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

Chapter 10. The Determination of Nitrite 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 nitrite in seawater. This method is suitable for the assay of oceanic concentrations between 0.01 μmol l^{-1} to 2.5 μmol l^{-1}. This method is a modification of Strickland and Parsons (1968).

2.0 Definition

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

3.0 Principle of Analysis

The determination of nitrite is based on the method of Strickland and Parsons (1968). Nitrite reacts with sulfanilamide in an acid solution resulting in a diazonium compound. This is then coupled with N-(1-Naphthyl)-ethylenediamine dihydrochloride to form a colored azo dye, the extinction of which can be measured spectrophotometrically.

4.0 Apparatus

Spectrophotometer

5.0 Reagents

5.1 *Sulfanilamide solution*: 5 g reagent grade sulfanilamide dissolved in a mixture of 50 ml concentrated hydrochloric acid and 300 ml Milli-Q water. This solution is then diluted to 500 ml with Milli-Q water and stored in a glass bottle. It is stable for many months.

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

6.0 Preparation for sampling

6.1 Samples are collected in 250 ml polyethylene bottles for analysis of nitrate and nitrite. 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 filtered water. 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 analysed.

7.2 Prolonged storage of samples is avoided.

8.0 Procedures

8.1 *Sample analysis*

8.1.1 Samples should be thawed and at a temperature between 15°C and 30°C for analysis. Once thawed, analysis should proceed as soon as possible.

8.1.2 The 125 ml Erlenmeyer flasks and 50 ml measuring cylinder to be used in this analysis should be rinsed twice with the sample seawater and shaken dry.

8.1.3 50 ml of the sample is measured into a 125 ml Erlenmeyer flask.

8.1.4 1.0 ml of the sulfanilamide solution is added to each flask, mixed and allowed to react for 2 - 8 minutes.

8.1.5 1.0 ml of the N-(1-Naphthyl) - ethylenediamine dihydrochloride solution is added and mixed immediately.

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

8.2 Reagent blank determination

8.2.1 The reagent blank is determined using Milli-Q water as sample instead of seawater, following the procedure outlined in Section 8.1. This should be done in duplicate. A reagent blank should not exceed 0.03 and should be determined for each batch of samples.

8.3 *Standardization*

8.3.1 *Primary nitrite standard*: 0.345 g dried anhydrous reagent grade sodium nitrite dissolved in 1000 ml Milli-Q water. 1 ml = 5 µmol. This solution is stored in a dark bottle with 1 ml of chloroform as a preservative and is stable for 1-2 months.

8.3.2 *Working nitrite standard*: 10.0 ml of the primary standard solution diluted to 1000 ml with Milli-Q water (1 ml = 0.05 µmol).

8.3.3 *Standard solutions*: Four standard solutions are prepared by diluting 2.0 ml of working nitrite standard up to 50 ml in Milli-Q water. Nitrite determinations of each standard are carried out as described above in Section 8.1.

9.0 Calculation and expression of results

9.1 A standardization factor F can be calculated as:

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

Where:

20 µmol/kg	=	concentration of the standard
E_s	=	mean absorbance of the standards
E_b	=	mean absorbance of the blanks

9.2 The nitrite concentration is calculated by:

$$\mu M\ NO_2 = \text{corrected absorbance} \bullet F$$

Where:

F = standardization factor

corrected absorbance = sample absorbance - reagent blank

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

10.0 References

Strickland, J.D.H., and Parsons, T.R. (1968). Determination of reactive nitrite. In: *A Practical Handbook of Seawater Analysis*. Fisheries Research Board of Canada, Bulletin **167**, 71–75.