

### 3. 3. 4 Nutrients

#### (1) Personnel

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#### (2) Objectives

To monitor the long-term variability of nutrient concentrations along 110° E transect.

#### (3) Parameters

The determinants are nitrate, nitrite, silicate and phosphate.

#### (4) Summary of nutrients analysis

We made 6 QuAAtro runs for the water columns sample at 6 stations during UM-14-08. The total amount of layers of the seawater sample reached up to 148. We made duplicate sampling at 6 layers and measurement.

The reference material of nutrients in seawater was used for calibration standard in UM-14-08. This calibration method was same as CLIVER cruise by JAMSTEC.

#### (5) Instrument and method

Analytical detail using QUAATRO 2-HR systems (BL-Tech).

Nitrate + nitrite and nitrite are analyzed according to the modification method of Grasshoff (1970). The sample nitrate is reduced to nitrite in a cadmium tube inside of which is coated with metallic copper. The sample stream with its equivalent nitrite is treated with an acidic, sulfanilamide reagent and the nitrite forms nitrous acid which reacts with the sulfanilamide to produce a diazonium ion. N-1-Naphthylethylene-diamine added to the sample stream then couples with the diazonium ion to produce a red, azo dye. With reduction of the nitrate to nitrite, both nitrate and nitrite react and are measured; without reduction, only nitrite reacts. Thus, for the nitrite analysis, no reduction is performed and the alkaline buffer is not necessary. Nitrate is computed by difference.

The silicate method is analogous to that described for phosphate. The method used is essentially that of Grasshoff et al. (1983), wherein silicomolybdic acid is first formed from the silicate in the sample and added molybdic acid; then the silicomolybdic acid is reduced to silicomolybdous acid, or "molybdenum blue," using ascorbic acid as the reductant. The analytical methods of the nutrients, nitrate, nitrite, silicate and phosphate, during this cruise are same as the methods used in (Kawano et al. 2009).

The phosphate analysis is a modification of the procedure of Murphy and Riley (1962). Molybdic acid is added to the seawater sample to form phosphomolybdic acid which is in turn reduced to phosphomolybdous acid using L-ascorbic acid as the reductant.

The flow diagrams and reagents for each parameter are shown in Figures 3.3.4-1 to 3.3.4-4.

a. Nitrate + Nitrite reagents

Imidazole (buffer), 0.06 M (0.4% w/v)

Dissolve 4 g imidazole,  $C_3H_4N_2$ , in ca. 1000 ml DIW; add 2 ml concentrated HCl. After mixing, 1 ml Triton(R)X-100 (50% solution in ethanol) is added.

Sulfanilamide, 0.06 M (1% w/v) in 1.2M HCl

Dissolve 10 g sulfanilamide,  $4-NH_2C_6H_4SO_3H$ , in 900 ml of DIW, add 100 ml concentrated HCl. After mixing, 2 ml Triton(R)X-100 (50% solution in ethanol) is added.

N-1-Naphthylethylene-diamine dihydrochloride, 0.004 M (0.1% w/v)

Dissolve 1 g NED,  $C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$ , in 1000 ml of DIW and add 10 ml concentrated HCl.

