Nutrients

TN376 - Balch 2020

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**Summary of Analysis**

• 1291 samples from 73 ctd stations, 15 trace metal stations, 4 trace metal incubations, and 18 underway samples.

• The cruise started with new pump tubes and they were changed 3 times.

• 3 sets of nitrate, phosphate, and silicate Primary/Secondary standards were made up over the course of the

cruise.

• 1 set of primary and 30 sets of secondary nitrite standards were made up over the course of the cruise.

• 2 sets of primary and 30 sets of secondary ammonia standards were made up over the course of the cruise.

• The cadmium column efficiency was checked periodically and ranged between 98%-100%. A new column was

put on if the efficiency fell below 97% or injected with air.

**Equipment and Techniques**

Nutrient analyses (phosphate, silicate, nitrate+nitrite, and nitrite) were performed on a Seal Analytical continuous-flow AutoAnalyzer 3 (AA3). The methods used are described by Gordon et al [Gordon1992] Hager et al. [Hager1972], and Atlas et al. [Atlas1971]. Details of modification of analytical methods used in this cruise are also compatible with the methods described in the nutrient section of the GO-SHIP repeat hydrography manual (Hydes et al., 2010) [Hydes2010].

**Nitrate/Nitrite Analysis**

A modification of the Armstrong et al. (1967) [Armstrong1967] procedure was used for the analysis of nitrate and

nitrite. For nitrate analysis, a seawater sample was passed through a cadmium column where the nitrate was reduced to nitrite. This nitrite was then diazotized with sulfanilamide and coupled with N-(1-naphthyl)-ethylenediamine to form a red dye. The sample was then passed through a 10mm flowcell and absorbance measured at 540nm. The procedure was the same for the nitrite analysis but without the cadmium column.

**REAGENTS**

**Sulfanilamide** Dissolve 10g sulfamilamide in 1.2N HCl and bring to 1 liter volume. Add 2 drops of 40% surfynol

465/485 surfactant. Store at room temperature in a dark poly bottle.

Note: 40% Surfynol 465/485 is 20% 465 plus 20% 485 in DIW.

**N-(1-Naphthyl)-ethylenediamine dihydrochloride (N-1-N)** Dissolve 1g N-1-N in DIW, bring to 1 liter volume.

Add 2 drops 40% surfynol 465/485 surfactant. Store at room temperature in a dark poly bottle. Discard if the solution turns dark reddish brown.

**Imidazole Buffer** Dissolve 13.6g imidazole in ~3.8 liters DIW. Stir for at least 30 minutes to completely dissolve.

Add 60 ml of CuSO4 + NH4Cl mix (see below). Add 4 drops 40% Surfynol 465/485 surfactant. Let sit overnight before proceeding. Using a calibrated pH meter, adjust to pH of 7.83-7.85 with 10% (1.2N) HCl (about 10 ml of acid, depending on exact strength). Bring final solution to 4L with DIW. Store at room temperature.

**NH4Cl + CuSO4** mix Dissolve 2g cupric sulfate in DIW, bring to 100 m1 volume (2%). Dissolve 250g ammonium

chloride in DIW, bring to l liter volume. Add 5ml of 2% CuSO4 solution to this NH4Cl stock. This should last many months.

**Phosphate Analysis**

Ortho-Phosphate was analyzed using a modification of the Bernhardt and Wilhelms (1967) [Bernhardt1967] method. Acidified ammonium molybdate was added to a seawater sample to produce phosphomolybdic acid, which was then reduced to phosphomolybdous acid (a blue compound) following the addition of dihydrazine sulfate. The sample was passed through a 10mm flowcell and absorbance measured at 820nm.

**REAGENTS**

**Ammonium Molybdate** H2SO4 sol’n Pour 420 ml of DIW into a 2 liter Ehrlenmeyer flask or beaker, place this

flask or beaker into an ice bath. SLOWLY add 330 ml of conc H2SO4. This solution gets VERY HOT!!

Cool in the ice bath. 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 3 drops of 15% DDS surfactant. Store in a dark poly bottle.

