

Method for Nitrite determination on Low-Range Samples

1. PRINCIPLE

This method is designed for the determination of nitrite in soil, water and other forms of samples. The sample is prepared offline if necessary and then introduced to the FIAlab analyzer. Nitrite ions react with sulfanilamide and couple to N- (1-Naphthyl) ethylenediamine dihydrochloride to form a magenta colored azo dye.

2. SUMMARY

This method is designed for the determination of nitrite in high range samples of various matrices. The method is capable of detecting nitrite in the range of 0.002-2.5mg 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:

- **Phosphoric Acid**

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
- SMA-Z absorbance flow cell with 100 mm light path
- Spectrophotometer, Ocean Optics USB4000-VIS/NIR, USB4000-UV/VIS, or corresponding
- Fiber Optic Cables (2)

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].
- Sulfanilamide, (H₂NC₆H₄SO₂NH₂) [CAS - 63-74-1]. Sigma-Aldrich P/N S-9251 or corresponding.
- 85% Phosphoric acid, (H₃PO₄) [CAS - 7664-38-2]. Sigma-Aldrich P/N 79606 or corresponding.
- N-1-Naphthylethylene diamine dihydrochloride, (C₁₀H₇NHCH₂CH₂NH₂ · 2HCl) [CAS - 1465-25-4]. Sigma-Aldrich P/N 222488 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: Sulfanilamide Solution (1L)

- Mix 100ml of 85% phosphoric acid into 800ml of deionized water.
- Dissolve 40g of sulfanilamide and 1.0g N-1-Naphthylethylene diamine dihydrochloride to this solution.
- Add deionized water to a total volume of 1L.
- Mix well and store in a dark glass bottle.

Reagent 2: Unnecessary. Block R2 port on LOV with plug tubing

Note: Use of high quality laboratory glass bottles is important.
Reagent 1 should be prepared every four weeks

5.3 Preparation of standards

- Dilute the 1000 mg/L N-(NO₂) stock solution with deionized water to the desired range of nitrite 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.

7. INSTRUMENT SETUP – LOW-RANGE

Flow injection analysis apparatus parameters:

- Peristaltic pump tubing: Tygon, 1.02mm/0.04in ID (white/black), FIALab P/N 270300
- Sample injection loop: 140 μ L - 12.0in/30cm of Teflon capillary tubing with 0.03 in/0.75 mm ID, FIALab P/N 270040
- Reaction coil 1: 50 μ L - 4.0in/10cm of Teflon capillary tubing with 0.03 in/0.75 mm ID, FIALab P/N 270040
- Reaction coil 2: 750 μ L - 65.0in/165cm of Teflon capillary tubing with 0.03 in/0.75 mm ID, FIALab P/N 270040
- SMA-Z flow cell: light path -100mm
- Flow rate: 60% pump speed (approx. 2.0 mL/min flow rate per pump channel)
- Bridge: 40 μ L – 3.5in/9cm of Teflon capillary tubing with 0.03 in/0.75 mm ID, FIALab P/N 270040

Recommended spectrometer parameters:

