Trilogy™ Laboratory Fluorometer

**Application Note:**
**Nitrate/Nitrite Reagents and Test Procedures Adapted from Strickland and Parsons (1968)**

## Reagents Required

| | |
|---|---|
| **Ammonium Chloride** | **[$NH_4CL$]** |
| Sulphanilamide | [$C_6H_8N_2O_2S$] |
| Hydrochloric Acid | [HCL] |
| N-(1-Naphthyl)-Ethylenediamine Dihydrochloride | [$C_{12}H_{14}N_2 \cdot 2HCL$] |
| Cadmium (granular) | [Cd] |
| Copper Sulfate Pentahydrate | [$CuSO_4 \cdot 5H_2O$] |
| Potassium Nitrate | [$KNO_3$] |

## Reagent Preparation

### Procedures:

***Concentrated Ammonium Chloride***
Dissolve 125g of analytical reagent grade ammonium chloride in 500ml of distilled water. Store solution in glass or plastic bottle.

***Dilute Ammonium Chloride***
Dilute 50ml of concentrated ammonium chloride solution to 2000ml with distilled water. Store in glass or plastic bottle.

***Copper Cadmium Column***
Materials required may be purchased at Fisher Scientific:

- Glass cadmium reduction columns (unpacked)
- Cadmium granules that will pass a 2-mm sieve opening but be retained by a 0.5-mm opening
- 2% weight/volume solution of copper sulfate
- Copper wool

### Packing Procedure:

1. Stir 100g of cadmium granules in 500ml of copper sulfate solution until blue color disappears.
2. Place a small plug of copper wool in the bottom of the reduction column and fill the column with dilute ammonium chloride solution making sure no air bubbles are present in the column.
3. Gently pipette the cadmium-copper granules into the column to a height of 30cm.
   ***(Note: Granules should always be covered with dilute ammonium chloride so they will not dry out.)***
4. Adjust the flow rate of the column to 8-12 ml per minute. Flow rate can be adjusted by tapping the side of the column while washing with dilute ammonium chloride. If the flow rate is lower than this, the column needs to be repacked.
5. When optimal flow rate has been reached add a small plug of copper wool to the top of the column.

### Column Maintenance and Repacking:

1. If column's reduction efficiency has decreased, remove granules from the column.
2. Wash the granules with 5% weight/volume hydrochloric acid.
3. Then wash with distilled water until pH of the decanted solution is >5.
4. The granules can then be reactivated with copper sulfate and used to repack the column (see packing procedure).

***Sulfanilamide solution***
Dissolve 5g of sulfanilamide in a mixture of 50ml of concentrated hydrochloric acid and about 300ml of distilled water. Dilute to 500ml with water. This solution is stable for many months.

***N-(1-naphthyl)-ethylenediamine dihydrochloride solution***
Dissolve 0.5g of the dihyrochloride in 500ml distilled water. Store the solution in a dark bottle. The solution is stable for 1 month, unless strong brown coloration develops.

## Experimental Procedure (Strickland and Parsons 1968)

### *Reactive Nitrate ($NO_3^-$)*

**Blank Procedure:**

1. Insert the Nitrate/Nitrite Module (P/N: 7200-074) into the Trilogy unit (P/N: 7200-000).
2. Turn on the Trilogy unit using the switch on the back panel.
3. Choose "Absorbance" from the application menu.
4. Verify the module inserted is the Nitrate/Nitrite Absorbance Module.
5. Make sure the blank sample is at room temperature.
6. Place a collection flask under the collection tube of the column.
7. Add 2 ml of concentrated ammonium chloride to 100 ml of the blank sample in a 125 ml Erlenmeyer flask. Mix the solution and pour about 5 ml onto the top of the column and allow it to pass through.
8. Add the remainder of the blank sample to the column. Collect about 40 ml and discard; then collect 50 ml in a graduated cylinder and dispense into the Erlenmeyer flask that contained the original blank sample. Allow the flow to cease before adding the next sample.
9. Using a pipette, add 1ml of sulfanilamide solution to the 50 ml collected blank sample, mix and allow the reaction to occur for more than 2 minutes but not exceeding 8 minutes.
10. Then add 1ml of naphthylethylenediamine solution to the blank sample and mix immediately. Wait at least 10 minutes but not more than 2 hours.
11. Pipette 3 ml of the processed blank sample into a cuvette.
12. Put the cuvette into the module, close lid.
13. Press the "Calibrate" button and wait till blanking is complete. Once blanking is complete all subsequent samples or standards that are analyzed using the Trilogy with Nitrate/Nitrite Module will be blank corrected.

