Chapter 8. The Determination of Nitrite, Nitrate + Nitrite, Orthophosphate and Reactive Silicate in Sea Water using Continuous Flow Analysis

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1.0   Scope and field of application

This procedure describes the methods for the determination of nitrite, nitrate + nitrite, phosphate and reactive silicate in sea water using Continuous Flow Analysis (CFA). These methods and manifold configurations are suitable for assays of oceanic nutrient levels (e.g. LOD of 0.005 μmol kg^{-1} for nitrite, 0.03 μmol kg^{-1} for nitrate + nitrite, 0.01 μmol kg^{-1} for phosphate and 0.2 μmol kg^{-1} for reactive silicate). Detection ranges are a function of colorimeter calibration settings and sample:dilution volumes used. Therefore, modification of the following methods will allow for expansion or reduction of the detection ranges. Linearity checks should be performed for all methods.

2.0   Definition

The nutrient concentrations of sea water are defined as:

$$NO_2^-, NO_3^-, HPO_4^{2-}, Si(OH)_4 = \mu mol\ kg^{-1}\ in\ seawater$$
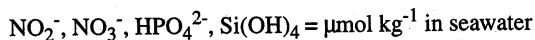
3.0   Principle of Analysis

3.1   Continuous Flow Analysis is performed on the Technicon AutoAnalyzer II or equivalent system. By utilizing a continuous mechanically driven stream of samples and reagents, the AutoAnalyzer automates colorimetric analysis, reduces technician error, and allows samples and standards to be treated exactly alike. As the air segmented stream flows through a system of glass coils, the sample-reagent mixture reacts colorimetrically for photometric analysis downstream.

3.2   The photometric determination of nitrite and nitrate + nitrite in sea water is based on the reaction of nitrite with an aromatic amine (i.e. sulfanilamide) which results in the formation of a diazonium compound. This is then coupled with a second aromatic amine (i.e. NEDA) to yield a red azo dye. Determination of nitrate + nitrite is accom-

plished with the same procedure as nitrite after the nitrate is reduced to nitrite by passing the sample stream through a column of copperized cadmium.

3.3 The photometric determination of phosphate in sea water follows the Technicon Industrial Method No. 155-71WEPA, employing ascorbic acid as the reductant. Ammonium molybdate reacts with phosphate to give phosphomolybdic acid, which is in turn reduced to phosphomolybdous acid, yielding a blue color.

3.4 The photometric determination of reactive silicate is based on the formation of a silicomolybdenum blue complex. The sample is reacted with ammonium molybdate in a dilute acid forming silicomolybdic acid which is then reduced to silicomolybdous acid. Stannous chloride is used as the reductant. This method is non-linear at high silicate concentrations and its color yield is sensitive to environmental temperature fluctuations. Reaction coils should be insulated from drafts and the temperature at the reaction coils monitored for change throughout a run.

4.0 Apparatus

Continuous Flow Analysis is performed on the Technicon AutoAnalyzer II or equivalent system. Figures 4 and 5 illustrate the Flow Diagrams (manifolds) for the individual chemistries.

5.0 Reagents

Unless stated otherwise, reagents are stored at room temperature in dark airtight containers and never kept for longer than 1 month.

5.1 Nitrite, Nitrate + Nitrite:

5.1.1 *Ammonium Chloride* (Nitrite+Nitrate only): 85.0 g ammonium chloride dissolved in Milli-Q water to a total volume of 1000 ml (8.5% w/v).

5.1.2 *Sulfanilamide*: 2.5 g sulfanilamide dissolved in 1.2 N HCl to a total volume of 250 ml (1% w/v).

5.1.3 *N-Naphthylethylene-diamine dihydrochloride*: 0.25 g NEDA dissolved in Milli-Q water to a total volume of 250 ml (0.1% w/v).

5.1.4 *Cadmium Column* (nitrate + nitrite only):

5.1.4.1 Cadmium filings are washed several times with 1.2 N HCl followed by several Milli-Q water rinses. Filings are then treated with suc-

cessive rinses of 75-100 ml of 2% w/v $CuSO_4 \cdot 5H_2O$, each time allowing the blue color of the solution to disappear before decanting and adding fresh solution. Rinses are repeated until the solution remains blue.

5.1.4.2   The copper-plated filings are washed with Milli-Q water several times to remove all colloidal copper, until the water above the filings is clear (15-20 rinses are often necessary).