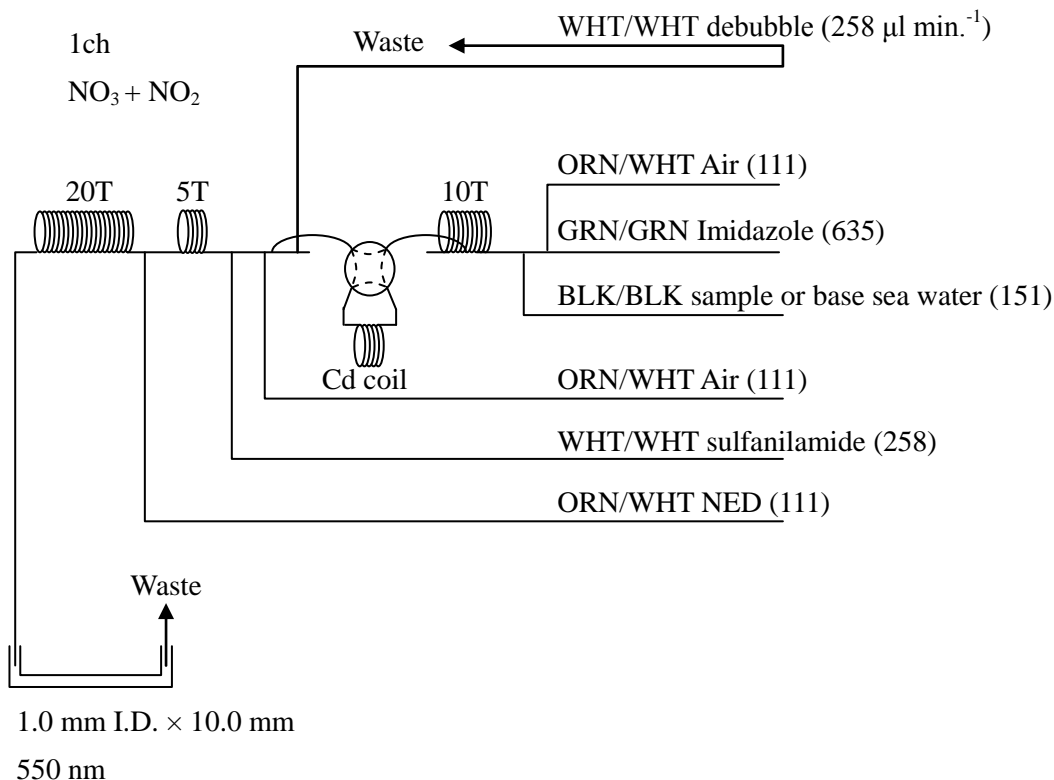


Figure 3.3.4-1 NO<sub>3</sub>+NO<sub>2</sub> (1ch.) Flow diagram.

Sulfanilamide, 0.06 M (1% w/v) in 1.2 M HCl

N-1-Napthylethylene-diamine dihydrochloride, 0.004 M (0.1% w/v)

2ch  
NO<sub>2</sub>

20T 10T 5T

10T

WHT/WHT debubble (258  $\mu\text{l min.}^{-1}$ )

ORN/WHT Air (111)

RED/RED sulfanilamide (323)

GRN/GRN sample or base sea water (635)

ORN/WHT Air (111)

RED/RED NED (323)

Waste

Waste

1.0 mm I.D.  $\times$  30.0 mm

550 nm

### c. Silicate reagents

Molybdic acid, 0.06 M (2% w/v)

Dissolve 15 g Disodium Molybdate(VI) Dihydrate,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , in 980 ml DIW, add 8 ml concentrated  $\text{H}_2\text{SO}_4$ . After mixing, 20 ml sodium dodecyl sulphate (15% solution in water) is added.

Oxalic acid, 0.6 M (5% w/v)

Dissolve 50 g Oxalic Acid Anhydrous,  $\text{HOOC:COOH}$ , in 950 ml of DIW.

Ascorbic acid, 0.01M (3% w/v)

Dissolve 2.5 g L (+)-Ascorbic Acid,  $\text{C}_6\text{H}_8\text{O}_6$ , in 100 ml of DIW. Freshly prepared before every measurement.

