spike duplicate (MS/MSD, Section 9.3), resulting in a minimum of five analyses (1 sample, 1 LRB, 1 LCS, 1 MS, and 1 MSD) and a maximum of 14 analyses (10 samples, 1 LRB, 1 LCS, 1 MS, and 1 MSD) in the batch. If more than 10 samples are analyzed at one time, the samples must be separated into analytical batches of 10 or fewer samples.

9.2 Initial Demonstration of Laboratory Capability

9.2.1 Method Detection Limit (MDL)—To establish the ability to detect ammonia at low levels, the analyst shall determine the MDL per the procedure in 40 CFR 136, Appendix B (Reference 15.1) using the apparatus, reagents, and standards that will be used in the practice of this method. An MDL less than or equal to the MDL listed in Section 1.2 must be achieved prior to practice of this method.

9.2.2 Initial Precision and Recovery (IPR)—To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:

9.2.2.1 Analyze four samples of the LCS (Section 9.6) according to the procedure beginning in Section 10.0.

9.2.2.2 Using the results of the set of the four analyses, compute the average percent recovery (x) and the standard deviation of the percent recovery (s) for ammonia. Use Equation 2 for the calculation of the standard deviation of the percent recovery (s).

EQUATION 2

$$s = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}}$$

Where:
s = Standard deviation
n = Number of samples
x = Percent recovery in each sample

9.2.2.3 Compare *s* and *x* with the precision and percent recovery acceptance criteria specified in Section 13.0. If the value of *s* exceeds the precision limit or the value of *x* falls outside the range for recovery, system performance is unacceptable and the problem must be found and corrected before the analysis may continue.

9.3 Matrix Spike/Matrix Spike Duplicate (MS/MSD)—The laboratory shall spike, in duplicate, a minimum of 10% of all samples (one sample in duplicate in each batch of 10 samples) from a given sampling site.

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of ammonia in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at one to five times higher than the background concentration of the sample (determined in Section 9.3.2), whichever concentration is higher.

9.3.1.2 If the concentration of ammonia in a sample is not being checked against a limit, the spike shall be at the concentration of the LCS or at least four times greater than the MDL.

9.3.2 Analyze one sample aliquot out of each set of 10 samples from each site or discharge according to the procedure beginning in Section 10.0 to determine the background concentration of ammonia.

9.3.2.1 If necessary, prepare a stock solution appropriate to produce a concentration level in the sample at the regulatory compliance limit or at one to five times the background concentration of ammonia (Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with the spiking solution (Section 9.3.2.1) and analyze these aliquots to determine the concentration after spiking.

9.3.3 Calculate the percent recovery of ammonia in each aliquot using Equation 3.

EQUATION 3

$$P = \frac{A - B}{T} \times 100$$

Where:
P = Percent recovery
A = Measured concentration of ammonia after spiking (Section 9.3.2.2)
B = Measured background concentration of ammonia (Section 9.3.2)
T = True concentration of the spike

9.3.4 Compare the recovery to the QC acceptance criteria in Section 13.0. If percent recovery is outside of the acceptance criteria, and the recovery of the LCS in the ongoing precision and recovery test (Section 9.6) for the analytical batch is within the acceptance criteria, an interference is present. In this case, the result may not be reported for regulatory compliance purposes.

9.3.5 If the results of both the MS/MSD and the LCS test fail the acceptance criteria, the analytical system is judged to be out of control. In this case, the problem shall be identified and corrected, and the analytical batch must be reanalyzed.

9.3.6 Compute the relative percent difference (RPD) between the two spiked sample results (Section 9.3.2.2, not between the two percent recoveries) using Equation 4.

EQUATION 4

$$RPD = \left[\frac{|D_1 - D_2|}{(D_1 + D_2) / 2}\right] \times 100$$

Where:
RPD = Relative percent difference
D_1 = *Concentration of ammonia in the spiked sample*
D_2 = *Concentration of ammonia in the spiked duplicate sample*

9.3.7 If the RPD is greater than 10%, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected. The analytical batch must be reanalyzed.