5.1.4.3   Filings are then covered with dilute ammonium chloride (~5 % NH_4Cl). They should be kept covered at all times to avoid any further exposure of the Cd-Cu filings to air.

5.1.4.4   A small plug of copper thread is inserted at one end of the reduction column. A 50 ml syringe containing dilute ammonium chloride is attached to this end of the column and the column is filled with dilute NH_4Cl to prevent trapping of air bubbles when it is loaded. A small funnel is attached to the end opposite the syringe in order to facilitate loading of the column with the Cd-Cu filings. About 50 g of cadmium filings are required to pack a column. Care is taken on transferring the Cd-Cu filings to the column to avoid extended exposure to air. Small sequential additions of filings are made while drawing on the syringe and tapping the column down. This ensures that the column is evenly and compactly packed. Another small plug of copper thread is inserted when the column is full.

5.1.4.5   The column is attached to the two-way valve (see manifold configuration, Figure 4). The column is conditioned by pumping through the NH_4Cl reagent and approximately 50 ml of 100 μM nitrate standard, followed by at least 10 minutes flushing with Milli-Q water. New columns can show an initial rapid increase in reduction efficiency, so additional conditioning may be necessary where high nitrate standards are passed through until a constant efficiency is obtained.

5.1.4.6   Columns are stored containing dilute ammonium chloride (~5%). Columns remain effective for hundreds of samples, but are checked for efficiency before and after each run with nitrite and nitrate standards. If air bubbles enter the column the Cd-Cu fillings are removed and the column repacked, as air will lead to rapid oxidation of the copperized cadmium and subsequent nitrate reduction efficiency loss.

5.2 Phosphate:

5.2.1 *Ammonium Molybdate Stock:* 40 g ammonium molybdate dissolved in Milli-Q water to a total volume of 1000 ml. This reagent is stable for several months if stored in the dark.

5.2.2 *Antimony Potassium Tartrate Stock*: 3.0 g antimony potassium tartrate dissolved in Milli-Q water to a total volume of 1000 ml. This reagent is stable.

5.2.3 *Ascorbic Acid*: 3.6 g ascorbic acid dissolved in Milli-Q water to a total volume of 200 ml. This solution is made up fresh each day.

5.2.4 *Sodium Lauryl Sulfate* (SLS): 3.0 g SLS dissolved in 100 ml Milli-Q water. SLS serves as a surfactant and can be omitted, though this is not recommended.

5.2.5 *Working Reagent A*: a combination of 150 ml sulfuric acid (4.9N), 45 ml ammonium molybdate stock, 15 ml antimony potassium tartrate stock and 6.0 ml SLS. New working reagent is made up daily.

5.2.6 *Working Reagent B*: 200 ml ascorbic acid with 1.0 ml SLS. This is made up each day.

5.3 Reactive Silicate:

5.3.1 *Ammonium Molybdate Stock*: 25 g ammonium molybdate dissolved in Milli-Q water to a total volume of 500 ml. It is stored in a dark bottle in the refrigerator.

5.3.2 *Ammonium Molybdate Working Solution*: 100 ml ammonium molybdate stock diluted to 250 ml with 10% v/v HCl. Note: a precipitate forms but redissolves.

5.3.3 *Tartaric Acid*: 25 g tartaric acid diluted to a total volume of 250 ml in Milli-Q water.

5.3.4 *Stannous Chloride Stock*: 40 g stannous chloride dissolved in 5 N HCl to a total volume of 100 ml. This stock solution is stored in the freezer.

5.3.5 *Stannous Chloride Working*: 5 ml of stannous chloride stock solution diluted to 200 ml with 1.2 N HCl. This reagent is very unstable and is replaced daily.

6.0   Preparation for sampling

6.1   Samples are collected in 60 ml amber bottles (Nalgene® HDPE). 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, then 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 filter holder containing a 0.8 μm Nuclepore filter is connected to the OTE bottle. The spigot is opened and two sets of samples (primary and back-up samples) are collected from the water as it filters. Each bottle is rinsed three times and then filled to just below the shoulder. Care is taken to avoid overfilling of samples. Both sample sets are transferred to a freezer (-20°C) and kept frozen until analysis. Five additional samples are collected from one of the two 3000 m and from the 4000 m OTE bottles and frozen. These deep water replicate samples are analyzed during the primary runs to provide additional data points for nutrient concentration variability analysis.