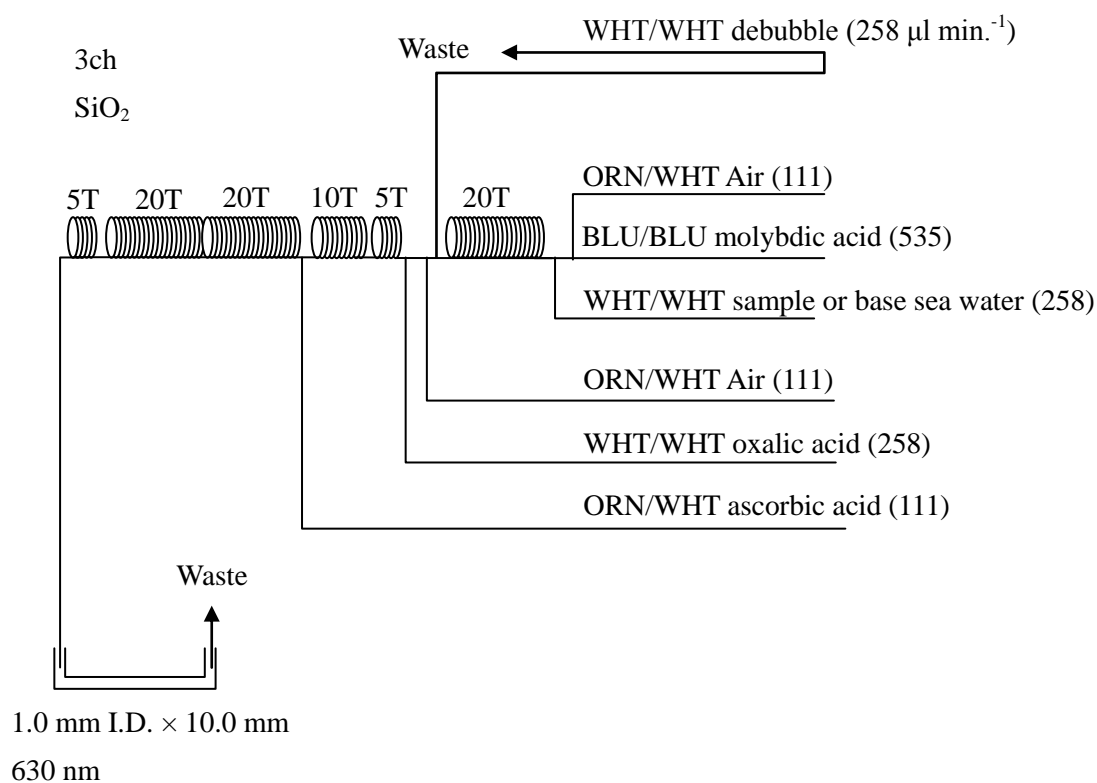


Figure 3.3.4-3  $\text{SiO}_2$  (3ch.) Flow diagram.

#### d. Phosphate reagents

Stock molybdate solution, 0.03M (0.8% w/v)

Dissolve 8 g Disodium Molybdate(VI) Dihydrate,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , and 0.17 g Antimony Potassium Tartrate,  $\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2 \cdot 3\text{H}_2\text{O}$ , in 950 ml of DIW and add 50 ml concentrated  $\text{H}_2\text{SO}_4$ .

#### Mixed Reagent

Dissolve 1.2 g L (+)-Ascorbic Acid,  $\text{C}_6\text{H}_8\text{O}_6$ , in 150 ml of stock molybdate solution. After mixing, 3 ml sodium dodecyl sulphate (15% solution in water) is added. Freshly prepared before every measurement.

