

## Method for Ammonia Determination by Salicylate in Water and TKN Samples, Version 2.0

### 1 PRINCIPLE

This method is designed for the determination of ammonia in water, as well as samples digested by the Total Kjeldahl Nitrogen (TKN) method or the Total Protein Method. The sample is prepared offline if necessary and then introduced to the FIALab analyzer.

After injection, the sample is made alkaline and subsequently introduced to a gas diffusion cell. Liberated ammonia passes through the membrane of the gas diffusion cell and is absorbed into a stream of hypochlorite. Hypochlorite and sodium salicylate react with ammonia in a two-step reaction, converting it to 5-aminosalicylate. The aminosalicylate is finally oxidized in the presence of sodium nitroferricyanide to form a blue-green colored dye.

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

### 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 0.05-50mg N/L. For more information, see the section on performance metrics.

### 3 COMPLIANCE

This method follows EPA 351.2

## 4 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
- Sodium hypochlorite (bleach)

## 5 EQUIPMENT AND SUPPLIES

### ▪ Equipment:

- Balance, analytical, with a 0.01 g resolution
- Pipettes for making standards

### ▪ Glassware:

- Class A volumetric flask, 1L, QTY 4
- Graduated cylinder, 100mL, QTY 1
- Polyethylene storage container, 1L, clear, QTY 4
- Glass storage container, 1L, brown tinted, QTY 1

### ▪ Autosampler (for high sample loads):

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

## 6 REAGENTS AND STANDARDS

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

Chemical	Chem. Formula	CAS#	Supplier PN
Deionized Water	H <sub>2</sub> O	7732-18-5	
Sodium hypochlorite solution (6%) Clorox household bleach or equivalent	NaOCl	7681-52-9	13440
Sodium hydroxide	NaOH	1310-73-2	221465
Sodium salicylate	HOC <sub>6</sub> H <sub>4</sub> COONa	54-21-7	S3007
Sodium nitroferricyanide (III) dihydrate	Na <sub>2</sub> [Fe(CN) <sub>5</sub> NO]•2H <sub>2</sub> O	13755-38-9	228710
Diethylenetriaminepentaacetic acid (DTPA)	[(HOOCCH <sub>2</sub> ) <sub>2</sub> NCH <sub>2</sub> CH <sub>2</sub> ] <sub>2</sub> NCH <sub>2</sub> COOH	67-43-6	32319
Brij <sup>®</sup> L23	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>10</sub> CH <sub>2</sub> (OCH <sub>2</sub> CH <sub>2</sub> ) <sub>n</sub> OH		16005
Potassium Sulfate	K <sub>2</sub> SO <sub>4</sub>	7778-80-5	223492

**■ Preparation of Reagents**

- **Carrier:** Matrix match carrier to samples. Salt solution to seawater samples, extraction solution for soil samples, etc.
- **Reagent 1:** 3M Sodium Hydroxide – 50mM DTPA – 0.2M K<sub>2</sub>SO<sub>4</sub> Solution (1 L)
  - Dissolve 120 g of sodium hydroxide in 800 mL deionized water.
  - Add 15 g DTPA and stir until dissolved.
  - Add 35 g potassium sulfate.
  - Stir until dissolved and fill container to 1000mL mark using deionized water.
  - Store in a plastic container.
  - The solution should appear colorless.
- **Reagent 2:** Hypochlorite Solution (1 L)
  - Mix 60 mL of 6% sodium hypochlorite solution into 700 mL deionized water.
  - Fill container to the 1 L mark with DI water and store in a glass bottle.
  - Store in a plastic container.
  - The solution should appear colorless.
- **Reagent 3:** Salicylate Solution (1 L)
  - Add 150 g of sodium salicylate to 700 ml of deionized water and stir until dissolved.
  - Add 0.3 g of sodium nitroferricyanide (III) dihydrate and stir until dissolved.
  - Fill container to the 1 L mark with DI water and store in a glass bottle.
  - Store in a dark glass container.
  - The solution should appear orange-yellow (color of apple juice). A perceptible red tint indicates a bad solution.
- **Probe Wash Solution:** Water with 0.1% Brij L23 (1L)
  - Add 3.3mL Brij L23 to 800mL of deionized water, dilute to 1L.
  - Mix well and store in a glass bottle.
  - The wash solution should be clear with no particles in it.

**■ Notes on reagent shelf life:**

- Use of high quality laboratory glass and plastic bottles is important.
- Standards should be prepared fresh daily.
- Reagent 1 should be prepared every 4 weeks.
- Reagent 2 should be prepared every day.
- Reagent 3 should be prepared every 4 weeks.

**■ Preparation of Standards**

- Dilute 1000 mg/L N-(NH<sub>4</sub>) stock solution with carrier to the desired range of nitrate standards.

## 7 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.

## 8 INSTRUMENT SETUP – MID-RANGE

- 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	Tygon, 1.02 mm/0.04 in ID (white/white)	*Inquire
Peristaltic pump tubing	Tygon, 0.89 mm/0.03 in ID (orange/orange)	*Inquire
Peristaltic pump tubing	Tygon, 0.64 mm/0.03 in ID (orange/white)	*Inquire
Peristaltic pump tubing	Tygon, 0.51 mm/0.02 in ID (orange/yellow)	*Inquire
Sample injection loop	35 $\mu$ L – 3.0 in/ 5.7 cm of Teflon capillary tubing with 0.03 in/0.75 mm ID	*Inquire
Heated reaction coil	1,800 $\mu$ L – 155in/400 cm of Teflon capillary tubing with 0.03 in/0.75 mm ID	*Inquire
Back pressure coils	20 ft / 600 cm of Teflon capillary tubing with 0.02 in/0.50 mm ID	*Inquire

