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Leaders in Flow Injection Technology

Method for Ammonia Determination by Salicylate, Version 2.2

1 PRINCIPLE

This method is designed for the determination of ammonia in water, soil, 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.

This method is designed to be run on the FIAlyzer-1000.

2 SUMMARY

This method is designed for the determination of ammonia in water, soil, and other forms of samples. The method is capable of detecting ammonia in the range of 0.02-50 mg $N\text{-}NH_4$ / L. For more information, see the section on performance metrics.

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, local, and any other applicable 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) dehydrate**

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4 EQUIPMENT AND SUPPLIES

- **Equipment:**
  - Balance, analytical, with a 0.01 g resolution
  - Pipettes for making standards
- **Glassware:**
  - Class A volumetric flask, 1 L, QTY 2
  - Graduated cylinder, 100 mL, QTY 1
  - Glass storage container, 1 L , clear, QTY 1
  - Glass storage container, 1 L, brown tinted, QTY 1

- **Autosampler (for high sample loads):**

  - Cetac ASX-280/560 (recommended) or AIM-3200/3300

5 REAGENTS AND STANDARDS

Chemical part numbers refer to Sigma-Aldrich unless noted otherwise.

Chemical	Chem. Formula	CAS#	Supplier PN
Deionized Water	H_2O	7732-18-5	
Sodium hypochlorite solution (6%)	NaOCl	7681-52-9	13440
Sodium hydroxide	NaOH	1310-73-2	221465
Sodium salicylate	HOC_6H_4COONa	54-21-7	S3007
Sodium nitroferricyanide (III) dihydrate	$(Na_2[Fe(CN)_5NO]*2H_2O)$	13755-38-9	228710
Brij L23 Solution (30%)	$C_{12}E_{23}$	9002-92-0	B4184

- **Preparation of Reagents**

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

  - **Reagent 1:** Hypochlorite Solution (1 L)
    - Mix 50 mL of sodium hypochlorite (6%) solution into 700mL of deionized water.
    - Dissolve 5 g of sodium hydroxide into this solution.
    - Add 3 mL of Brij® L23 and stir until completely dissolved.
    - Fill container to the 1 L mark with DI water and store in a glass bottle.
    - The reagent should be clear with no particles in it.

  - **Reagent 2:** Salicylate/Catalyst Solution (1 L)
    - Add 100 g of sodium salicylate to 700mL of DI water and stir until dissolved.
    - Add 2.0 g of sodium nitroferricyanide (III) dihydrate and stir until dissolved.
    - Add 5.0 g of sodium hydroxide into this solution and stir until dissolved.
    - Add 3 mL of Brij® L23 solution and mix well.
    - Fill container to the 1 L mark with DI water and store in a glass bottle.
    - Reagent should be a light orange color, approximately the color of apple juice.

  - **Probe Wash Solution:** Water with 0.1% Brij L23 (1 L)
    - Add 3.3 mL Brij L23 to 800mL of deionized water, dilute to 1 L.
    - Mix well and store in a polyethylene bottle.
    - The wash solution should be clear with no particles in it.

- **Notes on reagent shelf life:**
  - Use of high quality laboratory glass bottles is important.
  - Reagent 1 degrades with time and should be prepared daily.
  - Reagent 2 should be prepared every week.

- **Preparation of Standards**
  - Dilute 1000 mg/L N-NH_4 / L stock solution with carrier to the desired range of ammonia standards.

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.

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7 INSTRUMENT SETUP – STANDARD

- **Flow injection analysis apparatus parameters:**

Component	Specifications	FIAlab PN
Analyzer	FIAlyzer-1000 or FIAlyzer-FLEX flow injection analyzer	*Inquire
Spectrometer	FLAME-T-VIS-NIR Spectrometer. 350-1000 nm.	64019
Light Source	HL-2000-LL. Tungsten-halogen, 360-2000 nm.	17041
Peristaltic Pump Tubing	1.02 mm ID (white/white)	*Inquire
Sample Injection Loop	35 µL – 3.0in/7.6cm of Teflon capillary tubing with 0.03in/0.71mm ID	*Inquire
Reaction coil 1	750 µL – 65in/165cm of Teflon capillary tubing with 0.03in/0.76mm ID	79209
Reaction coil 2, heated	800 µL - 69.0in/175cm of Teflon capillary tubing with 0.03 in/0.75 mm ID – heated	77030
Waste/backpressure coil	600 µL - 120.0in/300cm of Teflon capillary tubing with 0.02 in/0.51mm ID	270160
SMA-Z Flow Cell	10 mm light path	79028

