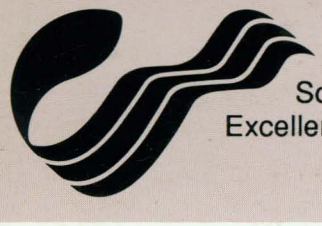

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INSTITUTE OF OCEAN SCIENCES NUTRIENT METHODS AND ANALYSIS

by

J. Barwell-Clarke and F. Whitney

Institute of Ocean Sciences
Department of Fisheries and Oceans
Sidney, B.C.

1996

CANADIAN TECHNICAL REPORT OF HYDROGRAPHY AND OCEAN SCIENCES NO. 182

Fisheries and Oceans Pêches et Océans

Canada

Canadian Technical Report of

Hydrography and Ocean Sciences 182

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Cat. No. 0711-6764 ISSN Fs 97-18/182E

Correct citation for this publication:
Barwell-Clarke, J. and F. Whitney, 1996. Institute of Ocean Sciences Nutrient Methods and Analysis. Can. Tech. Rep. Hydrogr. Ocean Sci. 182: vi + 43 p.

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ABSTRACT

Barwell-Clarke, J. and F. Whitney, 1996. Institute of Ocean Sciences Nutrient Methods and Analysis. Can. Tech. Rep. Hydrogr. Ocean. Sci. 182: vi + 43 p.

Nutrient samples have been analyzed at the Institute of Ocean Sciences (IOS) using continuous flow autoanalyzers (CFAs) for almost 20 years. This report provides details of current procedures employed at IOS, as modified to meet the requirements for high quality data of the World Ocean Circulation Experiment (WOCE). Two systems are currently in use at IOS in support of North Pacific, Arctic and coastal programs. This manual covers the analysis of nitrate + nitrite, silicate, and phosphate plus a brief outline of the ammonia method recently in use on selected projects. A description of the CFA and labware is followed by details of sampling and storage, calibration, analytical methods (including schematics) instrument set-up, data collection and processing. A trouble shooting guide is included to cover some of the more common problems associated with this instrument.

Key words: ammonia, analysis, continuous flow autoanalyzer, nitrate, nitrite, nutrients, silicate, oceanography, phosphate, sampling, storage.

RÉSUMÉ

Barwell-Clarke, J. and F. Whitney, 1996. Institute of Ocean Sciences Nutrient Methods and Analysis. Can. Tech. Rep. Hydrogr. Ocean. Sci. 182: vi + 43 p.

Des échantillons des éléments nutritifs ont été analysés à l'Institut des Sciences de la Mer (ISM) en utilisant CFAs (Continuous Flow Autoanalyzers) pendant 20 ans. Ce rapport donne des détails sur les procédures actuelles utilisées à l'ISM, améliorées afin de satisfaire les exigences de qualité de WOCE (World Ocean Circulation Experiment). Deux systèmes sont maintenant utilisés a l'ISM pour les programmes du Pacifique Nord, de l'Arctique et des eaux côtières. Ce manuel traite de l'analyse de nitrate + nitrite, de silicate et de phosphate et fournit un exposé sommaire de la méthode de l'ammoniac utilisée récemment pour des certains projets. Une déscription du CFA et de l'équipement de laboratoire est suivie par des détails de la technique d'échantillonnage et de l'emmagasinage, de la calibration, des méthodes analytiques (y compris des diagrammes), de l'arrangement des instruments, de la collecte de données et du traitement de données. Un guide est inclu pour aider à résoudre des problèmes communs associés à cet instrument.

Mots-clés: ammoniac, continuous flow autoanalyzer, échantillonnage, éléments nutritifs, emmagasinage, nitrate, nitrite, océanographie, phosphate, silicate.

1. INTRODUCTION

Nutrient concentrations are commonly measured using Continuous Flow Analyzers (CFAs). Chemistries are controlled by a multi-channel peristaltic pump which regulates the flow rate of samples and reagents throughout the analytical procedures. The sample flow is segmented with air bubbles to enhance mixing of reagents and sample, and to reduce smearing of the sample. The sample-reagent mixture reacts chemically to produce colour in proportion to the concentration of the nutrient in the sample. The colour intensity of the mixture is measured in a flow-through colorimeter fitted with interference filters specific to the method; the output is an analog voltage which is proportional to light absorbance.