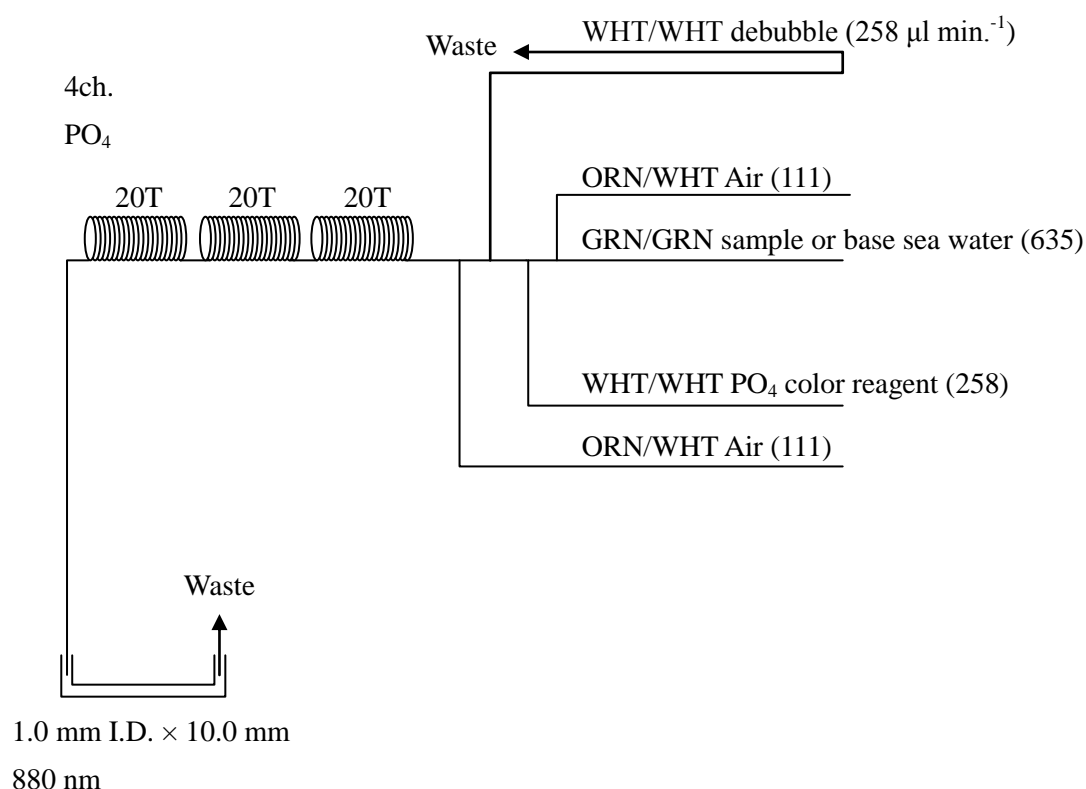


Figure 3.3.4-4 PO<sub>4</sub> (4ch.) Flow diagram.

#### e. Sampling procedures

Sampling of nutrients followed that oxygen and salinity. Samples were drawn into a virgin 10 ml polyacrylates vials without sample drawing tubes. These were rinsed three times before filling and vials were capped immediately after the drawing. The vials are put on room temperature, ca.  $24 \pm 2$  deg C, before use to stabilize the temperature of samples.

No transfer was made and the vials were set an auto sampler tray directly. Samples were analyzed after collection basically within 24 hours.

#### f. Data processing

Raw data from QuAAtro 2-HR was treated as follows:

- Checked baseline shift.
- Checked the shape of each peak and positions of peak values taken, and then changed the positions of peak values taken if necessary.
- Carry-over correction and baseline drift correction were applied to peak heights of each samples followed by sensitivity correction.

- Baseline correction and sensitivity correction were done basically using liner regression.
- Loaded pressure and salinity from CTD data to calculate density of seawater.
- Calibration curves to get nutrients concentration were assumed second order equations.

#### (6) Nutrients standards

##### a. Volumetric laboratory ware of in-house standards

All volumetric polypropylene (PP) ware used was gravimetrically calibrated. Plastic volumetric flasks were gravimetrically calibrated at the temperature of use within 0 to 4 K.

##### Volumetric flasks

Volumetric flasks of PP are used because their nominal tolerances are 0.05% or less over the size ranges likely to be used in this work. Because of their larger temperature coefficients of cubical expansion and lack of tables constructed for these materials, the plastic volumetric flasks were gravimetrically calibrated over the temperature range of intended use and used at the temperature of calibration within 0 to 4 K. The weights obtained in the calibration weightings were corrected for the density of water and air buoyancy.

##### Pipettes and pipettors

All pipettes have nominal calibration tolerances of 0.1% or better. These were gravimetrically calibrated in order to verify and improve upon this nominal tolerance.

##### b. Reagents, general considerations

##### Specifications

For nitrate standard, “potassium nitrate 99.995 suprapur®” provided by Merck, Lot. B0771365211, CAS No.: 7757-91-1, was used.

For nitrite standard, “sodium nitrite” provided by Wako, CAS No.: 7632-00-0, was used. And assay of nitrite was determined according JIS K8019 and assays of nitrite salts were 98.53 %. We use that value to adjust the weights taken.

For the silicate standard, we use “Silicon standard solution SiO<sub>2</sub> in NaOH 0.5 mol/l CertiPUR®” provided by Merck, CAS No.: 1310-73-2, of which lot number is HC382250 are used. The silicate concentration is certified by NIST-SRM3150 with the uncertainty of 0.5 %. HC382250 is certified as 1000±5 mg L<sup>-1</sup>, however, our direct comparison between two Merck standards and estimation based on 5 lots of RMNS gave us a factor of 973 mg L<sup>-1</sup> for HC382250 which was exceed 7 mg of certification by Merck. We use this factor throughout UM-14-08 to keep comparability for silicate concentration.

