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Nutrient Analysis, Nitrate, Nitrite, Silicate, Phosphate and Ammonium

SUMMARY: The phytoplankton macro nutrients nitrate, nitrite, silicate, and phosphate in seawater are analyzed using colorimetric assays. Ammonium concentrations are determined using a fluorometric assay.

1. Principle

Nutrient analysis is performed on a QuAAtro continuous segmented flow autoanalyzer (SEAL Analytical). A sample of seawater enters a reagent stream within a manifold on the analyzer where it undergoes a series of reactions that ultimately produce a colored compound. These compounds absorb light at a specific wavelength. A monochromatic beam of light is passed through the sample and the absorbance is measured. The machine is calibrated with a series of known standards and a standard curve is produced. The intensity of the color produced by the unknown sample is proportional to the concentration of the analyte present. The product of the ammonia method is a fluorescent species; however the same basic principle applies, where the intensity of the fluorescence is directly related to concentration. The methods for silicate and total oxidized nitrogen (TON) are modified versions of those described by used Armstrong et al. (1967) and Gordon et al. (1992). The phosphate determination employs a modification of the method described by Murphy and Riley (1962), and ammonia is analyzed based on the Kerouel and Aminot (1997) fluorometric method.

2. Method Description

Silicate (SiO_2)

Silicate is analyzed using a modified technique of Armstrong (1967). An acidic solution of ammonium molybdate is added to a seawater sample to produce silicomolybdic acid which is then reduced to a blue silicomolybdous acid following the addition of ascorbic acid. The amount of blue color produced is proportional to the amount of dissolved reactive silicate in the sample. Oxalic acid is added to inhibit PO_4 color interference. The sample is passed through a 10mm flow cell and the absorbance is measured at 820nm.

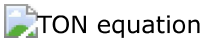
Nitrate and Nitrite (NO_3 and NO_2)

A modification of the Armstrong (1967) procedure is used for the analysis of nitrate plus nitrite, or total oxidized nitrogen (TON). For this analysis, the seawater sample is passed through a cadmium reduction coil where nitrate is reduced to nitrite. The efficiency of this reduction is determined by running two equimolar solutions, one containing only nitrate and one containing only nitrite, through the coil. The percent of nitrate converted to nitrite yields the coil efficiency, a factor used to calculate the nitrate concentration. Sulfanilamide is

introduced to the sample stream followed by N-(1-naphthyl) ethylenediamine dihydrochloride which complexes with nitrite to form a red azo dye. The stream is then passed through a 10 mm flowcell and the absorbance measured at 520nm.

The same method is employed for nitrite analysis, except the cadmium column is not present. Consequently, only nitrite already present in the sample is measured.

The nitrate concentration is calculated on a "virtual channel" by the following equation, using the coil efficiency, TON, and NO2:



NO3=Nitrate (in sample)

NO2= Nitrite (in sample)

TON=Total oxidized nitrogen (NO3+NO2) in sample

A= NO3 concentration in mixed calibrant

B= NO2 concentration in mixed calibrant

Recovery = Coil efficiency (expressed as a decimal)

Phosphate (PO_4)

Phosphate is analyzed using a modification of the Murphy and Riley (1962) technique. Similar to the silicate method, an acidic solution of ammonium molybdate is added to the sample to produce phosphomolybdic acid that is subsequently reduced to blue phosphomolybdous acid following the addition of ascorbic acid. Color intensity is directly related to the concentration of dissolved phosphate in the sample. The reaction product is then passed through a 10mm flow cell and the absorbance measured at 880nm.

Ammonia (NH_3)

Ammonia is measured fluorometrically using a modification of the method described by Kerouel and Aminot (1997). In the presence of a borate buffer, samples are reacted with o-pthalaldehyde (OPA) to form a fluorescent complex that is excited at 370nm and emits at 460nm. The reaction takes place at 75°C. Sodium sulfite is added to the working reagent to reduce sensitivity to dissolved amino acids.

3. Method Notes

Ammonium is a difficult parameter to measure accurately due to its insidious nature and problems with contamination. Phosphorus and nitrogen compounds are also potential sources of contamination with poor sampling technique. Care must be taken during sampling to insure there is no contamination (e.g. touching the inside of the tube or the cap with fingers, smoking near rosette).

4. Water Sampling

Nutrient samples are drawn into 30 ml polypropylene screw-capped centrifuge tubes.

The tubes and caps are cleaned with 10% HCl and rinsed 3 times with sample before filling.

Samples that are not analyzed immediately are refrigerated and analyzed within 16 hours of collection. All samples are allowed sufficient time to reach room temperature. The centrifuge tubes fit directly onto the sampler.

5. Calculations

All data is reported in micro-moles/liter. The main calculations for concentration on the QuAAtro are run through the required AACE software interface. These calculations still follow the principle of other instruments, where:

[X] micro moles/liter =(Absorbance-blank) x F1 (Response Factor)

Values are corrected for drift based on changes in beginning and end standards, ultra pure water and the relative position of samples in the run. Corrections for linearity are performed, if necessary, based on a set of absorbances and concentrations; deviations from Beer's law can be plotted to reveal a polynomial function that can be applied to correct sample values accordingly. Improvements in optics in the QuAAtro instrument have resulted in marked improvement in linearity and reduction of blank values for nitrate and silicate and phosphate.

6. Quality Control

A sample of reference material for nutrients in seawater (RMNS), produced by KANSO technos (www.kanso.co.jp (http://www.kanso.co.jp)) is included in every run and those data are monitored for consistency.
An aliquot from a large volume of stable deep seawater is run once a day as an additional check. The stability of the deep seawater check is aided by the addition of mercuric chloride as a poison.

The efficiency of the cadmium column used for nitrate reduction is monitored throughout the cruise and usually ranges from 97.0-100.0%.

NO_3, PO_4, NO_2, and NH_4 are reported to two decimals places and SiO_2 to one.

Accuracy is based on the quality of the standards; the levels in micro moles/liter (µM) are:

- NO_3 = 0.05
- PO_4 = 0.004
- SiO_2 = 2-4
- NO_2 = 0.05
- NH_3 = 0.03

The precision of the instrument for NO_3, PO_4, and NH_4 is 0.01 µM and 1.0µM for silicate and 0.01µM for NO_2.

The detection limits in micro moles/liter for the instrumentation are:

- NO_3+NO_2 = 0.02
- PO_4 = 0.02
- SiO_2 = 0.5
- NO_2 = 0.02
- NH_3 = 0.04

7. Equipment/Supplies

Seal Analytical continuous-flow QuAAtro run by IOD since CalCOFI 1203SH; AutoAnalyzer 3 (AA3) run by ODF on cruises prior to 1203SH. Distributed by Bran and Luebbe, http://www.seal-analytical.com/ (http://www.seal-analytical.com/Products/QuAAtro39AutoAnalyzer/tabid/814/language/en-US/Default.aspx)

30 ml centrifuge tubes and test tube racks, 8 sets color coded and numbered

Barnstead Nanopure purified water system or equivalent polished water source

Sundry laboratory glassware

8. References

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Keuroul, R. and A. Aminot, (1997). "Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis," Marine Chemistry Vol. 57, no. 3-4, pp.265-275.

Murphy, J. and J.P. Riley, (1962). A modified single solution method for the determination of phosphate in natural waters. Analytica Chimia Acta, Vol. 27 pp.31-36.