Analysts at the Institute of Ocean Sciences (IOS) have proved the reliability of their procedures through participation in the ICES nutrient intercomparison exercises in 1989 and 1993. As labs number 10 and 11 of the 1993 ICES study, both IOS analytical systems placed among the top dozen of 132 worldwide labs that analyzed nitrate + nitrite, phosphate and silicate in seawater (based on the index of consensus standard deviation shown in Aminot and Kirkwood, 1995). This is in part due to the broad experience garnered by analysts at IOS, and to the continued use of commercial standards that are integral to our data quality checks.

2. APPARATUS

2.1. CONTINUOUS FLOW ANALYZER (CFA)

A Technicon AutoAnalyzer™ II (AA-II) has been used by IOS to determine seawater concentrations of dissolved silica (referred to as silicate, in this manual), phosphate, nitrate + nitrite since its inception in 1978. Ammonia and nitrite procedures have been tested more recently.

The AA-II consists of 6 components: automated sampler, peristaltic pump, analytical cartridges, colorimeters, chart recorders (optional), and an IBM compatible personal computer (PC). The automated sampler introduces seawater samples into a zero nutrient solution of 3.2 % NaCl (w/v) in deionized water at precise intervals determined by a timing cam. Samples are separated by the 3.2 % NaCl "wash" at the rate of 20 samples per hour - 1.5 minutes of sample and 1.5 minutes wash. The peristaltic pump simultaneously pumps reagents and samples through the analytical cartridges which consist of injection fittings, mixing coils and heating baths (phosphate and ammonia). The sample then flows through the colorimeter in an unsegmented (bubble free) stream. Light passes through the flowcell and is detected on a phototube. Colorimeters provide two outputs: one signal that is used to drive a chart recorder (0 to 5 V dc) and one that connects to an analog to digital converter and computer which will overrange to 8 V dc.

The components must be arranged such that tubing lengths between sampler, pump, and analytical cartridges are as short as possible. This applies particularly to parts of the flow stream that are not segmented by bubbles, e.g. between the sampler and the pump, and from the cadmium column to the pump. This avoids excessive mixing between adjacent samples and wash water.

2.2. DEIONIZED WATER

Reliable deionized water is essential for quality nutrient analysis. There are two types of systems used to produce 18 Megohm-cm water at IOS: a Millipore™ system, fed by a reverse-osmosis purifier, which produces large volumes of Milli-Q (MQ) and double run Milli-Q (DMQ) water, and smaller bench top Nanopure™ systems which can be taken to sea. The Nanopure™ systems require the use of a prefilter, as water quality onboard ships and in labs is variable. Several carboys of DMQ water should be taken on cruises to monitor the quality of the Nanopure™ water being produced at sea and provide good quality water if shipboard cartridges fail. Ships' water supplies have been fouled by high particle load from shore water and organics arising from the fresh epoxy coating of holding tanks in past cruises.

2.3. LAB WARE

All labware including beakers, graduated cylinders, sample test tubes, pipette tips etc. must be acid cleaned, covered or stored upside down in a cupboard to avoid contamination from stock standards, soap (phosphate), nitric acid vapours (nitrate), reagents (phosphate and ammonia) and glassware (silicate).

Labware is first rinsed with MQ water to remove dissolved salts or concentrated reagents, soaked in 10 % HCl (v/v) for 2 - 4 hours or more, then rinsed twice with MQ and once with DMQ and air dried. All labware in the nutrient labs must be acid washed in this manner before placing in the lab cupboards. Use separate graduated cylinders for sulphuric and phosphoric acid (labeled H_2SO_4 and H_3PO_4) and rinse well before acid cleaning. Glass and plastic test tube caps must be separated before washing. This prevents the caps from nesting inside each other while being acid washed and rinsed.

* No soap or nitric acid is allowed in nutrient work areas. *

When preparing reagents and standards for silicate analysis, avoid the use of glassware whenever possible or keep contact to a minimum.