For phosphate standard, “potassium dihydrogen phosphate anhydrous 99.995 suprapur®”

provided by Merck, Lot. B0691108204, CAS No.: 7778-77-0, was used.

#### Treatment of silicate standard due to high alkalinity

Since the silicon standard solution Merck CertiPUR® is in NaOH 0.5 mol/l, we need to dilute and neutralize to avoid make precipitation of  $\text{MgOH}_2$  etc. When we make B standard, silicon standard solution is diluted by factor 12 with pure water and neutralized by HCl 1.0 mol  $\text{L}^{-1}$  to be about 7. After that B standard solution is used to prepare C standards.

#### Ultra pure water

Ultra pure water (Milli-Q) freshly drawn was used for preparation of reagent, standard solutions and for measurement of reagent and system blanks.

#### Low-nutrients seawater (LNSW)

Surface water having low nutrient concentration was taken and filtered using 0.45  $\mu\text{m}$  pore size membrane filter. This water is stored in 20 liter cubitainer with paper box. The concentrations of nutrient of this water were measured carefully in August 2012.

#### c. Concentrations of nutrients for A, B and C standards

Concentrations of nutrients for A, B and C standards are set as shown in Table 3.3.4-1. The C standard is prepared according recipes as shown in Table 3.3.4-2. All volumetric laboratory tools were calibrated prior the cruise as stated in chapter (6) a. Then the actual concentration of nutrients in each fresh standard was calculated based on the ambient, solution temperature and determined factors of volumetric laboratory wares.

The calibration curves for each run were obtained using 6 levels, C-1, C-2, C-3, C-4, C-5 and C-6. C-1, C-2, C-3, C-4 and C-5 were the reference material of nutrients in seawater and C-6 was in-house standard.

Table 3.3.4-1 Nominal concentrations of nutrients for A, B and C standards.

	A	B	C-1	C-2	C-3	C-4	C-5	C-6
$\text{NO}_3$ ( $\mu\text{M}$ )	22500	900	BY	BU	CA	BW	BZ	45
$\text{NO}_2$ ( $\mu\text{M}$ )	4000	20	BY	BU	CA	BW	BZ	1.0
$\text{SiO}_2$ ( $\mu\text{M}$ )	35000	2100	BY	BU	CA	BW	BZ	105
$\text{PO}_4$ ( $\mu\text{M}$ )	3000	60	BY	BU	CA	BW	BZ	3.0

Table 3.3.4-2 Working calibration standard recipes.

C Std.	B-1 Std.	B-2 Std.
C-6	25 ml	25 ml

B-1 Std.: Mixture of nitrate, silicate and phosphate

B-2 Std.: Nitrite

## d. Renewal of in-house standard solutions

In-house standard solutions as stated in paragraph c were renewed as shown in Table 3.3.4-3(a) to (c).

Table 3.3.4-3(a) Timing of renewal of in-house standards.

NO <sub>3</sub> , NO <sub>2</sub> , SiO <sub>2</sub> , PO <sub>4</sub>	Renewal
A-1 Std. (NO <sub>3</sub> )	maximum a month
A-2 Std. (NO <sub>2</sub> )	maximum a month
A-3 Std. (SiO <sub>2</sub> )	commercial prepared solution
A-4 Std. (PO <sub>4</sub> )	maximum a month
B-1 Std. (mixture of NO <sub>3</sub> , SiO <sub>2</sub> , PO <sub>4</sub> )	maximum a week
B-2 Std. (NO <sub>2</sub> )	maximum a week

Table 3.3.4-3(b) Timing of renewal of working calibration standards.

Working standards	Renewal
C-6 Std. (mixture of B-1 , B-2 Std.)	every 24 hours

Table 3.3.4-3(c) Timing of renewal of in-house standards for reduction estimation.