- Software parameters:

Parameter	Value
Pump Speed	100%
Primary Spectrometer Wavelength	660nm
Reference Wavelength	570nm

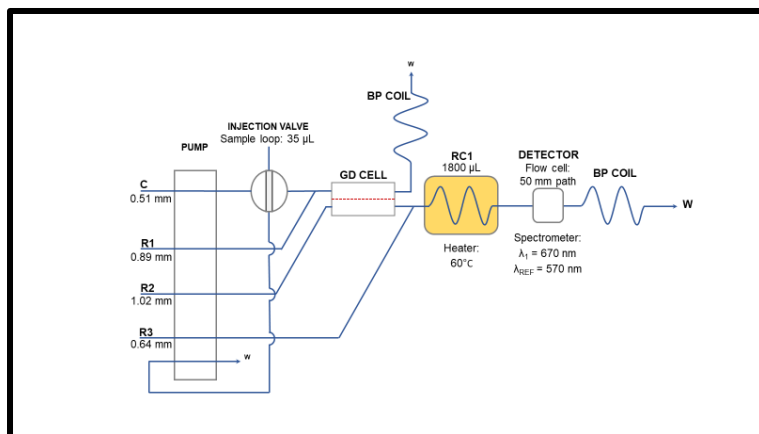


Fig. 1- Flow schematic for ammonia determination.

## 9 FIASOFT METHOD

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

### ' NH4 method.

FIALab Injection Valve Sample Load  
 Delay (sec) 10  
 Next Sample  
 Delay (sec) 55

Loop Start

Autosampler Wash  
 Analyte New Sample  
 Next Sample  
 FIALab Injection Valve Sample Inject  
 Delay (sec) 6  
 FIALab Injection Valve Sample Load

Spectrometer Reference Scan  
 Spectrometer Absorbance Scanning  
 Delay (sec) 100  
 Spectrometer Stop Scanning

Loop End

## 10 PERFORMANCE METRICS

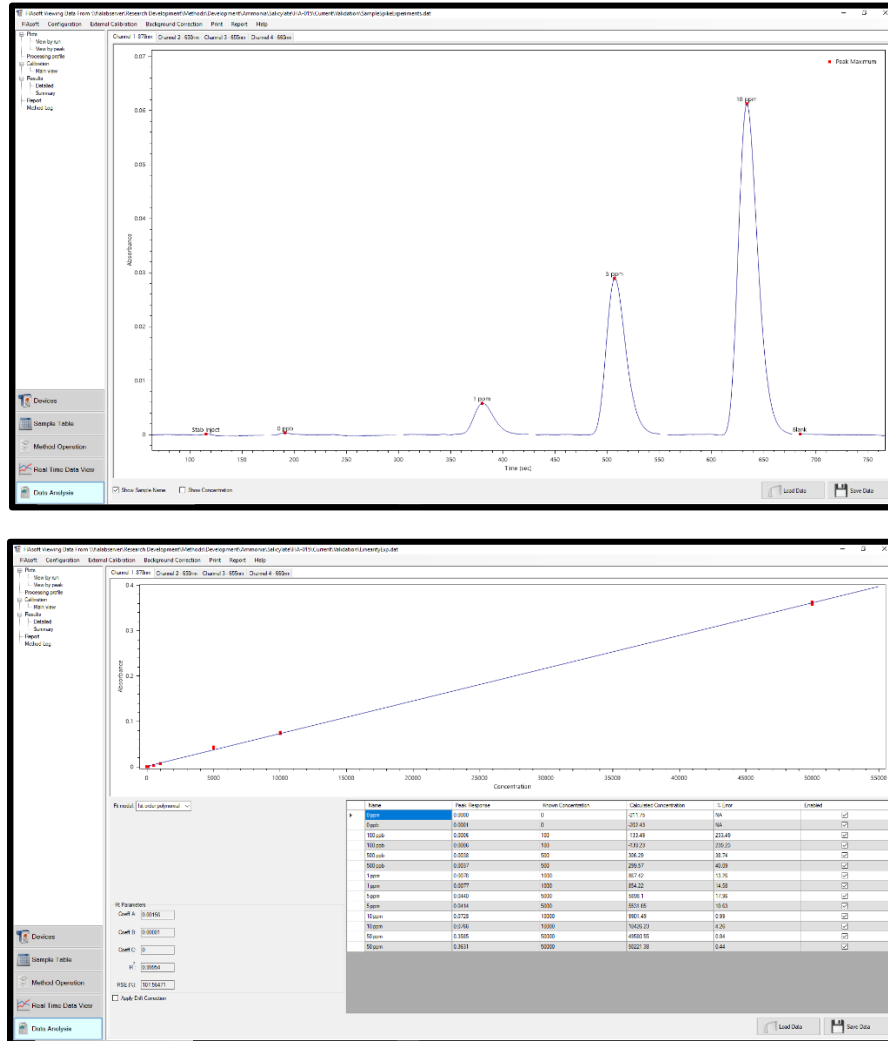


Fig. 2 – Example plot and calibration data for ammonia.

- Lower limit of detection: 0.50 mg/L –  $3.6 \cdot 10^{-6}$  mol N/L
- Lower limit of quantification: 0.150 mg/L –  $1.1 \cdot 10^{-5}$  molN/L
- Upper limit of detection: 0.05 - 10 mg/L or  $3.6 \cdot 10^{-6}$  molN/L –  $7.1 \cdot 10^{-4}$  molN/L
- Sample throughput: 30 samples / hour
- Startup + Calibration: 10 minutes
- Shutdown: 5 minutes