9.3.8 As part of the QC program for the laboratory, method precision and accuracy for samples should be assessed and records should be maintained. After the analysis of five spiked samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (P_a) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from P_a–$2s_p$ to P_a+$2s_p$. For example, if P_a = 90% and s_p = 10% for five analyses, the accuracy interval is expressed as 70–110%. Update the accuracy assessment on a regular basis (e.g., after each 5–10 new accuracy measurements).

9.4 Laboratory Reagent Blanks (LRB)—Laboratory reagent blanks are analyzed to demonstrate freedom from contamination.

9.4.1 Analyze an LRB initially (i.e., with the tests in Section 9.2) and with each analytical batch. The LRB must be subjected to the exact same procedural steps as a sample.

9.4.2 If ammonia is detected in the LRB at a concentration greater than the ML, analysis of samples is halted until the source of contamination is eliminated and consequent analysis of another LRB shows no evidence of contamination.

9.5 Calibration Verification—Verify calibration of the analytical equipment before and after each analytical batch of 14 or fewer measurements. (The 14 measurements will normally be 10 samples, 1 LRB, 1 LCS, 1 MS, and 1 MSD). This can be accomplished by analyzing the midrange calibration standard and verifying that it is within the QC acceptance criteria for recovery in Section 13.0. (The concentration of the calibration verification depends on the calibration range being used.) Failure to attain recoveries within the acceptance criteria requires recalibration of the analytical system (Section 10.4).

9.6 Laboratory Control Sample (LCS)—To demonstrate that the analytical system is in control and acceptable precision and accuracy is being maintained with each analytical batch, the analyst shall perform the following operations:

9.6.1 Analyze an LCS with each analytical batch according to the procedure in Section 10.0.

9.6.2 If the precision and recovery for the LCS are within the acceptance criteria specified in Section 13.0, analysis of the batch may continue. If, however, the concentration is not within this range, the analytical process is not in control. In this event, correct the problem, repeat the LCS test, and reanalyze the batch.

9.6.3 The laboratory should add results that pass the specification in Section 9.6.2 to IPR and previous LCS data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory should also develop a statement of laboratory data quality for ammonia by calculating the average percent recovery (R) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from R–$2s_r$ to R+$2s_r$. For example, if R = 95% and s_r = 5%, the accuracy is 85–105%.

9.7 Reference Sample—To demonstrate that the analytical system is in control, the laboratory may wish to periodically test an external reference sample, such as a Standard Reference Material (SRM) available from the National Institute of Standards and Technology (NIST). Corrective action should be taken if the measured concentration significantly differs from the stated concentration.

10.0 Configuration and Start-up

10.1 Instrument Configuration

10.1.1 Configure the OI Analytical Flow Solution 3000 Analyzer according to the Operator's Manual and verify that each module is properly powered on.

10.1.2 Verify that the Ammonia Nitrogen, USEPA Cartridge (Part #A002053) is configured as illustrated in the flow diagram shown in Section 17.0.

10.1.3 Connect the appropriate pump tubes to the cartridge and to their appropriate reagent containers according to the flow diagram.

10.2 Instrument Stabilization

10.2.1 Connect the reagent pump tubes to a reagent bottle containing the start-up solution (Section 7.2.2). Start the pump at low speed, allowing the start-up solution to flow through the entire system.

10.2.2 Make sure that the flowcell of each detector is purged of all bubbles and the flow is stable and free from surging.

10.2.3 Verify that a stable flow has been achieved and that the flowcell is free of bubbles before proceeding.

10.3 Baseline Verification

10.3.1 Create and save a Method in WinFLOW. Refer to the WinFLOW Operator's Manual (Reference 15.8) for help on creating a Method.

