Abstracted from Laboratory Operations, Pg. 99, Section 4.5.10. Ammonium by Flow Injection Analysis Colorimetry
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Ammonium by Flow Injection Analysis Colorimetry (FIA)

Go To: Calibration, Quality Control, Procedures, Troubleshooting

Calibration

Reagents and Solutions (CAPMoN, 2013)

i. DI water – the carrier - purity is highly important. It should be filtered and deionized to a resistivity of >18.0 MΩ.
ii. 20% H_2SO_4
iii. helium (UHP grade)
iv. sodium phenolate
   a. liquid phenol (ASC grade)
   b. sodium hydroxide (ASC grade)
v. disodium ethylenediamine-tetraacetate of EDTA (ASC grade)
vi. sodium hypochlorite (ASC grade) Do not use household bleach.
vii. sodium nitroprusside (ASC grade)

Sparging with Helium

Bubble helium vigorously through the sodium hypochlorite, disodium EDTA and sodium nitroprusside for a minimum of 1 minute to remove ammonium. Reduce the gas flow if the solution foams up and threatens to spill over.

20% H_2SO_4 (Scrubber)

Dilute 200 mL concentrated H_2SO_4 (95%-97% GR grade) in 800 mL of DI water. Note in Figure 4.39 how this H_2SO_4 scrubbing solution is vented to all reagent containers to cleanse any air in these containers of incidental ammonia gas. Refresh the scrubber solution every three months.

Sodium Phenolate

1) In a 2 L volumetric flask dissolve 188 g liquid phenol (C_6H_5OH) (ASC grade) in approximately 1 L of DI water. While stirring, slowly add 64 g sodium hydroxide. Continue stirring until the sodium hydroxide (NaOH) pellets are dissolved.

2) Let the solution cool. Dilute to the 2 L mark with DI water. Cap the flask and invert it three times to mix the solution thoroughly. Do not sparge this solution.

3) Store at room temperature in two darkened 1 L Nalgene bottles. Stable for one month.

Sodium Hypochlorite

Attention: Prepare one day in advance of the day of analysis.

1) Dilute 300 mL 5% sodium hypochlorite (NaOCl) (reagent grade) to 500 mL with DI water in a volumetric flask. Cap and mix by inversion.

2) Sparge with helium. Store at room temperature in a 1 L Nalgene bottle. Stable for 24 hours.

Disodium Ethylenediamine-Tetraacetate (EDTA)

1) In a 2 L volumetric flask, dissolve 110.8 g Na_2EDTA and 11.0 g NaOH in 1 L of DI water. Dilute to the 2 L mark with DI water. Cap and mix by inversion.

2) Sonicate the flask for approximately 1 hour to dissolve the solutes, or insert a stirring magnet and mix on a magnetic stirrer until the solution is clear.

3) Sparge with helium. Store the solution at room temperature in a 2 L Nalgene bottle. Stable for one month.

Sodium Nitroprusside

1) In a 2 L volumetric flask, dissolve 7.0 g sodium nitroprusside (sodium nitroferricyanide) $[Na_2[Fe(CN)_5NO]\cdot 2H_2O]$ in 1 L of DI water. Dilute to the 2 L mark with DI water. Cap and mix by inversion to dissolve the crystals.

2) Sparge with helium. Store the solution at room temperature in a 2 L Nalgene bottle. Stable for one month.

Calibration

Calibrate the FIA after every 40 to 50 injections. FIA calibration curves generally are linear; however, the curves will tend to flatten at very high concentrations. Use only the linear portion of the curve. The expected analytical range for this method is 0.005 mg L^{-1} to 1.000 mg L^{-1}.

Stock Standard Solution (1000 mg L^{-1})

To prepare the ammonium chloride solution, place approximately 4 g of NH_4Cl in a glass crucible and dry it overnight at 110ºC.

New flasks and bottles are conditioned by soaking in DI water over night. Following this soak, rinse the flasks and bottles with DI water three times and dry in a warm oven. Flasks and bottles need only be conditioned once when they are new and before putting them into service. See Appendix C for calibration of flasks and analytical balances and for glassware storage details.

1) Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly. Use containers that are dedicated to the preparation and storage of stock standard solutions.

2) It is highly recommended that stock standard solutions be prepared by weighing the DI water volume. Calibrate the receiving flasks by dispensing DI water by weight into the flask and then marking the flask at the fluid line.

3) Gently tap approximately 2.972 g of dry ammonium chloride into a weigh boat. Record the weight to 3 decimal places in your calibration logbook.

4) In a 1 L calibrated and conditioned volumetric flask dissolve the NH_4Cl powder in 800 mL of DI water. Dilute to the 1 L mark with DI water. Cap the flask and invert it three times.

