Method for Ammonia Determination on Mid-Range Samples
1 PRINCIPLE

This method is designed for the determination of ammonia in soil, water and other forms of samples. The sample is prepared offline if necessary and then introduced to the FIAlab analyzer. In a phenol free variation of the Berthelot method, sodium salicylate reacts with ammonia in a two-step reaction, converting it to 5-aminosalicylate. The aminosalicylate is then oxidized in the presence of sodium nitroferricyanide to form a blue-green colored dye.

2 SUMMARY

This method is designed for the determination of ammonia in high-range samples of various matrices. The method is capable of detecting ammonia in the range of 0.1-70mg N / L.

3 SAFETY

The toxicity or carcinogenicity of all reagents used in this method must be taken into account and therefore each chemical listed below should be handled accordingly.

Each laboratory is responsible for maintaining compliance with OSHA regulations regarding the safe handling of the chemicals specified in this method. Material Safety Data Sheets (MSDS) should be made available to all personnel using the method.

All waste materials should be disposed of in a responsible manner, in accordance with federal, state and other local regulations.

The following chemicals have the potential to be highly toxic or highly hazardous, for detailed explanations consult the MSDS:

- Sodium hydroxide
- Sodium nitroferricyanide (III) dihydrate
4 EQUIPMENT AND SUPPLIES

Balance:
- Analytical, with a 0.01 g resolution

Glassware:
- Class A volumetric flasks of at least 50 ml
- Pipettes and appropriate beakers
- Tinted glass storage containers

Flow injection analysis apparatus:
- FIAlab-2500 flow injection analyzer
- Tungsten-halogen light source, Ocean Optics HL-2000-LL or corresponding
- FIAlab SMA-Z absorbance flow cell with 10 mm light path
- Spectrophotometer, Ocean Optics USB4000-VIS/NIR or corresponding
- FIAlab Fiber optic cables (2)
- FIAlab FT Heater

Autosampler (for high sample loads):
- Cetac ASX-260/520 or AIM-3200/3300

5 REAGENTS AND STANDARDS

5.1 List of chemicals
- Deionized water, (H$_2$O) [CAS - 7732-18-5].
- Sodium hypochlorite solution (6%), (NaOCl) [CAS - 7681-52-9]. Sigma-Aldrich P/N 13440 or corresponding.
- Sodium hydroxide, (NaOH) [CAS – 1310-73-2]. Sigma-Aldrich P/N 221465 or corresponding.
- Sodium salicylate (HOC$_6$H$_4$COONa) [CAS – 54-21-7]. Sigma-Aldrich P/N S3007 or corresponding.
- Sodium nitroferricyanide (III) dihydrate (Na$_2$[Fe(CN)$_5$NO]*2H$_2$O) [CAS – 13755-38-9]. Sigma-Aldrich P/N 228710 or corresponding.
- Brij® L23 (CH$_3$(CH$_2$)$_{10}$CH$_2$(OCH$_2$CH$_2$)$_n$OH) [CAS – 9002-92-0]. Sigma-Aldrich P/N 16005 or corresponding.
5.2 Preparation of reagents

**Carrier:** Matrix match carrier to samples. Salt solution to seawater samples, extraction solution for soil samples, etc.

**Reagent 1:** Hypochlorite Solution (1L)
- Mix 50ml of 6% sodium hypochlorite solution into 700ml of deionized water
- Dissolve 5g of sodium hydroxide into this solution.
- Add 1g of Brj® L23 and stir until completely dissolved.
- Fill container to the 1L mark with DI water and store in a glass bottle.

**Reagent 2:** Salicylate/Catalyst Solution (1L)
- Add 100g of sodium salicylate to 700ml of deionized water and stir until dissolved.
- Add 2.0g of Sodium nitroferricyanide (III) dihydrate and stir until dissolved.
- Dissolve 5g of sodium hydroxide into this solution.
- Add 1g of Brj® L23 and stir until dissolved.
- Fill container to the 1L mark with DI water and store in a glass bottle.