10.3.2 Create and save a Sample Table in WinFLOW that will be used to generate a calibration curve using at least three calibrants that cover the full range of expected concentrations in the samples to be analyzed. This Sample Table should also be used to analyze all necessary QC samples as well as the analytical batch of samples to be analyzed. For help on creating a Sample Table, refer to the WinFLOW Operator's Manual (Reference 15.8).

10.3.3 Select **Collect Data** in the WinFLOW main window, enter the user's identification, select the appropriate Method and Sample Table, and begin to collect baseline data. Very sharp fluctuations in the baseline and/or consistent drifting are typically signs of bubbles in the flowcell. The flowcell must be free of bubbles prior to beginning analysis.

10.4 Calibration and Standardization

10.4.1 Prepare a series of at least three working calibrants using the stock solutions (Section 7.3) according to Equation 1, covering the desired analysis range.

10.4.2 Place the calibrants in the autosampler in order of increasing concentration. Each calibrant should be analyzed according to the analytical procedures in Section 11.0. A calibration curve will be calculated by the WinFLOW software.

10.4.3 Acceptance or control limits for the calibration results should be established using the difference between the measured value of each calibrant and the corresponding “true” concentration.

10.4.4 Each calibration curve should be verified by analysis of a Laboratory Control Sample (LCS, Section 9.5). Using WinFLOW software, calibration, verification, and sample analysis may be performed in one continuous analysis.

11.0 Procedure

11.1 Analysis

11.1.1 Begin pump flow with the start-up solution (Section 7.2.2). Once the heater unit has reached 50°C, verify a stable baseline (Section 10.3).

11.1.2 After the baseline has been verified, place all reagents on-line and allow to pump at least 10–15 minutes. Verify there are no bubbles in the flowcell. Obtain a stable baseline at 660 nm and autozero the baseline before beginning analysis.

11.1.3 Load the sampler tray with calibrants, blanks, samples, and QC samples.

Note: The matrix of the working standards, blanks, and QC samples should match that of the samples being analyzed.

11.1.4 Using the Method and Sample Table created for the analytical batch to be analyzed and with the baseline verified to be stable, begin the analysis by selecting the “Fast Forward” button on the left side of the Data Analysis window in WinFLOW. This will initiate the sequential analysis of samples as defined in the Sample Table.

11.1.5 When analysis is complete, pump start-up solution through the system for at least 10–15 minutes. Stop the pump, release the tension on all pump tubes, and power off the system.

11.2 Operating Notes

11.2.1 Precipitation occurring after adding alkaline phenol may indicate poor reagent quality, especially the EDTA, or too much Brij-35.

11.2.2 Precipitation following addition of alkaline phenol may also occur if the samples contain calcium or magnesium in amounts that exceed the complexing capacity of the EDTA. Increase the amount of EDTA in the working complexing reagent (Section 7.2.4).

11.2.3 Clean precipitates from the system by pumping Kleenflow Acidic through the sample and reagent lines. Wash the system thoroughly with start-up solution (Section 7.2.2) before proceeding with the analysis.

11.2.4 The pH of the flowcell waste line should be approximately 11 when checked with pH paper.

11.2.5 To enhance the performance at lower concentrations:

11.2.5.1 Increase the size of the sample loop.

11.2.5.2 Prepare a 1:10 dilution of the working complexing reagent.

11.2.5.3 Decrease the range of calibrants to closely match the concentration of samples.

11.2.6 To enhance the performance at higher concentrations:

11.2.6.1 Decrease the size of the sample loop.

11.2.6.2 Increase the cycle duration time in the WinFLOW Timed Events Table.

11.2.7 Reduce background noise by cleaning the manifold weekly or as needed. To clean the manifold, pump the following solutions through all of the lines:

11.2.7.1 Start-up solution—5 minutes

11.2.7.2 Kleenflow Acidic—10 minutes

11.2.7.3 Kleenflow Basic—5 minutes

11.2.7.4 Start-up Solution—10 minutes

12.0 Data Analysis and Calculations

12.1 The calibration curve allows for accurate quantitation of the concentration in each sample.

12.2 WinFLOW software reports the concentration of each sample relative to the calibration curve.

13.0 Method Performance

Range:	0.01–25 mg/L N
Throughput:	51 samples/hour
Precision:	
1.5 mg/L	<1% RSD
Method Detection Limit (MDL):	0.002 mg/L N

14.0 Pollution Prevention and Waste Management

14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land-disposal restrictions. In addition, it is the laboratory's responsibility to protect air, water, and land resources by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations.