2.4. REAGENTS

All reagents used must be high purity reagent or analytical grade. When mixing or weighing out reagents for a cruise, information detailing stock and lot number, company name, analyst (initials), and date weighed are entered in a nutrient lab book. This lab book also contains all information pertaining to the particular AutoAnalyzer™ including reagent preparation, tubing changes, maintenance, lamp replacement, samples analyzed etc.. Pre-weighed reagents are also labeled with the above information and this label should be taped to the lab notebook once the reagent has been prepared. The analyst also notes the time and date of reagent preparation and when the reagent was introduced on-line in the analysis. Avoid adding a reagent in mid-run by ensuring you have enough solution to last for all analyses. A complete list of labels (including those for standards) is provided in Appendix 1.

2.5. LOW NUTRIENT SEAWATER (LNSW)

At IOS, 3.2 % NaCl is used to prepare standards and as the wash water to establish the analytical baseline. Comparisons with standards prepared in Low Nutrient Seawater (LNSW) have shown no significant differences. Calibration standards are ideally prepared in LNSW (QUASIMEME, Bulletin No. 3, July 1995) and the wash water should consist of the same. Commercial standard kits that use LNSW are now available from Ocean Scientific International in Hampshire, U.K..

3.0 SAMPLING AND STORAGE

3.1. WATER SAMPLERS

Water samplers must be examined regularly to ensure they are free of rust and biological films. Rust (from corroded springs) can be removed with 8 N HCl and organic material can be removed with scouring and/or suitable (nutrient free) detergent solutions . Samplers constructed from PVC such as Niskin, GO-FLO, BOT and Scripps bottles, have proven reliable. Note that nylon fittings on sample bottles become brittle when soaked in HCl.

3.2. NUTRIENT SAMPLE TUBES

Autoanalyzers at IOS have been modified to handle 16 x 125 mm test tubes, so that sampling tubes can be placed directly onto the sample carousel. Samples that are going to be analyzed immediately can be collected in polystyrene tubes. However, phosphate may be adsorbed onto polystyrene when samples are stored more than 12 - 24 hr. All frozen samples should be collected both in polystyrene (for silicate and nitrate) and glass (for phosphate) tubes. Plumbing is typically set up to analyze silicate, nitrate + nitrite and phosphate from one polystyrene tube, or silicate and nitrate + nitrite from the plastic tube and phosphate from a glass tube. Ammonia is analyzed from glass test tubes only. Test tubes are acid washed before use, as noted in section 2.3, and should be discarded if fine fracture lines appear (polystyrene tubes) as these lead to sample contamination or loss. Discard any glass tubes that are chipped on the lip or thread.

Label test tubes with water and alcohol proof markers, keeping labels as simple as possible by including cruise number, cast/station ID, and sample bottle number/depth. An even simpler label includes only cruise ID and a unique sample number which corresponds to data in the cruise log. A cruise log of all sampling must be made available to the analyst.

A condensed version of sampling and storage techniques is listed in Appendix 2.

3.3. SAMPLING PROCEDURE

Nutrient samples are routinely collected from a ship's seawater supply, buckets used to sample surface waters, or sampling bottles. Nutrients are subsampled from water samplers following gas sampling (CFC's, oxygen, CO_2, etc.). When drawing nutrient samples, note that human skin is a significant source of contamination (Kérouel and Aminot, 1987). Avoid touching the spigot, inside of the cap or test tube with dirty fingers. Also avoid contaminating samples with raindrops or any other seawater dripping off the sampler. Duplicate test tubes (including the caps) are rinsed two or three times with sample and filled to within 2 cm of the top. DO NOT overfill the test tubes because it may be necessary to freeze samples (in the case of instrument failure or by program necessity) and the samples expand on freezing . If the sample is overfilled, this expansion will cause some of the sample to be lost through the cap and the remaining sample will be compromised. When analyzing frozen samples if salt crystals are present around the cap it must be noted in the analysis log as this usually indicates overfilling. Do not underfill the test tube as the sampling probe will not reach into the sample if the tube is less than 2/3 full.

