Bermuda Biological Station For Research, Inc.
Bermuda Atlantic Time-series Study

Chapter 9. The Determination of Nitrate in Sea Water

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Modified from: Strickland and Parsons (1968)

1.0 Scope and field of application

This procedure describes a method for the determination of reactive nitrate in seawater, suitable for the assay of concentrations between 0.05 μmol l^{-1} to 45 μmol l^{-1}. This method is a modification of Strickland and Parsons (1968).

2.0 Definition

The concentration of reactive nitrate is given in μmol kg^{-1} in seawater.

3.0 Principle of Analysis

The determination of nitrate is based on the method of Morris and Riley (1963) and modified by Strickland and Parsons (1968). Nitrate is reduced to nitrite using a cadmium-copper column. The nitrite produced reacts with sulfanilamide in an acid solution. The resulting diazonium compound is coupled with N-(1-Naphthyl)-ethylenediamine dihydrochloride to form a colored azo dye, the extinction of which can be measured spectrophotometrically. A correction must be made for any nitrite initially present in the sample.

The following stoichiometric equations apply.

3.1 Nitrate is reduced using a copper-cadmium column:

$$NO_3^- + Me_{(s)} + 2H^+ \rightarrow NO_2^- + Me^{2+} + H_2O$$

3.2 NO_3^- can easily be reduced further to NO due to the similar electromotive forces (E_o) of the reactions:

$$NO_3^- + 3H^+ + 2e^- \rightarrow HNO_2 + H_2O \ (E_o = 0.94\ V)$$

$$NO_3^- + 4H^+ + 3e^- \rightarrow NO + 2H_2O \ (E_o = 0.97 \text{ V})$$

3.3 To ensure that this does not occur, the reaction takes place in a neutral or slightly alkaline solution.

$$NO_3^- + H_2O + 2e^- \rightarrow NO_2^- + 2OH^- \ (E_o = 0.015 \text{ V})$$

3.4 Ammonium chloride in the sample stream acts as both a complexant and as a buffer.

$$2NH_4^+ \leftrightarrow 2NH_3 + 2H^+$$

$$Cd^{2+} + 2NH_3 \rightarrow [Cd(NH_3)_2]$$

4.0 Apparatus

Spectrophotometer

5.0 Reagents

5.1 *Concentrated ammonium chloride solution*: 125 g of reagent grade ammonium chloride (NH_4Cl) dissolved in 500 ml of Milli-Q water. This solution may be stored in a glass or plastic bottle.

5.2 *Dilute ammonium chloride solution*: 50 ml of the concentrated ammonium chloride (NH_4Cl) solution diluted to 2000 ml with Milli-Q water. This solution may be stored in a glass or plastic bottle.

5.3 *Sulfanilamide solution*: 5 g of sulfanilamide dissolved initially in a mixture of 50 ml of concentrated hydrochloric acid and about 300 ml Milli-Q water, then diluted to 500 ml with Milli-Q water. This solution is stable for many months.

5.4 *N-(1-Naphthyl) - ethylenediamine dihydrochloride solution*: 0.50 g of the dihydrochloride dissolved in 500 ml Milli-Q water. This solution is stored in a dark bottle and renewed monthly, or sooner if a brown coloration develops.

5.5 *Copper sulfate stock solution*: 20 g cupric sulfate pentahydrate, $CuSO_4 \cdot 5H_2O$ dissolved in 1 liter of Milli-Q water (2% w/v). This is stable at room temperature.

6.0 Preparation for sampling

6.1 Samples for analysis of both nitrate and nitrite are collected in 250 ml polyethylene bottles. Contamination is a major problem with nutrient samples, especially near the surface where the ambient concentrations are low. All the nutrient bottles are rigorously cleaned before use. New bottles are soaked for 2-3 days in 5 % Aquet and tap water, rinsed with tap water, then soaked for 2 -3 days in 10 % HCl. Bottles are then soaked overnight in Milli-Q water and rinsed 5 -6 times with Milli-Q water. After bottles have been seasoned they are cleaned between uses by soaking overnight in 5 % detergent, transferred to 10 % HCl overnight, and rinsed 5 -6 times with Milli-Q water.

6.2 Polycarbonate filter holders (Gelman) are used in the filtering of samples. Cleaning of these begins with an overnight soak in Aquet, followed by tap water rinsing, a soak in 5 % HCl for 1-2 hours, and 5-6 rinses with Milli-Q water.

7.0 Sampling

7.1 Samples are collected at 35 depths between the surface and 4200 m. A polycarbonate filter holder (Gelman) containing a 0.8 µm Nuclepore filter is connected to the OTE bottle. The spigot is opened and samples are collected from the water that filters. Each bottle is rinsed three times and then filled to just below the shoulder. Care is taken to avoid overfilling of samples. The samples are transferred to a freezer (-20°C) and kept frozen until analysis.