8.0   Procedures

8.1   Ideally, samples are run within a week of collection. Nutrient analysis is carried out on two separate runs (high and low colorimeter sensitivity settings). Each run has a corresponding set of standard calibration settings in order to maximize sensitivities within each range of expected values.

8.2   Sample are thawed in a warm water bath for 10 - 15 minutes, then allowed to equilibrate to room temperature (between 18 and 25°C) and shaken vigorously.

8.3   The colorimeters are allowed to warm up for at least 30 minutes while running Milli-Q water through the system and are set at the appropriate standard calibration setting for the run.

8.4 Once a stable baseline is established the sample stream is changed to Low Nutrient Sea Water (LNSW) until a new level baseline develops (about 5 min) and then returned to Milli-Q water. This is repeated three times or until two satisfactory baselines are obtained for the two sample streams. The difference between the two baselines constitutes the Refractive Index (RI), which will be used in calculating the blank. The RI test is finished with a baseline of Milli-Q water.

8.5 The ammonium chloride reagent is added to the sample stream, and after 5 minutes the CdCu column is opened.

8.6 Remaining reagents are added to the sample streams. For silicate analysis, ammonium molybdate is added last, after other reagents have been running for 5 minutes or more. Likewise, ammonium molybdate is removed first when shutting down silicate runs. Failure to do so results in the coating of the flow cell with a dark blue precipitate and requires scrubbing with a dilute NaOH solution.

8.7 Once all of the reagents have been added and running for 10 minutes with a good Milli-Q water baseline, the RI test is repeated by alternating between Milli-Q water and LNSW. The difference between the two constitutes both the RI and nutrient concentration of the LNSW (assuming the Milli-Q water is zero) which will be used in calculating the blank. This test is completed with a baseline of LNSW. Seawater blank determination is only necessary for silicate analysis, as nitrate, nitrite and phosphate concentrations in the seawater blank (0.2 µm filtered Sargasso surface seawater) are close to zero.

8.8 The Cd column efficiency is checked by running $10\mu m\ NO_3^-$ and $10\mu mNO_2^-$ standards at the beginning and end of each run. The values obtained for the two standards are compared, if the efficiency falls below 90% the Cd column must be replaced. Nitrate + nitrite values are corrected for Cd column efficiency.

8.9 The autosampler is loaded with the set of standards appropriate for the run type followed by samples. Standards and LNSW baseline checks are re-run after every 15-20 samples. Samples are processed at a rate of 20 per hour.

8.10 The run is completed with a long LNSW baseline, followed by a switch to Milli-Q water. At this point the colorimeters are turned off.

8.11 All of the reagents are removed except ammonium chloride. With silicate runs, ammonium molybdate is the first reagent removed.

8.12 The CdCu column is closed after 10 minutes, and then the ammonium chloride reagent is removed.

8.13 The system is washed by running Milli-Q water for 10 minutes, followed by a 5% NaOH solution for 10 minutes, another 10 minutes of Milli-Q water, a 10% HCl wash for 10 minutes and a final rinse with Milli-Q water for at least 10 minutes.

9.0 Standardization

9.1 Working standards are prepared in aged, filtered (0.2 μm) LNSW. Standards are prepared using Gilson Pipetman pipettes that are regularly calibrated. Standards for reactive silicate are prepared in Nalgene polypropylene volumetric flasks, while the other nutrient standards are prepared in Class A Pyrex volumetric flasks. Each newly made stock standard is individually checked for potential cross contamination of other nutrients when employing mixed standards.

9.2 The LNSW plus reagents baseline (*LR*) is taken as the standard blank since the standards are prepared in LNSW. The Response Factor (*F*) is calculated as follows:

$$F \left(\mu M \, chart \, unit^{-1} \right) = \frac{[STD]}{(PH - LR)}$$

Where:

$[STD]$ = the concentration of the standard (μM)

PH = the peak height of the standard (chart units)

LR = baseline of LNSW + reagents

The Response Factor (F) applied to the samples is the average of the bracketing standard sets for silicate, nitrite and phosphate runs, while a sample specific F is applied to the nitrate+nitrite samples by interpolating between the average F at the beginning and the average F at the end of the sample set based on sample peak position. The nitrate calculation uses interpolated F values to account for cadmium column efficiency loss throughout the course of a run.