3.4. SAMPLE STORAGE

3.4.1. Fresh Samples

For best results, nutrient samples should be analyzed as quickly as possible . Ideally, the AutoAnalyzer™ should be running with standards prepared as a hydro cast is being completed, so that nutrient samples can be placed directly on the sampler. However, samples can be stored in the dark in a 4°C refrigerator for at least 12 hours without measurable change. Cool storage for up to two days is preferable to freezing, although there will be some loss in data quality.

3.4.2. Frozen Samples

If storage is required for longer than 48 hours the samples should be frozen as quickly as possible. Freeze samples in wire or plastic racks in a chest freezer (do not use the frost free freezer above a refrigerator unless necessary). Samples must remain upright until frozen to avoid brine spillage. Retighten caps and store glass and plastic test tubes together for each station in marked plastic bags in the freezer. An even faster method of freezing is an alcohol filled freezing bath, but extra caution must be used to avoid contaminating the samples with alcohol. Keep samples upright, ensuring that their caps are above the level of the alcohol while freezing. Before storing frozen samples, wipe excess alcohol from tubes and re-tighten caps which may loosen during freezing.

For results of the highest accuracy and precision, prolonged storage of samples in the refrigerator or freezer should be avoided. This is evident from Table 1 from a comparison of the standard deviation of pairs of samples (Sp) analyzed fresh (Arctic), frozen (coastal), and frozen (Pacific).

TABLE 1. Standard deviation of pairs of fresh and frozen samples.

	Nitrate (µM)		Silicate (µM)		Phosphate (µM)		no. of pairs
	Sp	Range	Sp	Range	Sp	Range	
Fresh (Arctic)	0.01	0 - 17	0.4	3 - 46	0.02	0.5 - 2.3	221
Frozen (Coastal)	2.2	7 - 29	5.0	17 - 82	0.08	0.6 - 3.8	25
Frozen (Pacific)	0.7	0 - 36	1.1	0 - 64	0.11	0.0 - 2.4	50

3.5. THAWING FROZEN SAMPLES

Thaw frozen samples, about 10 at a time, using a tepid water bath (approx. 40°C) or an electric fan (the samples should thaw completely in less than 1/2 hour). Check that caps are tight and above water level to avoid contamination. Shake samples vigorously after thawing to mix the fresh and brine layers formed on freezing.

If silicate concentrations are greater than 40 µM or samples have been frozen for more than several weeks, thaw these samples (plastic tubes) for 24 hours before analysis. This allows polymerized silicate to redissolve into solution (Macdonald et al., 1986). Do not change the 1/2 hour thaw procedure for phosphate.

4.0 CALIBRATION

Caution: Labour Canada has instituted a policy that ensures all lab workers are warned of the dangers of working with noxious chemicals. Training in the Workplace Hazardous Materials Information System (WHMIS) is a prerequisite to working in labs at IOS.

A primary tool of an analyst is the gravimetric balance. Most measurements made of samples and standards are verified against weight, thus the careful use of a variety of balances is a basis for good chemistry. Periodic checks of balances, using standard weights, are crucial.

Volumetric glassware used to prepare stock standards must be gravimetrically calibrated Class A. Volumetric ware is calibrated at 20°C and deviations from this temperature during standard preparations require corrections to be made. An aqueous solution of 1.000 L weighs 998.23 g at 20°C and 997.07 g at 25°C.

Eppendorf Maxipettor™ tips used to prepare diluted standards should be individually calibrated by the analysts who will use them and these calibration results recorded in the AutoAnalyzer™

log book. Generally these tips are accurate within 0.5% but this can be improved upon with careful calibration.

4.1. STOCK STANDARD PREPARATION

Prior to a cruise, stock standard solutions are prepared in the lab using dried, high purity, crystalline chemicals. Dry the chemicals (see below for details) and allow them to cool in a desiccator for several hours. Weigh salts to the nearest 0.1 mg in acid washed and dried 5 mL bottles. Note the weight of the bottle before taring the balance and transferring the standard to a volumetric flask, to check for residue. Dissolve salts using DMQ that is at room temperature.