14.2 For further information on waste management, consult Section 13.6 of *Less is Better: Laboratory Chemical Management for Waste Reduction* (Reference 15.3).

15.0 References

15.1 *Code of Federal Regulations*, Part 136, Title 40, Appendix B, 1994.

15.2 *Handbook for Analytical Quality Control in Water and Wastewater Laboratories*; EPA-600/4-79-019; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1979.

15.3 *Less is Better: Laboratory Chemical Management for Waste Reduction.* Available from the American Chemical Society, Department of Government Regulations and Science Policy, 1155 16th Street, NW, Washington, DC, 20036.

15.4 Nitrogen, Ammonia. *Methods for Chemical Analysis of Water and Wastewater;* EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; Method 350.1.

15.5 Patton, C.J.; Crouch, S.R. *Analytical Chemistry* **1977**, *49* (3), 464–469.

15.6 Sample Preservation. *Methods for Chemical Analysis of Water and Wastes*; EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; xvii.

15.7 *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; American Public Health Association: Washington, D.C., 1998.

15.8 WinFLOW Software and Operator's Manual (Part #A002877). Available from OI Analytical, P.O. Box 9010, College Station, TX, 77842-9010.

16.0 Glossary of Definitions and Purposes

The definitions and purposes are specific to this method but have been conformed to common usage as much as possible.

16.1 Units of weights and measures and their abbreviations

16.1.1 Symbols

°C	degrees Celsius
%	percent
±	plus or minus
≥	greater than or equal to
≤	less than or equal to

16.1.2 Alphabetical characters

g	gram
L	liter
mg	milligram
mg/L	milligram per liter
µg	microgram
µg/L	microgram per liter
mL	milliliter
ppm	parts per million
ppb	parts per billion
M	molar solution
N	normal solution

16.2 Definitions

16.2.1 Initial Precision and Recovery (IPR)—Four aliquots of the LRB spiked with the analytes of interest and used to establish the ability to generate acceptable precision and accuracy. An IPR is performed the first time this method is used and any time the method or instrumentation is modified.

16.2.2 Laboratory Control Sample (LCS)—An aliquot of LRB to which a quantity of the analyte of interest is added in the laboratory. The LCS is analyzed like a sample. Its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

16.2.3 Laboratory Reagent Blank (LRB)—An aliquot of reagent water and other blank matrix that is treated like a sample, including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if the method analyte or other interferences are present in the laboratory environment, reagents, or apparatus.

16.2.4 Matrix Spike/Matrix Spike Duplicate (MS/MSD)—An aliquot of an environmental sample to which a quantity of the method analyte is added in the laboratory. The MS/MSD is analyzed like a sample. Its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analyte in the sample matrix must be determined in a separate aliquot, and the measured values in the MS/MSD must be corrected for the background concentration.

16.2.5 Method Detection Limit (MDL)—The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero.

16.2.6 Minimum Level (ML)—The level at which the entire analytical system will give a recognizable signal and acceptable calibration point, taking into account method-specific sample and injection volumes.