5) Calculate the concentration of NH_4^+ to 3 decimal places. Record the concentration and preparation date in your calibration logbook. This solution contains 1000.000 mg L^{-1} of NH_4^+.

6) Record the concentration and preparation date on a label and affix it to the flask.

7) To ensure consistency between old and new stock standard solutions, prepare a dilution of the new stock standard solution and analyze it as an unknown.

   i. Into a rinsed weigh boat dispense 0.500 gm of new stock standard solution.
   ii. Pour this solution into a clean, rinsed and calibrated 1 L volumetric flask.
   iii. Rinse the weigh boat into the flask and fill the flask to the mark with Type I DI water.
   iv. Mix well and allow the solution to equilibrate at least one hour.
   v. Analyze as a sample, using the old calibration set.
   vi. Results should be 0.500 mg L^{-1} and fall within the QC precision guidelines at that concentration.
   vii. 1000 mg L^{-1} standard is best kept in its original glass flask sealed with Parafilm® around the stopper. Store at 4°C. Stable for six months.

8) If the concentration of this new diluted stock standard solution does not fall within the expected range, discard the 1000 mg L^{-1} solution and prepare a new stock standard solution. Make sure the newly prepared stock standard solution has equilibrated for one hour before analysis.

Intermediate Working Standard (1.000 mg L^{-1})

1) Prepare this working standard fresh with each analytical run. Use a calibrated glass volumetric flask dedicated for the preparation and storage of this solution. A best practice is to use the same flask that was used to store the working standard from the previous run. This flask is conditioned for an NH_4Cl solution of the same strength. Empty this seasoned flask and rinse it three times with DI water. You can use DI water from the holding tank for the first two rinses, but preform the final rinse using fresh DI water from the point of use dispenser. Fresh DI water should be completely free of any dissolved ammonium.

2) Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly.

3) Dispense 1.000 g of stock standard solution into a weigh boat. Measure to 3 decimal places. Record the weight in your calibration logbook.

4) Transfer this aliquot into the 1 L calibrated, conditioned, dedicated volumetric flask and dilute to the 1 L mark with ***fresh DI water*** from the point of use dispenser. Cap the flask and invert it three times to ensure the solution is thoroughly mixed.

5) Calculate the concentration of NH_4^+ to 3 decimal places. Record the concentration in your calibration logbook. This solution contains approximately 1.000 mg L^{-1} NH_4^+. Use this intermediate working standard to make the rest of the standards as in table 4.39.

Calibration Standards

1) Weigh all volumes using an analytical balance. Rinse all weigh boats thoroughly. Use calibrated, dedicated glass volumetric flasks as a final reservoir. Do not use vessels from other procedures.

2) Use DI water from the dispensing gun as the tenth or zero calibration standard.

3) Table 4.39 lists a suggested calibration series. Figure 4.40 illustrates a calibration curve using these standards.

Table 4.39. NH_4^+ Calibration Standard Preparations (CAPMoN, 2013)

Calibration Standard #	Intermediate Working Standard Volume (mL)	Final Volume (mL)	Concentration (NH_4^+) (mg L^{-1})
1	Intermediate Working Standard		1.000
2	80	100	0.800
3	50	100	0.500
4	30	100	0.300
5	10	100	0.100
6	8	100	0.080
7	5	100	0.050
8	3	100	0.030
9	1	100	0.010
10	0	100	0.000

Quality Control

Ammonium deteriorates too rapidly in natural precipitation, even if refrigerated, to use for QC solutions. Other sources of QC solutions include:

- Certified Reference Material
- simulated samples made from an alternate standard material such as ammonium sulfate
- Pooled inter-laboratory comparison samples may be used if they have been sterilized by filtration; however, do not use this solution if a pipette has been dipped into it or if the bottle openings have been touched with hands, allowing for bacterial contamination.

Prepare at least three QC solutions in the standard range, perhaps similar to standards 9, 7 and 4 in Table 4.39. QC solutions should test the low, mid and high range of the calibration curve.

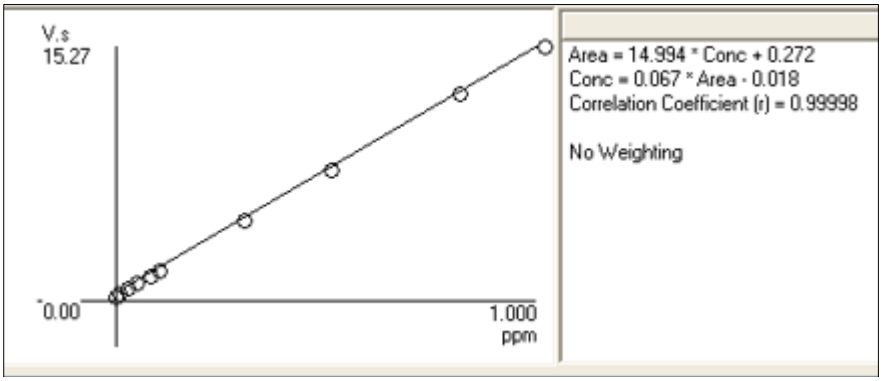

Figure 4.40. Ammonium Calibration Curve

Procedures

1) Turn on the computer, sample changer, peristaltic pump and system unit that houses the heating block and light source.