- **Software parameters:**

Parameter	Value
Pump Speed	45%
Heater	60°C
Primary Spectrometer Wavelength	670nm
Secondary Wavelength (optional – for extended upper range)	800nm
Reference Wavelength	525nm

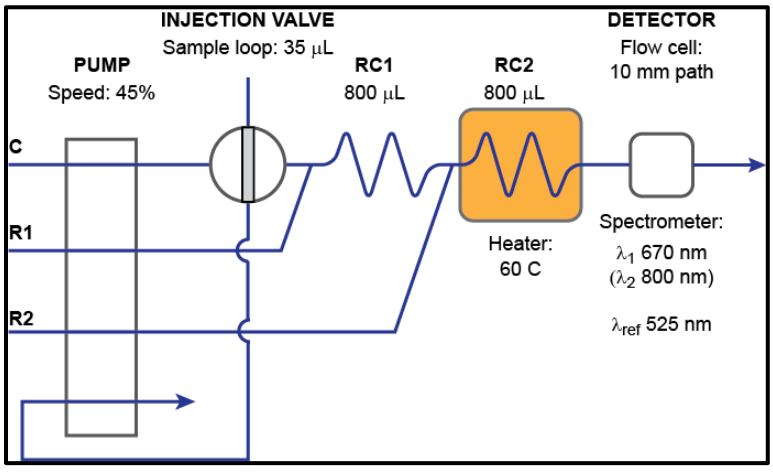

Fig. 1- Flow schematic for ammonia determination.

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8 FIASOFT METHOD

- The program script using an autosampler is shown below. For manual sample introduction see the FIAsoft manual.

```
FIAlab Injection Valve Sample Load
FIAlab Peristaltic Pump CounterClockwise(%) 45
Autosampler Wash
Next Sample
Delay (sec) 60

Loop Start

  Autosampler Wash
  Analyte New Sample
  Next Sample
  FIAlab Injection Valve Sample Inject
  Delay (sec) 3
  FIAlab Injection Valve Sample Load

  Spectrometer Reference Scan
  Spectrometer Absorbance Scanning
  Delay (sec) 30
  Spectrometer Stop Scanning

Loop End

Autosampler Rinse
Delay (sec) 60
Autosampler Wash
Delay (sec) 30
Autosave Data C:\Users\FIAlab\Desktop\Autosave Data\DateTime
FIAlab Peristaltic Pump Off
```