The stock standards are considered stable for six months. Never store the silicate standard in the refrigerator. The new stock standard concentration should be compared to the previous "old" batch and should agree within 1 %.

These standards are diluted with 3.2 % NaCl solution to prepare working standards.

Preparation of 3.2 % NaCl:

Dissolve 500 g NaCl into 15.6 L DMQ. Mix thoroughly to dissolve.

Standard labels are listed in Appendix 1.

4.1.1. Nitrate Stock Standard 2,500 µM NO_3^-

Quantitatively transfer 0.2528 g potassium nitrate (KNO_3, F.W. 101.11, dried by desiccation) to a 1000 mL volumetric flask and dissolve in DMQ. Dilute to exactly 1000 mL and record the temperature by measuring the temperature of a 50 mL aliquot of standard in a small beaker. Rinse a dark plastic bottle 3 times with about 15 mL of standard then bottle the remaining solution, adding 1 mL chloroform as a preservative (chloroform is a volatile compound that will not persist as a preservative over the expected life of the standard solution). One mL of this stock when diluted to 250 mL with 3.2% NaCl gives a working standard of 10.0 µM nitrate.

4.1.2. Nitrite Primary Stock Standard 2,000 µM NO_2^-

Quantitatively transfer 0.1380 g sodium nitrite (NaNO2, F.W. 69.00, dried at 105°C for 2 hours) to a 1000 mL volumetric flask and dissolve in DMQ. Dilute to exactly 1000 mL then record the temperature of a 50 mL aliquot transferred to a small beaker. Store in a dark plastic bottle that has been rinsed 3 times with 15 mL standard. Add 1 mL chloroform as a preservative.

4.1.2.1. Nitrite Stock Standard 100 µM NO_2^-

Using volumetric glassware dilute the primary stock standard twenty fold (e.g. 5 mL to 100 mL), to prepare the stock standard of 100 µM NO_2 used to prepare the working standards. One mL of this stock when diluted to 250 mL of 3.2 % NaCl gives a 0.40 µM nitrite standard. Add 1 mL chloroform as a preservative.

4.1.3. Silicate Stock Standards 7,500 and 5,000 µM Si

Quantitatively transfer 1.4105 g sodium silicofluoride (Na_2SiF_6, F.W. 188.06, dried at 105 °C for 2 hours) into a plastic beaker containing ~800 mL DMQ and stir for 4 - 6 hours (cover the beaker to prevent contamination). Transfer to a 1000 mL glass volumetric flask, rinsing the beaker 5 times, and quickly bring to 1000 mL with DMQ. Record the temperature of the standard and store it in an amber plastic bottle. Keep contact with glassware to a minimum. This stock standard is used for North Pacific Ocean Cruises. One mL of this stock when diluted to 250 mL of 3.2 % NaCl gives a working standard of 30.0 µM silicate.

To prepare 5,000 µM silicate standard (used for Arctic and surface samples) dissolve 0.9403 g sodium silicofluoride in 1000 mL DMQ as above. One mL of the Arctic stock when diluted to 250 mL of 3.2 % NaCl gives a 20.0 µM silicate standard.

4.1.4. Phosphate Primary Stock Standard 2,500 µM PO_4^{-3}

Quantitatively transfer 0.3402 g potassium dihydrogen phosphate (KH_2PO_4, F.W. 136.09, dried at 105 °C for 2 hours) to a 1000 mL volumetric flask and dissolve in DMQ. Dilute to exactly 1000 mL with DMQ, record temperature and store in a teflon (to avoid silicate contamination since standards are often combined) or an amber glass bottle. Add 1 mL chloroform as a preservative.

4.1.4.1. Phosphate Stock Standard 250 µM PO_4^{-3}

Using volumetric glassware dilute the primary stock standard ten fold, to prepare the stock standard of 250 µM phosphate used to prepare the working standards. One mL of this stock when diluted to 250 mL of 3.2 % NaCl gives a 1.00 µM phosphate standard. Add 1 mL chloroform as a preservative.