Primary wavelength: 540nm

Second wavelength: 580nm

Third wavelength: 595nm

Fourth wavelength: 600nm

Reference wavelength: 650nm

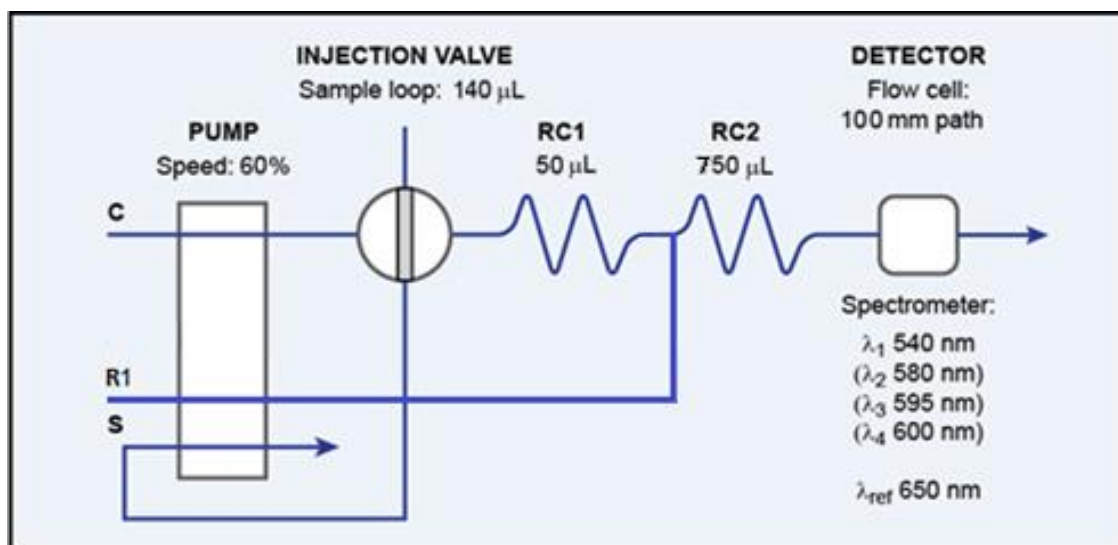


Fig. 1- Flow schematic for low-range nitrite determination on a FIALab-2500

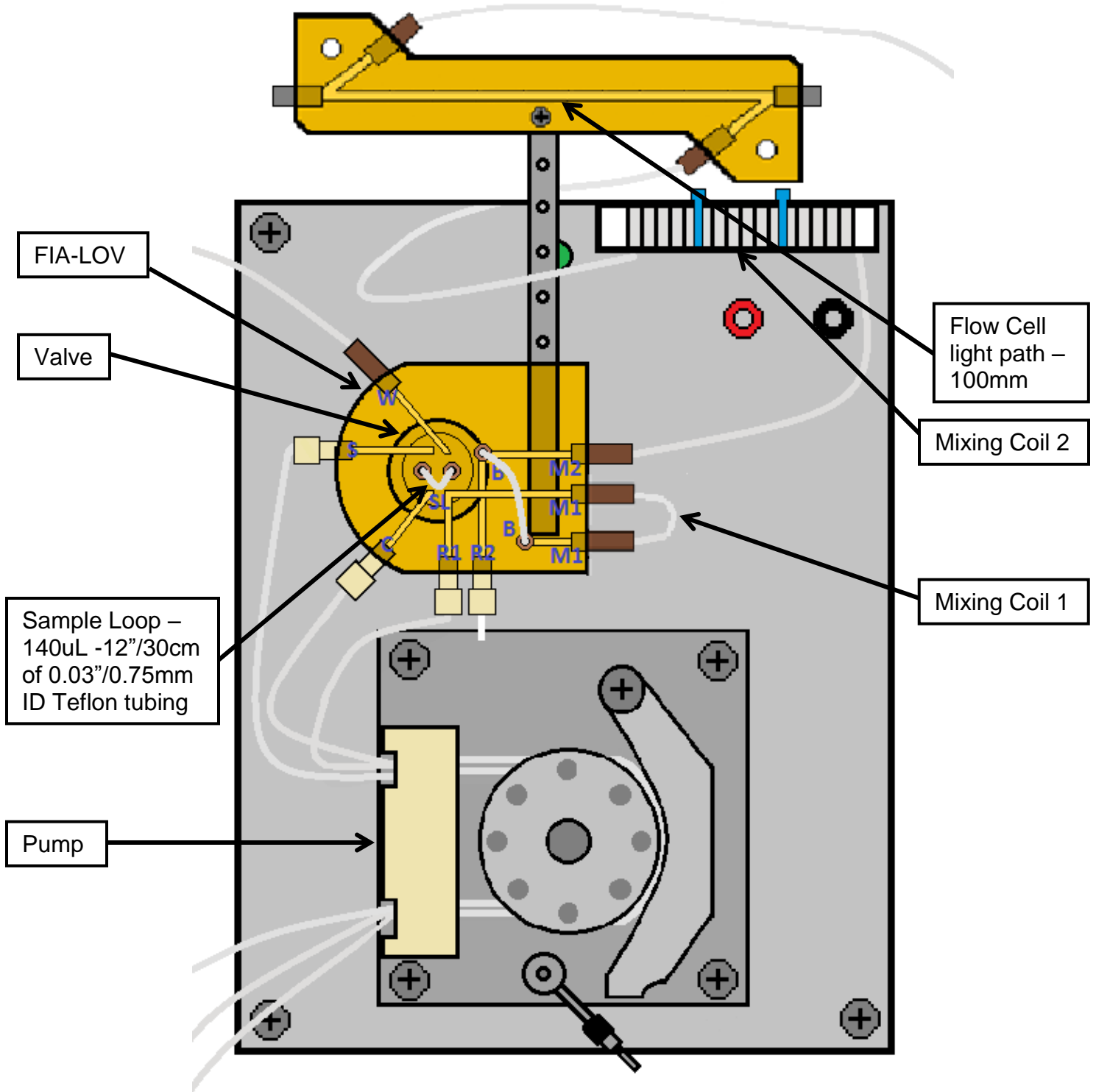


Fig. 2- Pictorial representation of low-range nitrite setup on a FIAlab-2500

The program script using an autosampler is shown below. For manual sample introduction see the FIAlab-2500 software manual.