Reduction estimation	Renewal
D-1 Std. (3600 µM NO <sub>3</sub> )	maximum a week
14 µM NO <sub>3</sub>	when C Std. renewed
16 µM NO <sub>2</sub>	when C Std. renewed

## (7) Reference material of nutrients in seawater

To get the more accurate and high quality nutrients data to achieve the objectives stated above, huge numbers of the bottles of the reference material of nutrients in seawater (hereafter RMNS) are prepared (Aoyama et al., 2006, 2007, 2008, 2009). In the previous worldwide expeditions, such as WOCE cruises, the higher reproducibility and precision of nutrients



measurements were required (Joyce and Corry, 1994). Since no standards were available for the measurement of nutrients in seawater at that time, the requirements were described in term of reproducibility. The required reproducibility was 1%, 1 to 2%, 1 to 3 % for nitrate, phosphate and silicate, respectively. Although nutrient data from the WOCE one-time survey was of unprecedented quality and coverage due to much care in sampling and measurements, the differences of nutrients concentration at crossover points are still found among the expeditions (Aoyama and Joyce, 1996, Mordy et al., 2000, Gouretski and Jancke, 2001). For instance, the mean offset of nitrate concentration at deep waters was  $0.5 \mu\text{mol kg}^{-1}$  for 345 crossovers at world oceans, though the maximum was  $1.7 \mu\text{mol kg}^{-1}$  (Gouretski and Jancke, 2001). At the 31 crossover points in the Pacific WHP one-time lines, the WOCE standard of reproducibility for nitrate of 1% was fulfilled at about half of the crossover points and the maximum difference was 7% at deeper layers below 1.6 deg C in potential temperature (Aoyama and Joyce, 1996).

a. RMNS for this cruise

RMNS lots BY, BU, CA, BW and BZ which cover full range of nutrients concentrations in the Antarctic Ocean were prepared for UM-14-08.

RMNS lot BV are prepared for this cruise. Lot BV was analyzed at all station to keep the comparability.

These RMNS assignment were completely done based on random number. The RMNS bottles were stored at the laboratory (B), where the temperature was maintained around ca. 24 deg C.

b. Assigned concentration for RMNSs

We assigned nutrients concentrations for RMNS lots BY, BU, CA, BW and BZ as shown in Table 3.3.4-4.

Table 3.3.4-4 Assigned concentration of RMNSs.

	unit: $\mu\text{mol kg}^{-1}$				
	Nitrate	Nitrite	Phosphate	Silicate	Assigned year
BY	0.07	0.03	0.041	1.54	2014
BU	3.96	0.07	0.348	20.27	2014
CA	19.65	0.07	1.423	35.66	2014
BW	24.59	0.08	1.545	58.18	2014
BZ	43.40	0.21	3.059	156.63	2014

## (8) Quality control

### a. Precision of nutrients analyses during the cruise

Precision of nutrients analyses during the cruise was evaluated based on the 6 measurements, which are measured every 6 to 9 samples, during a run at the concentration of C-6 std. Summary of precisions are shown as shown in Table 3.3.4-5. During this cruise, analytical precisions were 0.14% for nitrate, 0.09% for nitrite, 0.11% for silicate and 0.10% for phosphate in terms of median of precision, respectively.

Table 3.3.4-5 Summary of precision based on the replicate analyses.

	Nitrate	Nitrite	Silicate	Phosphate
	CV%	CV%	CV%	CV%
Median	0.14	0.09	0.11	0.10
Mean	0.13	0.09	0.10	0.10
Maximum	0.16	0.15	0.15	0.12
Minimum	0.05	0.04	0.03	0.07
N	6	6	6	6

### b. Carry-over

We can also summarize the magnitudes of carry-over throughout the cruise as shown in Table 3.3.4-6.

Table 3.3.4-6 Summary of carry-over throughout UM-14-08.

	Nitrate	Nitrite	Silicate	Phosphate
	CV %	CV %	CV %	CV %
Median	0.22	0.10	0.17	0.08
Mean	0.22	0.11	0.18	0.07
Maximum	0.27	0.19	0.22	0.11
Minimum	0.18	0.00	0.16	0.00
N	6	6	6	6

## (9) Data archive

All raw data were attached in appendix of this cruise report. Quality checked data are archived in National Institute of Polar Research (NIPR), and published as JARE data reports within one year.

## (10)References

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