4.1.5. Ammonia Primary Stock Standard 2500 µM NH_4^+

Quantitatively transfer 0.1337 g ammonium chloride (NH_4CL, F.W. 53.49, dried at 105 °C for 2 hours) to a 1000 mL volumetric flask and dissolve in DMQ. Dilute to exactly 1000 mL, record the temperature and store refrigerated in an amber glass bottle. Add 1 mL chloroform as a preservative.

4.1.5.1. Ammonia Stock Standard 125 µM NH_4^+

Using volumetric glassware dilute the primary stock standard twenty times, to prepare the stock standard of 125 µM NH_4 used to prepare the working standards. One mL of this stock when diluted to 250 mL of 3.2 % NaCl gives a 0.50 µM ammonia standard. Add 1 mL chloroform as a preservative. Store refrigerated in an amber glass bottle.

4.2. WORKING STANDARD PREPARATIONS

Mixed working standards are prepared by diluting 1 - X mL of the nitrate, silicate and phosphate stock standards in 250 mL plastic (nitrate, silicate and phosphate) or glass (phosphate/

ammonia) volumetric flasks with 3.2 % NaCl, to obtain the appropriate standard range for samples as listed in Table 2. Working standards will last two days (if refrigerated overnight) and are used to calculate the regressions used in computing sample concentrations.

For North Pacific cruises prepare five concentrations (one zero and four standards) to bracket the expected concentration range and analyze these in duplicate (total of 10 standard samples). For Arctic work prepare four concentrations (one zero and three standards) and run in triplicate (total of 12 standard samples). The program used to calculate results can handle a maximum of 12 standards.

For high precision work (i.e. WOCE) these standards are considered nominal and must be corrected for the temperature corrected volume of the 1000 mL flask and the volume delivered by pipette tips.

According to QUASIMEME Bulletin No. 3 (July 1995), natural low nutrient seawater (LNSW) of similar salinity to that of the samples is the only completely appropriate matrix for calibration purposes.

TABLE 2. Standard Concentration Ranges.

Area	Nitrate (µM)	Nitrite (µM)	Silicate (µM)*	Phosphate (µM)	Ammonia (µM)
North Pacific (to 6000 m)	0 - 48	0 - 2	0 - 180	0 - 3.6	0 - 2
Coastal/Arctic	0 - 30	N/A	0 - 60	0 - 3	0 - 5
Saanich Inlet (anoxic)	0 if H_2S present	N/A	0 - 180	3 - 7.5	0 - 25
Thermal Vents	0 - 50	N/A	0 - 200	0 - 4	N/A

* In the Pacific Ocean near thermal vents, the Bering Sea and the Deryugin Basin (Sea of Okhostk), silicate concentrations can exceed 200 µM.

4.3. EXTERNAL CALIBRATIONS

There are three commercial standards used to calibrate the primary standards prepared at IOS; Sagami Standards, Acculute Standards and the Marine Nutrients Standards Kit.

4.3.1. Sagami Standards

Sagami Standards are available from WACO Chemical (Richmond, VA) and are produced by Sagami Chemical of Japan. Nitrate and silicate are prepared in 30.5 % NaCl solution ready for analysis and appear to be stable for several years. Phosphate standards have proven unreliable due apparently to a short shelf life.

4.3.2. Acculute Standards

Acculute Standards for nitrate, silicate, and phosphate are available from Anachemia Scientific (Rouses Point, NY) and are diluted in DMQ to prepare concentrated stock standards. These are then diluted with 3.2 % NaCl and compared with standards prepared at IOS.

4.3.3. Marine Nutrients Standards Kit

Ocean Scientific International (Hampshire, U.K.) have recently produced a seawater calibration standard kit for dissolved nutrients (nitrate, nitrite, phosphate, and silicate). The kit contains concentrated nutrient standard solutions and low nutrient sea water (LNS). The standards are diluted in LNS to overcome any saline matrix effect.

4.4. QUALITY ASSURANCE DURING A CRUISE

A simple way to monitor day to day variability in analytical procedures is to routinely analyze a single bulk sample with each batch of collected samples. At the start of a cruise collect a 5 L carboy of deep water from the first hydro cast. Run a sample of this water with each station's samples, and keep a record of its concentration over the duration of the cruise. The concentration may drift slightly due to storage, but should not change erratically unless an analytical problem develops.