'Nitrate/Nitrite 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) 540
Hardware Settings Wavelength 2 (nm) 580
Hardware Settings Wavelength 3 (nm) 595
Hardware Settings Wavelength 4 (nm) 600
Reference Wavelength1 650
```

```
'Set delay time, start pump to prime lines
Valve Delay 5000      ' sample inject (msec)
Peristaltic Pump Clockwise(%) 60
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) 30
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

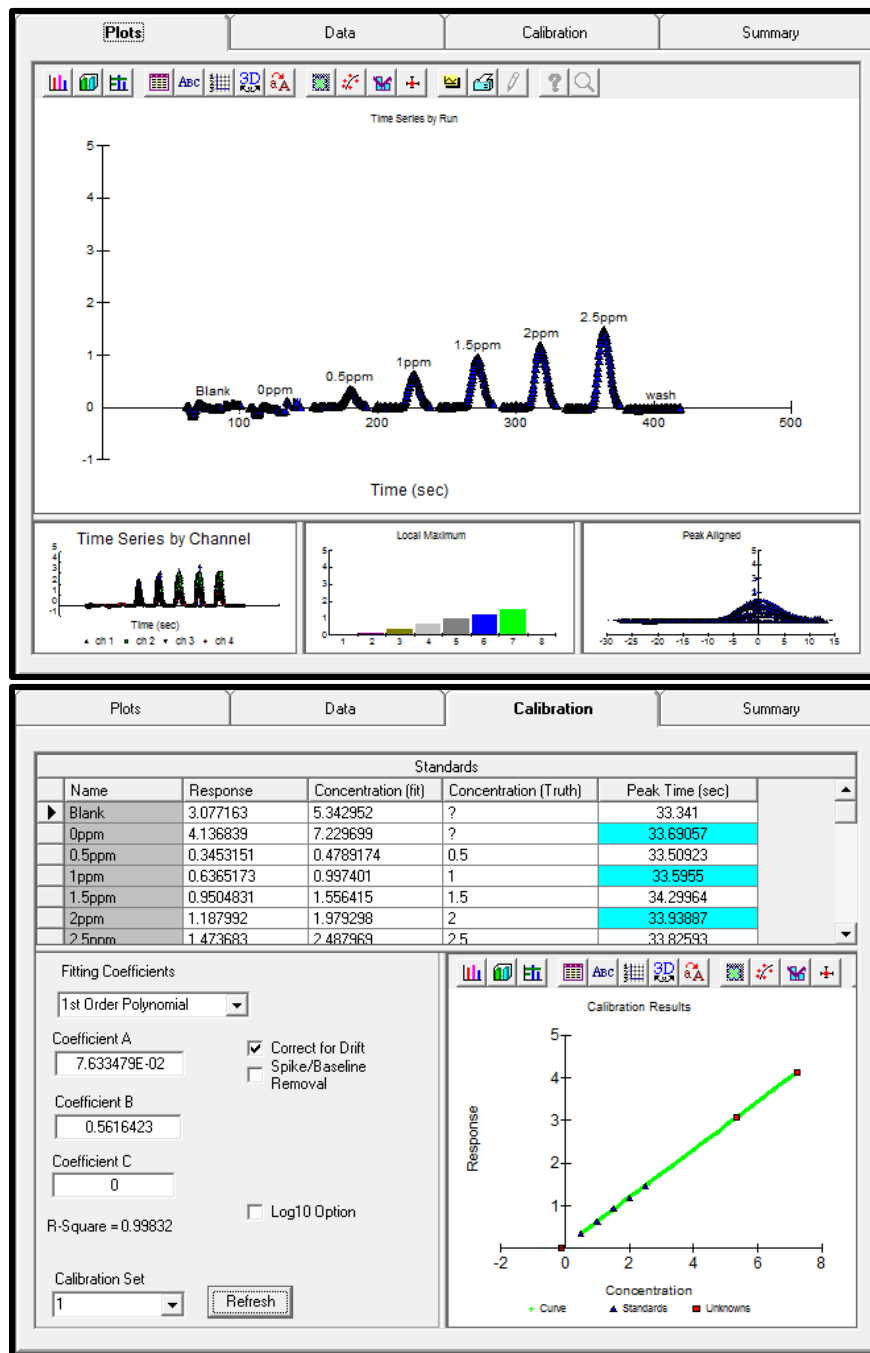


Fig. 3 – Example plot and calibration data for low-range nitrite at 600nm

Lower limit of detection: 0.002mg/L – 1.4×10^{-7} molN/L
 Upper limit of detection: 2.5mg/L – 1.8×10^{-4} molN/L
 Sample throughput: 100 samples/hr
 Startup: 5 minutes
 Shutdown: 5 minutes

FIAlab[®]



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