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9 PERFORMANCE METRICS

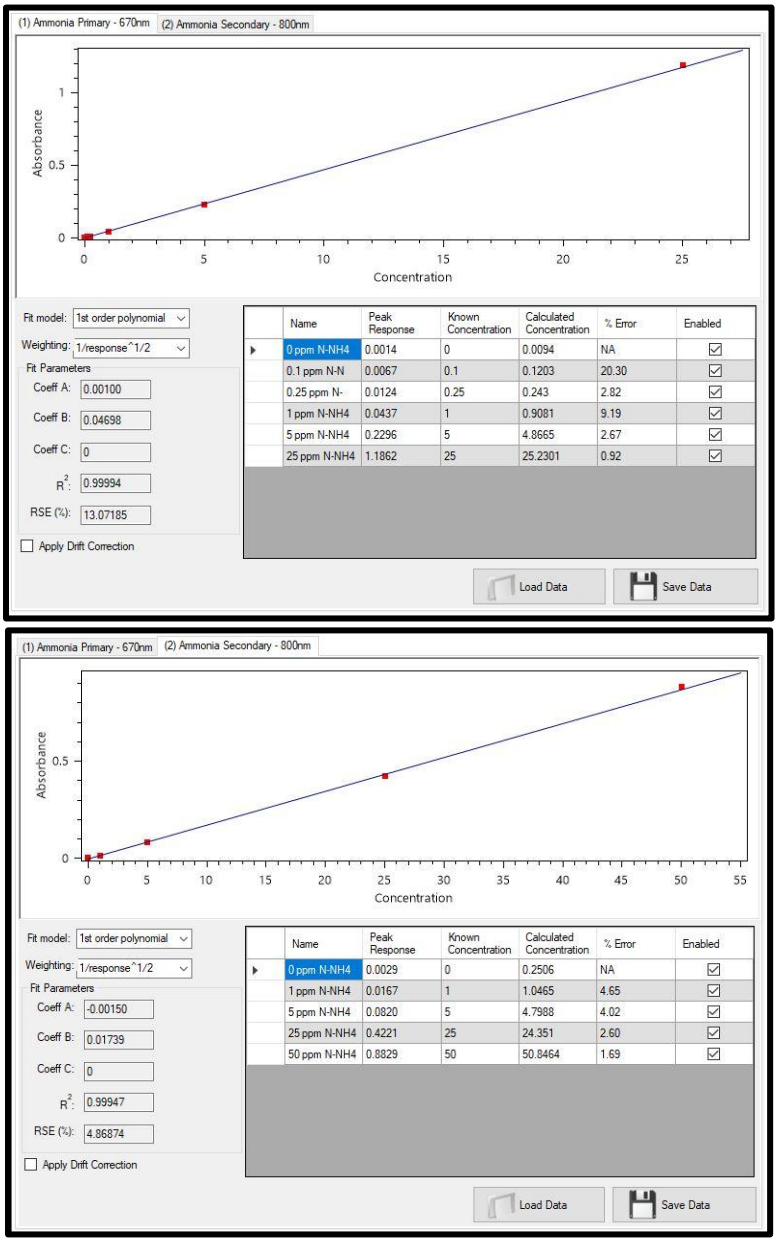

Fig. 2 – Example calibration data for standard ammonia configuration, primary and secondary wavelengths.

- Lower limit of detection: 0.075 mgN/L – 5.3×10^{-6}molN/L
- Upper limit of detection: 50 mg/L – 3.6×10^{-3}molN/L
- Sample throughput: 75 samples / hour
- Startup + Calibration: 10 minutes
- Shutdown: 5 minutes

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10 ALTERNATE CONFIGURATIONS

- **Low-Range Setup**

Component	Specifications	FIAlab PN
Sample Injection Loop	140 µL – 12.0in/30.5cm of Teflon capillary tubing with 0.03in/0.71mm ID	*Inquire
SMA-Z Flow Cell	100 mm light path	79038

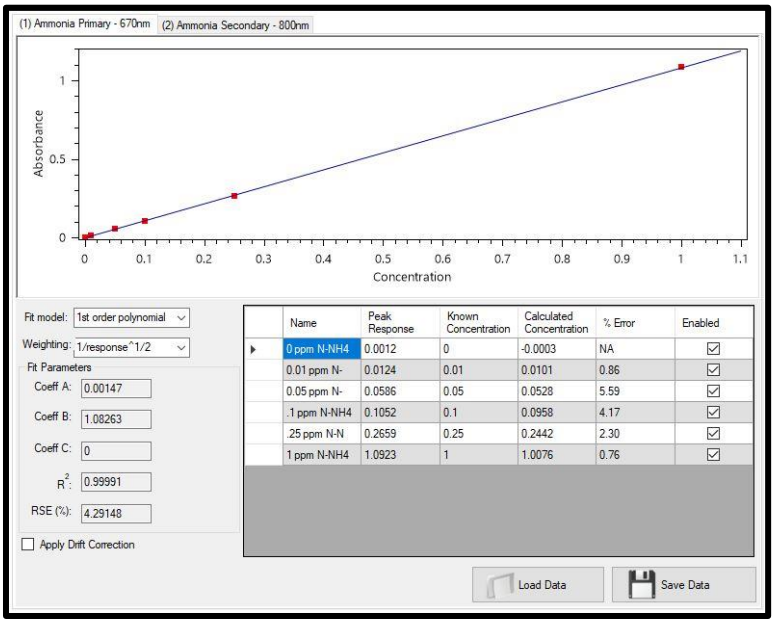

Fig. 3 – Example calibration data for low-range ammonia configuration, primary wavelength.

- Lower limit of detection: 0.02 mgN/L – 1.4×10^{-6}molN/L
- Upper limit of detection: 5 mg/L – 3.6×10^{-4}molN/L
- Sample throughput: 75 samples / hour
- Startup + Calibration: 10 minutes
- Shutdown: 5 minutes