**Sample Procedure: (Note: make sure Trilogy unit has been blanked before proceeding)**

1. For sample analysis, follow steps 5-12 from Reactive Nitrate Blank Procedure using collected samples in place of blank sample.
2. Press the "Measure Absorbance" button to measure sample absorbance.
3. Record absorbance value.
4. Use the following equation to calculate nitrate concentration in samples:

*Concentration of ($NO_3^-$) uM = [(m x + b) – 0.95 C]*

Where *m* and b are the slope and y-intercept, respectively, taken from the equation of regression curve, which is performed during the Trilogy unit's calibration (See Calibration Procedure). *x* is the absorbance reading of the sample, *C* is the concentration of nitrite as determined by the Reactive Nitrite Procedure, and *0.95* accounts for the nitrite retained by the column during sample reduction.

**Calibration Procedure:**

1. Purchase nitrate stock standard from Ricca Chemical Company (Cat. #: 5457-16).
2. Dilute stock standard to working standard concentration(s) in your working range using distilled water or artificial seawater.
3. Make at least 3 calibration points throughout your working range to increase accuracy of calculated concentrations. [Example, if working range = 15uM-35uM; choose at least 3 points spanning that range (i.e. 20, 25, 30 uM) and dilute stock standard to those working standard concentrations.]
4. Follow steps 1-3 from Sample Procedure using working standards.
5. Plot the concentrations of standards (uM) vs. absorbance values of the standards.
6. The equation from the linear regression through these points can be used to calculate nitrate standard concentrations:

*Concentration of ($NO_3^-$) uM = (m x + b)*

Where *m* is the slope of the regression, *b* is the y-intercept, *x* is the absorbance reading of the sample.

## Reactive Nitrite ($NO_2^-$)

### Blank Procedure:

1. Insert the Nitrate/Nitrite Module (P/N: 7200-074) into the Trilogy (P/N: 7200-000).
2. Turn on the Trilogy unit using the switch on the back panel.
3. Choose "Absorbance" from the application menu.
4. Verify the module inserted is the Nitrate/Nitrite Absorbance Module.
5. Make sure the blank sample is at room temperature.
6. Using a pipette, add 1ml of sulfanilamide solution to 50 ml of blank sample, mix and allow the reaction to occur for more than 2 minutes but not exceeding 8 minutes.
7. Then add 1ml of naphthylethylenediamine solution to the blank sample and mix immediately. Wait at least 10 minutes but not more than 2 hours.
8. Pipette 3 ml of the processed blank sample into a cuvette.
9. Put the cuvette into the module, close lid.
10. Press the "Calibrate" button and wait till blanking is complete. Once blanking is complete all subsequent samples or standards that are analyzed using the Trilogy with Nitrate/Nitrite Module will be blank corrected.

### Sample Procedure: (Note: make sure Trilogy unit has been blanked before proceeding)

1. For sample analysis, follow steps 5-9 from Reactive Nitrite Blank Procedure using collected samples in place of blank sample.
2. Press the "Measure Absorbance" button to measure sample absorbance.
3. Record absorbance value.
4. Calculate the nitrate concentration in samples using the following equation:

*Concentration of ($NO_2^-$) uM = (m x + b)*

Where *m* and *b* are the slope and y-intercept, respectively, taken from the equation of regression curve, which is performed during the Trilogy unit's calibration (See Calibration Procedure),
*x* is the absorbance reading of the sample.

## Calibration Procedure:

1. Sodium Nitrite (Cryst./Certified ACS) may be purchased from Fisher Scientific.
2. Dissolve 0.345g of dried sodium nitrite in 1000ml of distilled water. This solution should be stored in a dark bottle with 1ml of chloroform as a preservative. This solution is stable for several months.

   *Concentration of ($NO_2^-$) stock standard = 5000 uM*

3. Dilute stock standard to working standard concentration(s) in your working range using distilled water or artificial seawater.
4. Make at least 3 calibration points throughout your working range to increase accuracy of calculated concentrations. [Example, if working range = 5uM-25uM; choose at least 3 points spanning that range (i.e. 10, 15, 20 uM) and dilute stock standard to those working standard concentrations.]
5. Follow steps 1-3 from Sample Procedure using working standards.
6. Plot the concentration of standard (uM) vs. absorbance value of standard.
7. The equation from the linear regression through these points can be used to calculate nitrite concentrations:

*Concentration of ($NO_2^-$) uM = (m x + b)*

Where *m* is the slope of the regression,
*x* is the absorbance reading of the sample,
*b* is the y-intercept.

## References

J. D. H. Strickland and T. R. Parsons, 1972. A practical handbook of seawater analysis. Second Edition, Bulletin 167. Fisheries Research Board of Canada, Ottawa

Timothy R. Parsons, Maita Y. and Lalli C. M., 1984. A manual of chemical and biological methods for seawater analysis. First Edition. Pergamon Press Ltd., Great Britain

Standard Methods for the Examination of Water and Wastewater, 18th edition 1992. American Public Health Association, American Water Works Association, Water Environment Federation. Method 4500-E. ISBN 0-87553-207.