2) Place the waste line from the flow cell into a waste container. Connect the transmission tubing to the reagents. Waste contains phenol and other reagents and must not be poured down the sink. A container equipped with a charcoal filter on the air vent will help lessen the phenol odor. Dispose of waste responsibly following local regulations.

3) Place the reagent pump tubing under tension as per the manufacturer's specifications.

4) Start the peristaltic pump and condition the transmission tubing with the reagents and establish a baseline. Periodically check the display for any deviation from the baseline. This may indicate that there are air pockets in the tubing. Conditioning usually takes about half an hour. The analyst can make up standards during this warm-up period.

5) Run a DI water sample to check instrument stability. Usually only one to three water injections are required.

6) Start the calibration after a good baseline has been achieved and the temperature of the heating unit has reached 60°C.

Do not dispense samples to be analyzed until the system demonstrates a stable baseline. This will help to minimize exposure of the samples to laboratory air. Humans exhale ammonia and this gas has many other sources, including some cleaning solvents. It is important to isolate standards and samples from the ambient laboratory environment.

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7) Begin instrument calibration by dispensing about a 5 mL volume of the calibration standards into each tube. This is enough for two analyses from the same tube. Be aware that when tubes are labeled some markers may have ammonia in the ink. Use a wax pencil or labels with an adhesive backing. Place Parafilm® over each tube promptly after adding liquid to the tube. Ensure each tube has the minimum volume required for duplicate readings.

8) Run the calibration sequence followed immediately by a QCS. After all standards have been run, re-cap all standards. If the system does not stabilize and a troubleshooting period is required, dispense fresh standards again.

9) Continue with precipitation samples only after instrument stability and successful calibration have been verified.

10) Fill the sample rack according to the specified sample sequence. Ensure there is enough volume in each tube to perform the analysis without drawing air into the FIA system. A run of fifty samples (in duplicate) takes about 1.5 hours. Standards that have been capped can be used for the next calibration. Dispense standards fresh from the flasks every three hours.

Note: Do not discard calibration standards after analysis. Leave the standards in the flasks to condition the flasks.

11) Following the run recheck all calibration curves and QC results before reporting, collating or tabulating results.

12) Check all raw data points individually for correct integration and output. Comment on all anomalies and flag data accordingly.

13) Calculate final results against the appropriate calibration curve and report the correct number of decimal places. Apply detection limit notations as needed. Mark all samples that exceed upper calibration ranges for dilution and repeat analysis. Ensure missing samples are accounted for and contamination codes are applied as needed.

14) Export data from the FIA system and archive all parameters associated with the analysis, including calibration data, raw measurement data, sample identification and instrument audit trails. Audit trails include instrument parameter settings that may be useful when the baseline is found to have drifted. It may be necessary to repeat the analysis from the point where a problem was discovered in the FIA output. The signal trace from an FIA is called an FIAgram. Figure 4.41 is an example of an FIAgram.

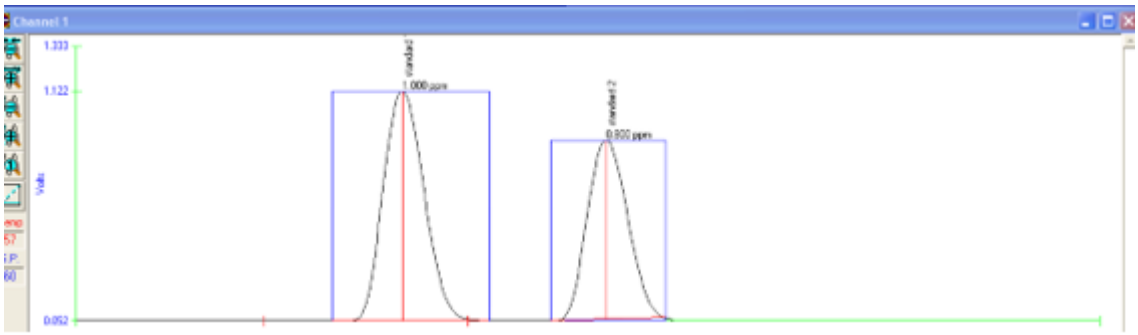

Figure 4.41: Example of FIAgram

Troubleshooting

Problem 1: Air spikes are observed on the real-time display as noisy irregular spikes in the baseline or sample peak.
Solution 1: Air bubbles will be introduced to the flow cell if reagents are not degassed or the transmission tubing is not securely connected. Ensure all reagents are sparged with helium in advance of analysis. Check the volume of sample in each tube to ensure there is enough for the analysis. If a tube cracks and the sample leaks out, the probe could draw in air causing air spikes.

