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

SUMMARY: The phytoplankton macro nutrients nitrate, nitrite, silicate, phosphate and ammonium are analyzed in seawater using a colorimetric assay in which light absorbance is measured versus known standards.

1. Principle

To analyze for nutrients (phosphate, silicate, nitrate+nitrite, nitrite, and ammonia) a Seal Analytical continuous-flow AutoAnalyzer 3 (AA3) is used. After each run, the charts are reviewed for any problems, any blank is subtracted, and final concentrations (micro moles/liter) are calculated. The methods used are described by Gordon et al. (1992) Hager et al. (1968) and Atlas et al. (1971).

2. Method Desciption

Silicate is analyzed using the 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 silicomolybdous acid (a blue compound) following the addition of stannous chloride. Tartaric acid is added to impede PO_4 color interference. The sample is passed through a 10mm flow cell and the absorbance measured at 660nm.

A modification of the Armstrong (1967) procedure is used for the analysis of nitrate plus nitrite. For this analysis, the seawater sample is passed through a cadmium reduction column where nitrate is quantitatively reduced to nitrite. Sulfanilamide is introduced to the sample stream followed by N-(1-naphthyl)ethylenediamine dihydrochloride which couples to form a red azo dye. The stream is then passed through a 10 mm flowcell and the absorbance measured at 520nm.

The same technique is employed for nitrite analysis, except the cadmium column is not present.

Nitrate concentration is calculated by subtracting the nitrite value from the combined Nitrate + Nitrite (N+N) value.

Phosphate is analyzed using a modification of the Bernhardt and Wilhelms technique. An acidic solution of ammonium molybdate is added to the sample to produce phosphomolybdic acid, then reduced to phosphomolybdous acid (a blue compound) following the addition of dihydrazine sulfate. The reaction product is heated to ~55C to enhance color development and then passed through a 10mm flow cell and the absorbance measured at 820m.

Ammonium is analyzed via the Berthelot reaction in which hypochlorous acid and phenol react with ammonium in an alkaline solution to form indophenol blue. The sample is passed through a 10 mm flow cell and measured at 660nm. This method is a modification of the procedure by Koroleff (1969,1970).

3. Method Notes

Make stannous chloride stock up daily - refrigerate when not in use in a dark poly bottle.

Minimize oxygen introduction into the N-(1-naphthyl) ethylenediamine dihydrochloride by swirling rather than shaking the solution. Discard if a white solution (oxychloride) forms.

Ammonium can be measured as the fifth species in a series of automated nutrient analysis. Ammonium is a difficult parameter to measure accurately due to its' insidious nature and problems with contamination.

4. Water Sampling

Nutrient samples are drawn into 40 ml polypropylene screw-capped centrifuge tubes.

The tubes and caps are cleaned with 10% HCl and rinsed once with de-ionized water and 3 times with sample before filling.

Samples are analyzed within 2-16 hours after sample collection, allowing sufficient time for all samples to reach room temperature. The centrifuge tubes fit directly onto the sampler.

5. Calculations

All data is reported in micro-moles/liter. The main calculation for concentration on the Bran and Luebbe AA3 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 absorbences 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 AA3 instrument have resulted in marked improvement in linearity and reduction of blank values for nitrate and silicate and phosphate.

The insidious nature of ammonium poses a particular problem in relation to reliable blank values. Upon expert recommendation, the true baseline blank is based on the value obtained from deep seawater samples (500m) where ammonium is thought to be zero. Carefully monitoring these values in relation to machine function enables them to be used as ammonium blanks and used to correct all depths.

6. Quality Control

An aliquot from a large volume of stable deep seawater is run with each set of samples 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 98.0-100.0%.

NO_3, PO_4, NO_2, and NH_4 are reported to two decimals places and SIL 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

Sil = 2-4

NO_2 = 0.05

NH4 = 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

Sil = 0.5

NO_2 = 0.02

NH_4 = 0.04

7. Equipment/Supplies

Seal Analytical continuous-flow AutoAnalyzer 3, Distributed by Bran and Luebbe, http://www.seal-analytical.com/

50 ml centrifuge tubes and test tube racks, 6 sets color coded and numbered

Milli-Q purified water system or equivalent polished water source

Sundry laboratory glassware

8. Reagents

Ammonium Molybdate (ACS Reagent Grade)

H2SO4 solution:

Pour 420 ml of dionized water (DIW) into a 2 liter Erlenmeyer flask or beaker, place this flask or beaker into an ice bath. Slowly add 330 ml of concentrated H_2SO_4. Be careful, this procedure is exothermic; it gets very hot. Make up as much as necessary in the above proportions.

Dissolve 27g ammonium molybdate in 250ml of DIW. Bring to 1 liter volume with the cooled sulfuric acid sol'n. Add 5 drops of 20% FFD6 surfactant. Store in a dark poly bottle and refrigerate.

Dihydrazine Sulfate (ACS Reagent Grade)

Dissolve 6.4g dihydrazine sulfate in DIW, bring to 1 liter volume and refrigerate.

Ammonium Assay:

Sodium Citrate (Complexing Reagent) (ACS Reagent Grade)

Dissolve 280gm Sodium Citrate in approximately 990ml DIW; adjust to pH7; bring to a 1 liter final volume with DIW. Store in a poly bottle.

Alkaline Phenol (ACS Reagent Grade)

Add 60ml 10N NaOH to 700ml DIW. Add 12ml or 12gm Phenol (liquid or solid). Dilute to 1L with DIW. Store in a dark poly bottle. Make up new as necessary if sensitivity drops off.

10N NaOH (ACS Reagent Grade)

Dissolve 400gm NaOH in 1L DIW

Sodium Hypochlorite (NaOCl) (household bleach)

Add 2.5ml of 5.25% NaOCl to 100ml DIW. Prepare daily.

Sodium Nitroprusside (ACS Reagent Grade)

Dissolve 0.5gm in 800ml DIW. Bring to 1L with DIW. Store in dark poly container. Keep away from light at all times.

9. References

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