**Dihydrazine Sulfate** Dissolve 6.4g dihydazine sulfate in DIW, bring to 1 liter volume and refrigerate.

**Silicate Analysis**

Silicate was analyzed using the basic method of Armstrong et al. (1967). Acidified ammonium molybdate was added to a seawater sample to produce silicomolybdic acid which was then reduced to silicomolybdous acid (a blue compound) following the addition of stannous chloride. The sample was passed through a 10mm flowcell and measured at 660nm.

**REAGENTS**

**Tartaric Acid** Dissolve 200g tartaric acid in DW and bring to 1 liter volume. Store at room temperature in a poly

bottle.

**Ammonium Molybdate** Dissolve 10.8g Ammonium Molybdate Tetrahydrate in 1000ml dilute H2SO4. (Dilute

H2SO4 = 2.8ml conc H2SO4 or 6.4ml of H2SO4 diluted for PO4 moly per liter DW) (dissolve powder, then add H2SO4) Add 3-5 drops 15% SDS surfactant per liter of solution.

**Stannous Chloride stock**: (as needed)Dissolve 40g of stannous chloride in 100 ml 5N HCl. Refrigerate in a poly

bottle.

NOTE: Minimize oxygen introduction by swirling rather than shaking the solution. Discard if a white solution (oxychloride) forms.

**working**: (every 24 hours) Bring 5 ml of stannous chloride stock to 200 ml final volume with 1.2N HCl.

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

**Ammonia Analysis**

Ammonia is analyzed using the method described by Kerouel and Aminot [Kero97]. The sample is combined with a working reagent made up of ortho-phthalaldehyde, sodium sulfite and borate buffer and heated to 75degC. Fluorescence proportional to the NH4 concentration is emitted at 460nm following excitation at 370nm.

**REAGENTS**

**Ortho-phthalaldehyde stock (OPH)** Dissolve 8g of ortho-phthalaldehyde in 200mls ethanol and mix thoroughly.

Store in a dark glass bottle and keep refrigerated.

**Sodium sulfite stock** Dissolve 0.8g sodium sulfite in DIW and dilute up to 100ml. Store in a glass bottle, replace

weekly.

**Borate buffer** Dissolve 120g disodium tetraborate in DIW and bring up to 4L volume.

**Working reagent** In the following order and proportions combine:

1L borate buffer

20ml stock orthophthalaldehyde,

2 ml stock sodium sulfite,

4 drops 40% Surfynol 465/485 surfactant and mix.

Store in a glass bottle and protect from light. Replace weekly. Make this up at least one day prior to use. Store in dark bottle and protect from outside air/nh4 contamination.

**Sampling**

Nutrient samples were drawn into 40 ml polypropylene screw-capped centrifuge tubes. The tubes and caps were

cleaned with 10% HCl and rinsed 2-3 times with sample before filling. Samples were analyzed within 1-12 hours after sample collection, allowing sufficient time for all samples to reach room temperature. If stored longer than 1 hour, samples were placed in the fridge and always protected from light. The centrifuge tubes fit directly onto the sampler.

**Data Collection and Processing**

Data collection and processing was done with the software (ACCE ver 6.10) provided with the instrument from Seal

Analytical. After each run, the charts were reviewed for any problems during the run, any blank was subtracted, and

final concentrations (micro moles/liter) were calculated, based on a linear curve fit. Once the run was reviewed and

concentrations calculated a text file was created. That text file was reviewed for possible problems and then converted to another text file with only sample identifiers and nutrient concentrations that was merged with other bottle data.

**Standards and Glassware Calibration**

Primary standards for silicate (Na2SiF6), nitrate (KNO3), nitrite (NaNO2), phosphate (KH2PO4), and ammonia ((NH4)2SO4) were obtained from Johnson Matthey Chemical Co. and/or Fisher Scientific. The supplier reports purities of >98%, 99.999%, 97%, and 99.999 respectively. All glass volumetric flasks and pipettes were gravimetrically calibrated prior to the cruise. The primary standards were dried and weighed out to 0.1mg prior to the cruise. The exact weight was noted for future reference. When primary standards were made, the flask volume at 20C, the weight of the powder, and the temperature of the solution were used to buoyancy-correct the weight, calculate the exact concentration of the solution, and determine how much of the primary was needed for the desired concentrations of secondary standard. Primary and secondary standards were made up every 7-10days. The new standards were compared to the old before use.