In addition, standards are embedded within station samples and treated as unknowns to test for problems with instrument drift, baseline variability or sample carry-over.

5.0 ANALYTICAL METHODS

This manual covers the analysis of unfiltered seawater samples only. Analysis of sediment pore waters or ice cores may require pre-treatment (e.g. filtering or diluting samples). Procedure modifications should be discussed with the scientist requesting analyses.

This section details analytical methods for each nutrient including flow schematics, notes about quirks and any known interferences for each procedure.

Reagents are pre-weighed into acid cleaned bottles and labeled with pertinent information (chemical Company Name, Lot No., date weighed, weighed by: initials). On preparing the reagent, the label is removed from the reagent bottle and glued into the notebook. The date and time of reagent preparation is also recorded in the notebook. See Appendix 1 for reagent and standard labels.

5.1. NITRATE + NITRITE

Since the procedure for analyzing NO_3 involves reducing this anion to NO_2 before reacting it with colour developing reagents, nitrite is also detected in this analysis. In most waters nitrite is

a minor nutrient, typically not exceeding 0.30 µM in surface North Pacific waters. In the deep ocean, nitrite is generally not detectable.

The nitrate + nitrite analysis described in Technicon Industrial Methods No. 158-71 W (revised August 1979) is a modification of the Armstrong et al. (1967) procedure. Nitrate is reduced to nitrite in a column of copperized cadmium. The nitrite ion is then reacted with sulfanilamide and N-Naphthylethylene-diamine to form a red azo dye.

The schematic for nitrate analysis is shown in Figure 1. For the AA-II a 50 mm flowcell (199-B007-01), 550 nm interference filters, and Technicon S-10 phototubes are used in the colorimeter. At high nitrate levels (up to 48 µM on North Pacific Ocean surveys) the sample injection tubing has been decreased from black/black (0.32 mL/min.) to orange/white (0.16 mL/min.) to increase linearity over the concentration range.

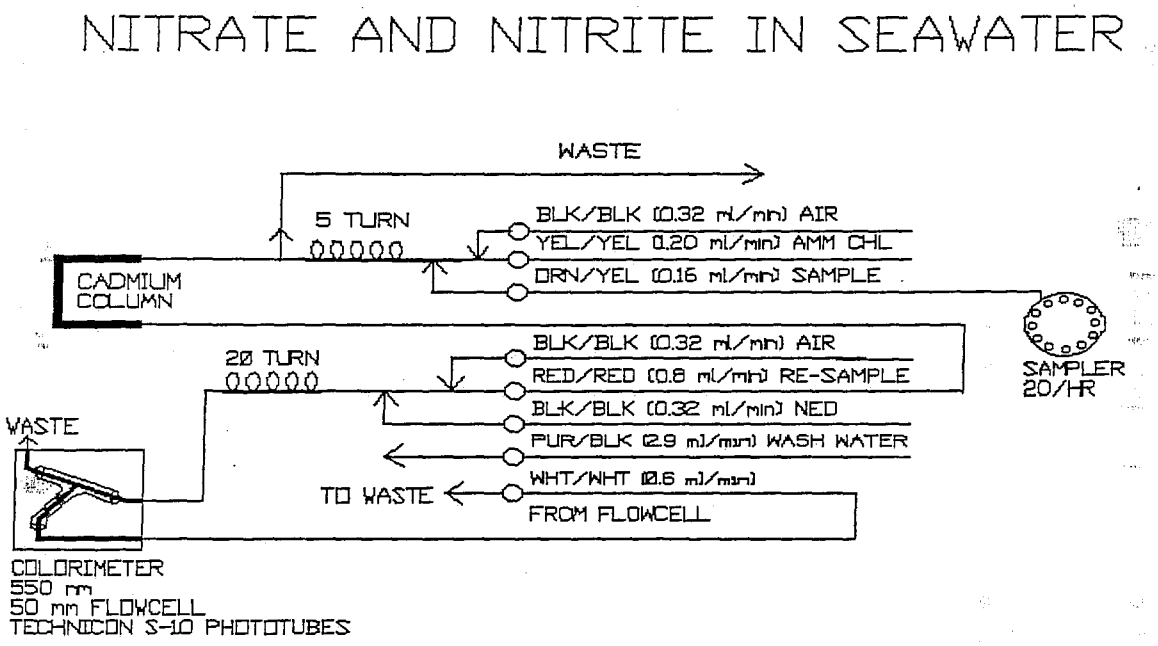

Figure 1. Schematic diagram for AA-II Nitrate method.