10.0 Calculations and Blank Determination

10.1 The baseline correction, BLC, applied to the LNSW baseline for each nutrient species is calculated as follows:

$$BLC = LR - (QR + RI)$$

Where:

LR = baseline of LNSW + reagents
QR = baseline of Milli-Q + reagents
RI = refractive index; LNSW baseline - Milli-Q water baseline

10.2 Calculation of the sample blank (LR - BLC) assumes that the Milli-Q water baseline plus reagents (QR) represents a nutrient concentration of zero. The BLC represents, in chart units, the contribution of the individual nutrients to the LNSW baseline. Generally, we find no detectable levels of nitrate, nitrite or phosphate in our LNSW, but reactive silicate is consistently calculated between 0.7 and 0.8 μM with this method.

10.3 Final nutrient concentrations are calculated using the response factor (F) as follows:

$$[\mu M] = F(PH - (LR - BLC))$$

Where:

F = response factor of standards
PH = peak height of sample
LR = baseline of LNSW + reagents
BLC = baseline correction

Note that the baseline LR incorporates a drift correction into all calculations.

10.4 The units of μmoles kg^{-1} are obtained by dividing the calculated nutritent concentrations by the density of the seawater at the time of analysis.

11.0   References

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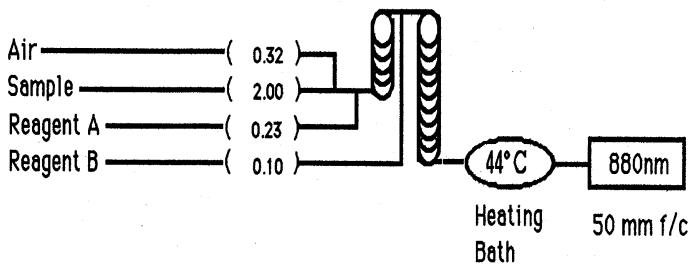Figure 4. Nitrite and Nitrate + Nitrite Manifold Configuration
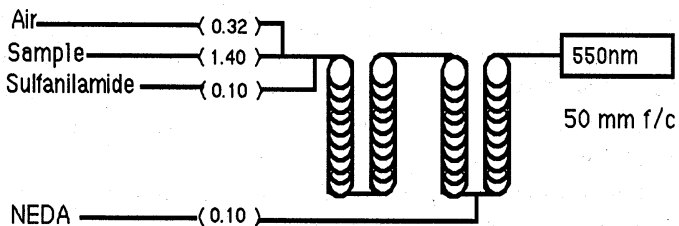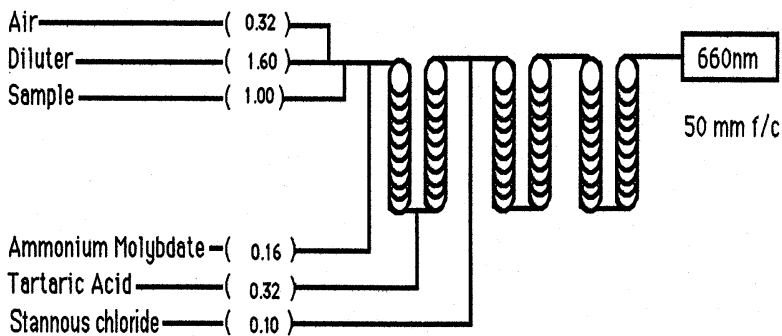

Nitrite Manifold Configuration
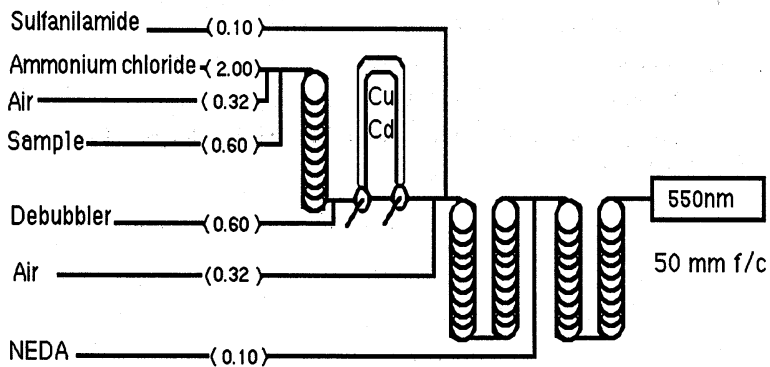
Nitrate + Nitrite Manifold Configuration

Figure 5. Phosphate and Silicate Manifold Configurations

Phosphate Manifold Configuration

Silicate Manifold Configuration