

Method for Ammonia Determination on High-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 2.5-175mg 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, (H₂O) [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 (HOC₆H₄COONa) [CAS – 54-21-7]. Sigma-Aldrich P/N S3007 or corresponding.
- Sodium nitroferricyanide (III) dihydrate (Na₂[Fe(CN)₅NO]*2H₂O) [CAS – 13755-38-9]. Sigma-Aldrich P/N 228710 or corresponding.
- Brij[®] L23 (CH₃(CH₂)₁₀CH₂(OCH₂CH₂)_nOH) [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 Brij[®] 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 0.8g of Sodium nitroferricyanide (III) dihydrate and stir until dissolved.
- Dissolve 5g of sodium hydroxide into this solution.
- Add 1g of Brij[®] 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.

7 INSTRUMENT SETUP – HIGH-RANGE

Flow injection analysis apparatus parameters:

- Peristaltic pump tubing: Tygon, 1.02mm/0.04in ID (white/black), FIALab P/N 270300
- Sample injection loop: 15µL – 3.0in/7.6cm of Teflon capillary tubing with 0.02 in/0.51mm ID, FIALab P/N 270160
- 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

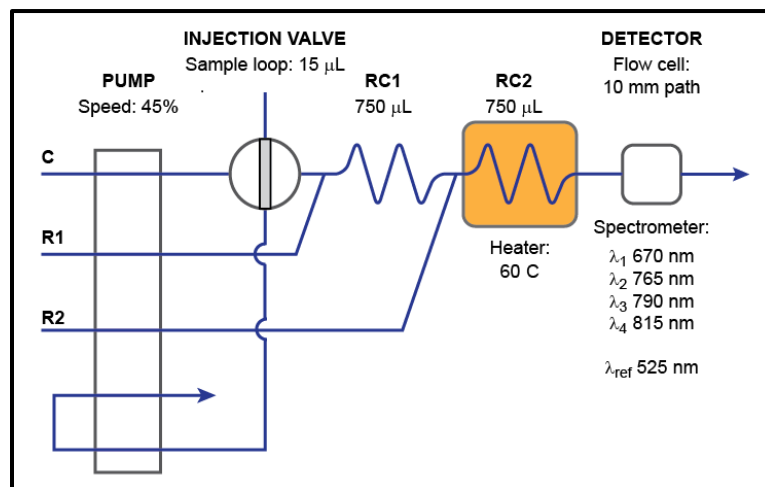


Fig. 1 – Flow Schematic for high-range ammonia determination on a FIALab-2500

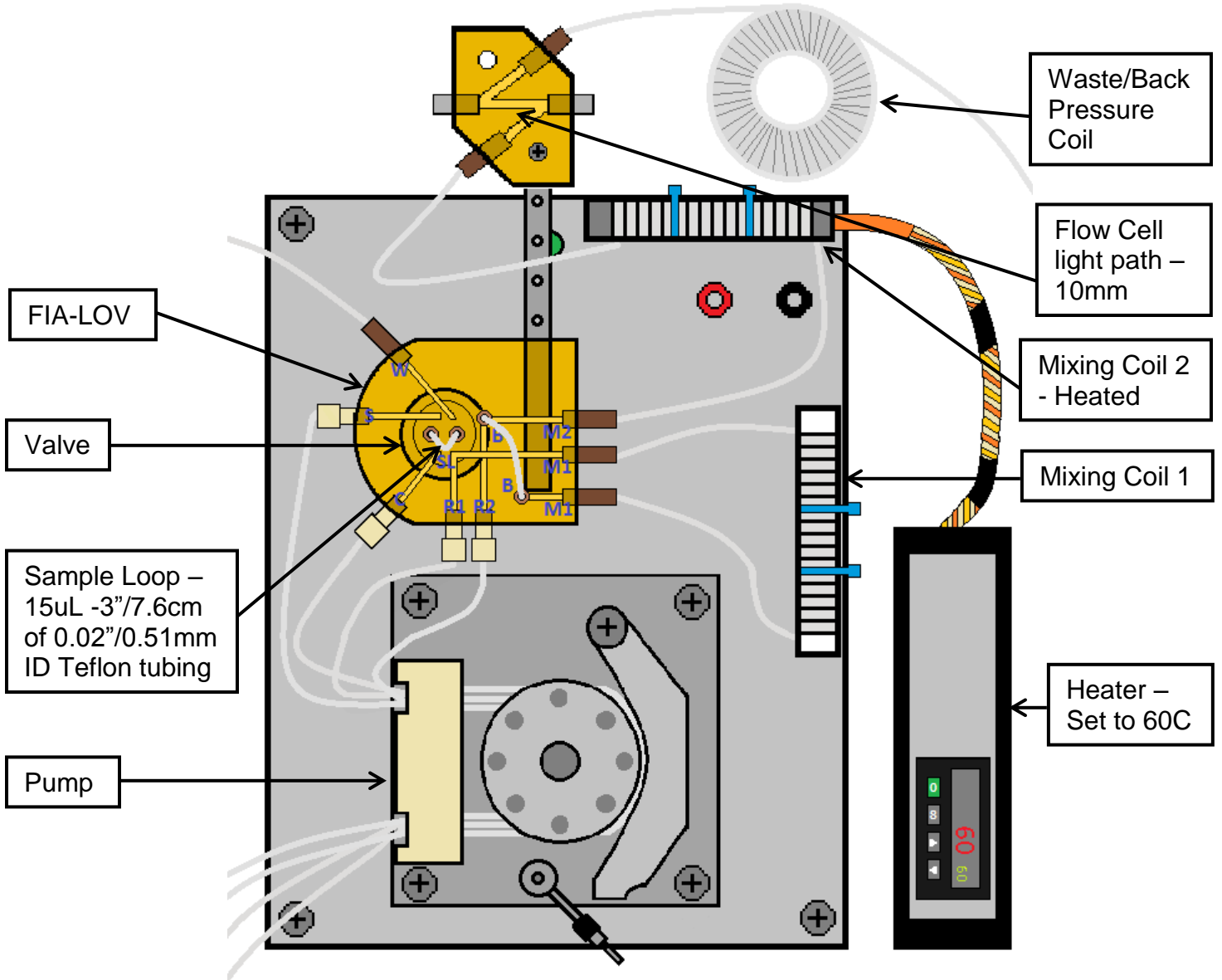


Fig. 2 – Pictorial representation of high-range ammonia setup on a FIAlab-2500

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

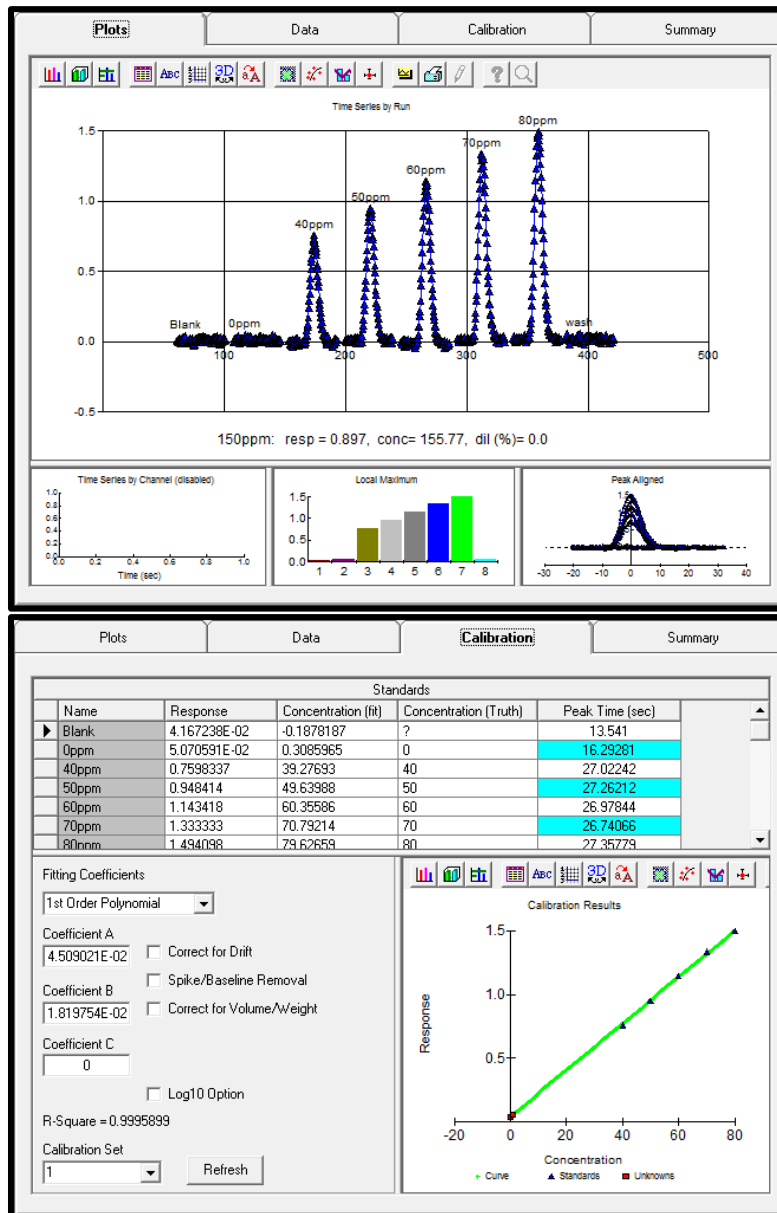


Fig. 3 – Example plot and calibration data for high-range ammonia at 765nm

Lower limit of detection: 2.5mg/L – $1.8 \cdot 10^{-4}$ mol/L
 Upper limit of detection: 175mg/L – $1.3 \cdot 10^{-2}$ mol/L
 Sample throughput: 75 samples/hr
 Startup: 5 minutes
 Shutdown: 5 minutes

FIAlab[®]



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