7.2 Prolonged storage of samples is avoided.

8.0 Procedures

8.1 *Cadmium - copper column material*: 100 g of acid-washed cadmium filings are stirred with 500 ml of a 2% (w/v) solution of copper sulphate pentahydrate, $CuSO_4 \cdot 5H_2O$, until all blue coloring has left the solution and copper particles enter the supernatant. This material is then used to pack the reduction columns, utilizing a small plug of copper "wool" at each end of the column. About 50 g of cadmium filings are required for a column of about 30 cm long. Columns should have a flow rate of about 10 ml min^{-1}. The columns are washed with dilute ammonium chloride solution and the column material completely covered by dilute ammonium chloride solution when not in use.

8.2 *Sample analysis*

8.2.1 Samples are thawed prior to analysis and should be at a temperature between 15°C and 30°C. Once thawed, analysis should proceed as soon as possible.

8.2.2 1.0 ml of concentrated ammonium chloride solution is added to 100 ± 2 ml of sample in a 125 ml Erlenmeyer flask, and the solution mixed

8.2.3 Approximately 5 ml of this solution is poured onto the top of the column and allowed to pass through.

8.2.4 The remainder of the sample is added to the column and the effluent collected. The first 40 ml or so of effluent is used to rinse the Erlenmeyer flask and a 50 ml graduated cylinder. A further 50 ml of effluent is collected in the graduated cylinder and poured into the flask. Remaining sample is allowed to drain out through the column.

8.2.5 There is no need to wash the column in between samples, but if the column is not to be used for over an hour, 50 ml of dilute ammonium chloride should be run through the system. This aids in extending the life of the column.

8.2.6 As soon as possible after the reduction, 1.0 ml of sulfanilamide solution is added to the sample in the flask, mixed and allowed to react for between 2 and 8 minutes.

8.2.7 1.0 ml of N-(1-Naphthyl)-ethylenediamine dihydrochloride solution is added to the flask and mixed.

8.2.8 The extinction of samples at 543 nm is measured between 10 minutes and 2 hours after the addition of the naphthylethylenediamine reagent. Absorbances of less than 0.1 in a 1 cm cell are re-read in a 10 cm cell.

Reagent Blank Determination: A reagent blank is barely significant when working with a 1 cm cell but gains considerable importance when a 10 cm cell is used. In either case it is checked throughout each analysis. The reagent blank is determined using Milli-Q water as sample and following the procedure outlined in section 8.2. The concentrated ammonium chloride solution is added to 100 ml of Milli-Q water in a clean Erlenmeyer flask and the column used is flushed with at least 50 ml dilute ammonium chloride solution just prior to use. The absorbance of the blank should not exceed 0.1 using a 10 cm cell.

8.3 Standardization

8.3.1 *Primary nitrate standard*:

1.01 g of analytical reagent quality potassium nitrate dissolved in 1000 ml of Milli-Q water; 1 ml = 10 µmol N.

8.3.2 *Working nitrate standard*:

4 ml of primary nitrate standard diluted to 2000 ml with low nutrient seawater (20 µM). A fresh standard is prepared in a dark bottle each day as needed.

Approximately 100 ml of working standard solution is run as described in Section 8.2. Initially, this is performed in triplicate for each column. Thereafter, standards are run with each batch of samples to check the efficiency of the reduction columns.

9.0 Calculation and expression of results

9.1 A standardization factor F can be calculated as:

$$F = \frac{20 \mu mol\ kg^{-1}}{E_s - E_b}$$

Where:

20 µmol kg^{-1}	=	concentration of the standard
E_s	=	mean absorbance of the standards
E_b	=	mean absorbance of the blanks

9.2 The nitrate concentration is calculated by:

$$\mu M\ NO_3 = \text{corrected absorbance} \bullet F - 0.95C$$

Where:

F	=	standardization factor
C	=	concentration of nitrite present in the sample

corrected absorbance = sample absorbance - reagent blank

With good columns, 5% of the nitrite is reduced leading to a correction of 0.95 times the nitrite concentration of the sample is made.

9.3 The units of µmoles kg^{-1} can be obtained by dividing the calculated nitrate concentration by the density of the seawater at the time of analysis.

10.0 Notes

10.1 *The cadmium-copper column*: The column deactivates through continual use. The addition of the ammonium chloride should slow this process. A well-packed column should be capable of reducing at least 100 samples.

10.2 Cadmium that has become inefficient at reduction may be regenerated by washing with 5% (v/v) hydrochloric acid (300 ml for the cadmium from four columns) and rinsing with 300 ml portions of Milli-Q water until the pH of the wash is greater than 5. The cadmium is then re-treated with the copper sulphate solution and re-packed.

10.3 Columns should be stored completely covered in dilute ammonium chloride.

11.0 References

Morris and Riley. (1963). *Analytica Chimica Acta*, **29**, 272–279.

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