Problem 2: Carry-over. Extremely high-concentration samples do not wash out of the sample lines completely, resulting in erroneously high readings for the following sample or samples.
Solution 2: Samples must be reanalyzed. Dilute the high-concentration sample and compare the result with the first analysis. Also, repeat the analyses of several samples that followed the high-concentration sample and by comparing the initial and re-analysis results ensure that any carry-over effects are eliminated. Do not analyze very contaminated samples. Samples contaminated with bird droppings will not produce a valid ammonium result and only contaminate the system. Report, code or comment that the sample was too contaminated for analysis.

Problem 3: Reagent line leakage.
Solution 3: Replace peristaltic pump lines frequently depending on use. Keep maintenance logs, review the logs and periodically check the condition of all of the lines. Replace transmission lines as needed. Sometimes the waste line can be blocked causing enough back pressure to loosen the reagent line connections or even cause them to become disconnected. Ensure the waste line is clear at all times. Look for crimps on the waste line and ensure lines are not tangled or constricted in any way.

Problem 4: Deterioration of QC measurements.
Solution 4: Check the age of the QCS. Prepare fresh QCS as needed. Prepare the ammonium hypochlorite on day before the day of analysis.

Problem 5: Low peak areas on calibration standards and controls compared to previous runs.
Solution 5: Check the age of all reagents and prepare fresh. Check sample lines for blockage.

Problem 6: Peaks show a small dip before each peak, i.e., a pre-peak dip.
Solution 6: If samples have been acidified as a preservation step, the pH in the reagent system will change. This pre-peak dip can be overcome by adding a proportional amount of acid to the DI water carrier, so that the pH of the mixture of reagents matches the pH of the sample matrix. See Figure 4.42 for an example of pre-peak dips.

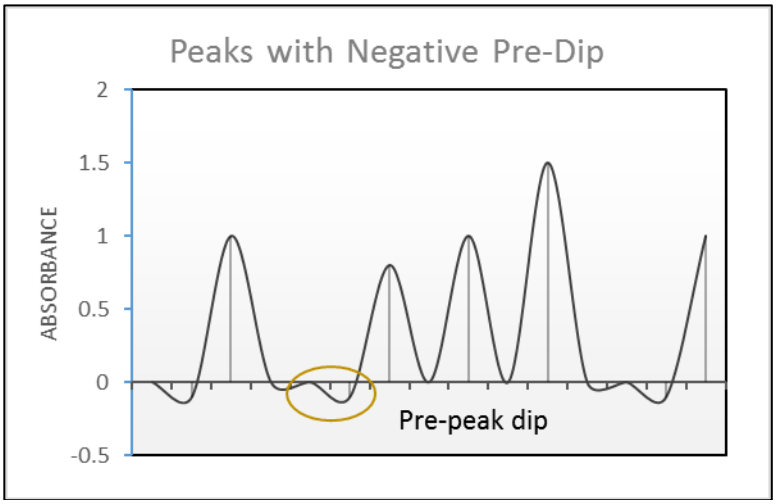

Figure 4.42: FIAgram with pre-peak dip

Tips to Improve Performance

1. Keep a log book that records maintenance procedures.
2. Maintain a log book for details of calibration solution preparations. Record dates of preparation, calculations, weights, volumes and the person who prepared the solutions.
3. Calibration solutions need dedicated glassware and containers. Do not use these containers for any other use.
4. Reserve an analytical balance for standard preparation and use another balance for reagents.
5. Cap samples immediately to minimize exposure to ambient air and minimize the head space in sample tubes.
6. Prepare standards fresh with each analytical run. Use calibrated flasks that have not been used for any other solutions. Weigh out calibration standard solutions.
7. Keep the area clean. Do not allow reagent spills to dry and form residue on laboratory benches.
8. Install the instrument in a low traffic area. Place in a well ventilated room.
9. Change the peristaltic pump tubing every 400 injections.
10. Change interconnecting tubing every three months.
11. Do not top up reagent bottles. Empty reagent bottles completely and rinse the container before putting new reagent in the bottle.
12. Always check that the sample line and injection valve is free of bacterial growth. Check that the waste line flows freely. T-junctions are places for residue to collect so regularly check them.
13. Do not extrapolate standard curves under any circumstances. Colorimetric calibration curves flatten out when saturated.

References

CAPMoN. (2013, November 26). Ammonium in Precipitation by Colourimetric Flow Injection Analysis (FIA). *Canadian Air and Precipitation Monitoring Network National Laboratory Standard Operating Procedure SOP 13*. Toronto, ON, Canada.