**Note:** Use of high quality laboratory glass bottles is important.
Reagent 1 degrades with time and should be prepared daily.
Reagent 2 should be prepared fresh weekly.

5.3 Preparation of standards

- Dilute the 1000 mg/L N-(NH₄) stock solution with deionized water to the desired range of ammonia standards. Prepare fresh daily.

6 SAMPLE COLLECTION AND PRETREATMENT

This protocol only covers the analysis process. Sample collection and pretreatment depends on the type of sample and will have to be determined separately.
Flow injection analysis apparatus parameters:

- Peristaltic pump tubing: Tygon, 1.02mm/0.04in ID (white/black), FIAlab P/N 270300
- Sample injection loop: 35µL – 3.0in/7.6cm of Teflon capillary tubing with 0.03 in/0.75mm ID, FIAlab P/N 270040
- Reaction coil 1: 750µL - 65.0in/165cm of Teflon capillary tubing with 0.03 in/0.75 mm ID, FIAlab P/N 270040
- Bridge: 40µL – 3.5in/9.0cm of Teflon capillary tubing with 0.03 in/0.75 mm ID, FIAlab P/N 270040
- Reaction coil 2: 750µL - 65.0in/165cm of Teflon capillary tubing with 0.03 in/0.75 mm ID, FIAlab P/N 270040. Heated.
- Waste/back pressure coil: 600µL - 120.0in/300cm of Teflon capillary tubing with 0.02 in/0.51mm ID - COILED, FIAlab P/N 270160
- SMA-Z flow cell: light path -10 mm
- Flow rate: 45% pump speed (approx. 2.0mL/min flow rate per pump channel)
- Heater: set to 60C

Recommended spectrometer parameters:

Primary wavelength: 670nm
Second wavelength: 765nm
Third wavelength: 790nm
Fourth wavelength: 815nm
Reference wavelength: 525nm

Fig. 1 – Flow Schematic for mid-range ammonia determination on a FIAlab-2500
Fig. 2 – Pictorial representation of mid-range ammonia setup on a FIAlab-2500
The program script using an autosampler is shown below. For manual sample introduction see the FIAlab for Windows software manual.

' Ammonia Assay

'FIA Template For FIAlab-2500 System
Global Logon 'logon to all components
Sample Description 'load sample description file
Injection Valve Sample Load
Optimize_FIAlab2500

'Set Wavelengths
Hardware Settings Wavelength 1 (nm) 670
Hardware Settings Wavelength 2 (nm) 765
Hardware Settings Wavelength 3 (nm) 790
Hardware Settings Wavelength 4 (nm) 815
Reference Wavelength1 525

'set delay time, start pump to prime lines
Valve Delay 8000 'sample inject (msec)
Peristaltic Pump Clockwise(%) 45
Injection Valve Sample Load
Delay (sec) 40
Hardware Settings Optimize Integration

'Put autosampler in first sample (usually a blank)
Next Sample
Delay (sec) 35
LoopStart (#) 5000

'inject sample, load next sample
'autosampler wash
Analyte New Sample
Next Sample
Injection Valve Sample Inject
Delay (sec) 3

'perform reference scan and start absorbance scans
Spectrometer Reference Scan
Spectrometer Absorbance Scanning
Delay (sec) 40
Spectrometer Stop Scanning

'Refresh plots and update concentrations
Refresh Plot

'method clean up (at end of run)
If sampleid < 0 Then
   autosampler Wash
   'Save Data Date-Time .dat
End If

Loop End
8 PERFORMANCE METRICS

Fig. 3 – Example plot and calibration data for mid-range ammonia at 670nm

Lower limit of detection: \( 0.1 \text{mg/L} - 7.1 \times 10^{-6} \text{molN/L} \)

Upper limit of detection: \( 70 \text{mg/L} - 5.0 \times 10^{-3} \text{molN/L} \)

Sample throughput: 75 samples/hr

Startup: 5 minutes

Shutdown: 5 minutes