16.2.7 Ongoing Precision and Recovery (OPR)—See Section 16.2.2, "Laboratory Control Sample."

17.0 Figures

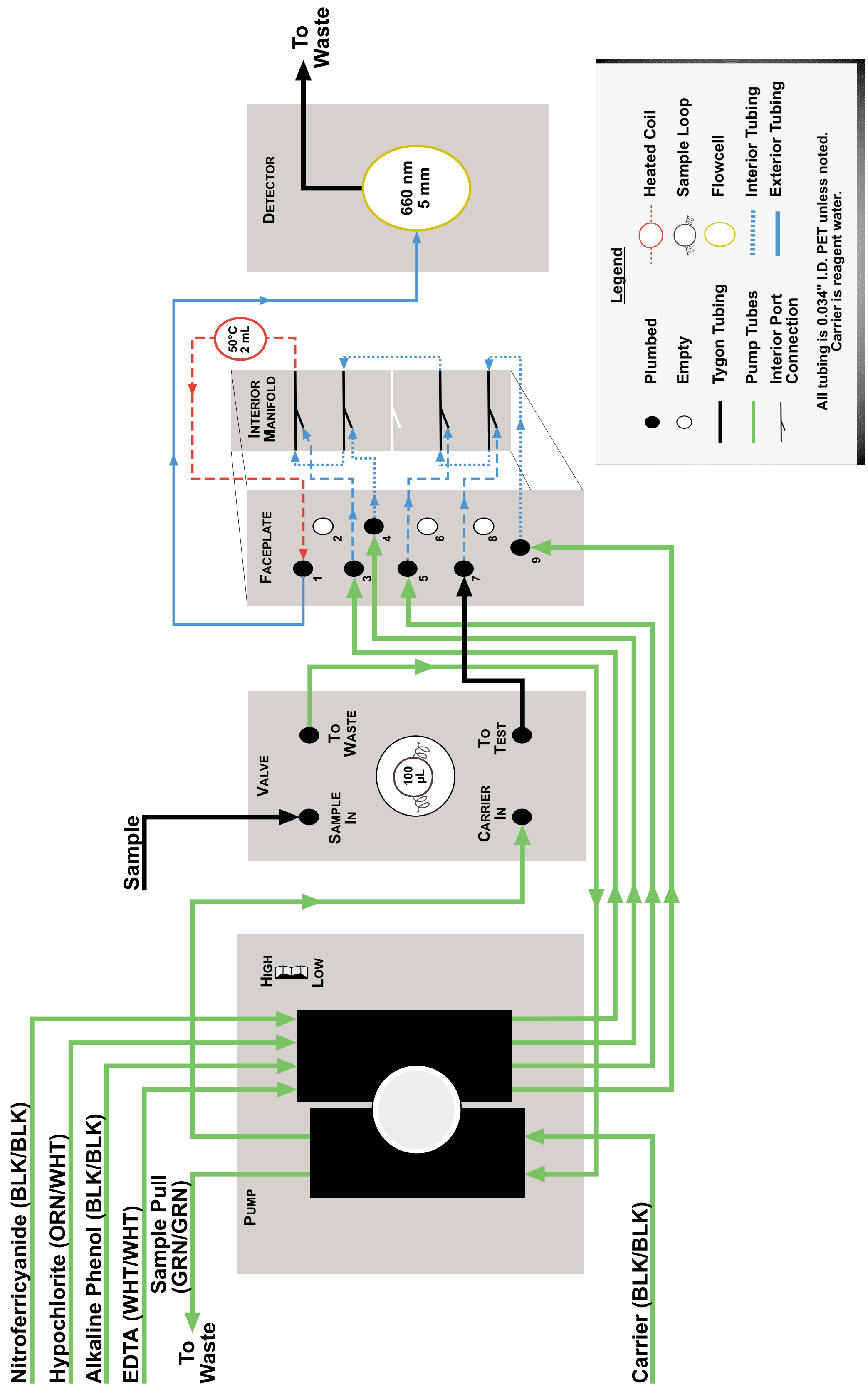


Figure 1. Detailed Flow Diagram for Ammonia Nitrogen, USEPA by FIA on a Flow Solution 3000, Cartridge Part #A002053

Results were obtained under optimal operating conditions. Actual results may vary depending on sample introduction, cleanliness of sample containers, reagent purity, operator skill, and maintenance of instruments.

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