All the reagent solutions, primary and secondary standards were made with fresh distilled deionized water (DIW).

Standardizations were performed at the beginning of each group of analyses with working standards prepared every

10-12 hours from a secondary. Working standards were made up in low nutrient seawater (LNSW). Two batches of

LNSW were used on the cruise. One was collected and filtered prior to the cruise. Another was collected from underway seawater in transit from Durban port stop. The actual concentration of nutrients in this water was empirically determined during the standardization calculations. The underway LNSW will be filtered and analyzed on shore as well.

A set of low concentration standards were used for CTD casts with a bottom sample at 800m or less. This accounted for all but 4 deep casts. The concentrations in micro-moles per liter of the working standards used were:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | N+N (uM) | PO4 (uM) | Sil (uM) | NO2 (uM) | NH4 (uM) |
| SW | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 2 | 7.25 | 0.6 | 30 | 0.25 | 1.0 |
| 3 | 15.50 | 1.2 | 60 | 0.5 | 2.0 |
| 4 | 22.75 | 1.8 | 90 | 0.75 | 3.0 |

A set of high concentration standards were used for CTD casts deeper than 800m. The concentrations in micro-moles per liter of the working standards used were:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | N+N (uM) | PO4 (uM) | Sil (uM) | NO2 (uM) | NH4 (uM) |
| SW | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | 15.50 | 1.2 | 60 | 0.50 | 2.0 |
| 5 | 31.00 | 2.4 | 120 | 1.00 | 4.0 |
| 7 | 46.50 | 3.6 | 180 | 1.50 | 6.0 |

**Quality Control**

All final data was reported in micro-moles/L. NO3, PO4, and NO2 were reported to two decimals places and SIL and NH4 to one. Accuracy is based on the quality of the standards the levels are:

NO3 0.05 μM

PO4 0.004 μM

SIL 2-4 μM

NO2 0.05 μM

As is standard ODF practice, a deep calibration “check” sample was run with each set of samples to estimate precision within the cruise. The data are tabulated below.

|  |  |  |
| --- | --- | --- |
| Parameter | Concentration (uM) | stddev |
| NO3 | 14.98 | 0.17 |
| PO4 | 1.08 | 0.02 |
| SIL | 6.4 | 0.38 |

Reference materials for nutrients in seawater (RMNS) were also used as a check sample run once a day. The

RMNS preparation, verification, and suggested protocol for use of the material are described by [Aoyama2006]

[Aoyama2007], [Aoyama2008] and Sato [Sato2010]. RMNS batch CG was used on this cruise, with each bottle

being used once or twice before being discarded and a new one opened. Data are tabulated below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Concentration (uM) | stddev | Assigned conc. (uM) | Assigned Uncertainty (uM) |
| NO3 | 24.10 | 0.11 | 24.27 | 0.2 |
| PO4 | 1.75 | 0.02 | 1.74 | 0.02 |
| SIL | 57.6 | 0.6 | 57.7 | 0.5 |
| NO2 | 0.07 | 0.01 | 0.06 | 0.03 |
| NH4 | 1.02 | 0.11 | n/a | n/a |

**Analytical Problems**

Two issues arose during the cruise that resulted in 29 silicate and 58 nitrate measurements flagged as questionable. The flagged data is still reasonable with max uncertainty estimated to increase to ~1.0uM silicate and ~0.2uM nitrate.

The DW system onboard required a complete filter change twice during the 35 day cruise. This is unusual and indicative of high contaminants in source water from the still. The overall impact on nutrient analysis is likely minimal since filters were changed when resistivity fell below 17ohm (optimal is 18.2ohm). However, a few runs of N+N data were flagged questionable after using ~15ohm DW before a DW filter change. The N+N standard curves were noticeably non-linear and instrument lines required significant cleaning and changing of Cd columns to address the issue. Samples of DW at low resistivity were shipped to shore for trace metal analysis (contaminant is suspected to be rust).

A failed photometer circuit caused increasing frequency of acquisition software crashes over the course of 36 hours mid cruise. The issue was located in the silicate and nitrite photometer and swapped for spare. In order to keep up with analysis, samples were measured before silicate channel was fully primed. The resulting runs flagged questionable contained several baseline jumps and therefore estimated increased uncertainty to ~1.0 uM.

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