5.1.1. Nitrate Reagents: Technicon Industrial Methods No. 158-71W

Ammonium Chloride: Need 750 mL/10 hr

Dissolve 100 g NH_4Cl in DMQ and dilute to 10 L. Adjust pH to 8.5 with NH_4OH (requires between 15 and 20 mL conc. NH_4OH). The ammonium chloride must be buffered to obviate the need for salinity corrections and to maintain a uniform and high reduction efficiency in the Cd column (Collos et al., 1992).

Stable many months.

Colour Reagent: Need 200 mL/10 hr

To 1500 mL DMQ add 200 mL conc. H_3PO_4 and 20.0 g sulphanilamide. Dissolve completely (do not heat). Add 1.0 g N-1-naphthylethylenediamine dihydrogen chloride and dissolve. Dilute to 2 L with DMQ and add 1 mL Brij-35. Store in amber plastic and refrigerate. Stable for about 2 months.

NOTE: The use of phosphoric acid in the nitrate colour reagent is a potential source of phosphate contamination and should be avoided (Aminot, 1995). It is possible to modify the nitrate technique by using hydrochloric acid as in Armstrong et. al., (1967) and more recently Koroleff and Grasshoff (1983).

Nitrate Reagents: Koroleff and Grasshoff (1983)

Ammonium chloride:

Dissolve 75 g ammonium chloride in 5 L DMQ. Adjust to pH 8.5 with about 12 mL NH_4OH.

Sulphanilamide:

Dissolve 5 g sulphanilamide and 50 mL concentrated hydrochloric acid in 500 mL DMQ and bring to 1L with DMQ. Store refrigerated in amber bottle.

Coupling reagent:

Dissolve 0.5 g N-1-napthylethylendiamine dihyrochloride (NED) in 1 L DMQ and add 1 mL Brij 35. Store refrigerated in amber bottle.

Note: The Koroleff and Grasshoff method requires some plumbing changes to the nitrate manifold.

5.1.2. Cadmium Column Preparation and Storage

Figure 2 shows a packed Cd-Cu column. The reductor column tube is a U-shaped 14 cm length of 2.0 mm ID glass tubing. It takes approximately 10 g of cadmium filings to fill one column. Prepare several columns at once - they can be stored for several months. Before filling the column, prepare the cadmium as follows:

1. Wash new or used Cadmium granules (Pulse Instrumentation part T11-5063-62) several times with 1.2 N HCl to remove grease and dirt. Stir gently with a glass stirring rod. Rinse well with DMQ.

2. Decant off the DMQ and slowly add approximately 100 mL of 2 % $CuSO_4.5H_20$ for each 10 g cadmium. Add the $CuSO_4.5H_20$ in 10 to 20 mL aliquots while stirring gently with a glass rod until no blue colour remains in solution and reddish particles appear. Stop adding $CuSO_4.5H_20$ at this point. The cadmium will be a dark gray/brown colour.

3. Decant and wash thoroughly at least 10 times with DMQ to remove all colloidal copper. Do not expose the cadmium filings to air beyond this point. Always cover them with DMQ or ammonium chloride reagent. As colloidal copper is a major source of contamination, make sure to rinse the treated cadmium thoroughly with DMQ before packing the column.

4. Fill the reductor column with ammonium chloride solution and transfer the prepared cadmium particles to the column using either a Pasteur pipette or small spatula, taking care to

always cover the cadmium particles with ammonium chloride reagent. Gently tap the column while filling to tightly pack the granules (or use a vibrating tool) and fill to within 2 cm of the top. The column should be well packed for optimum performance.

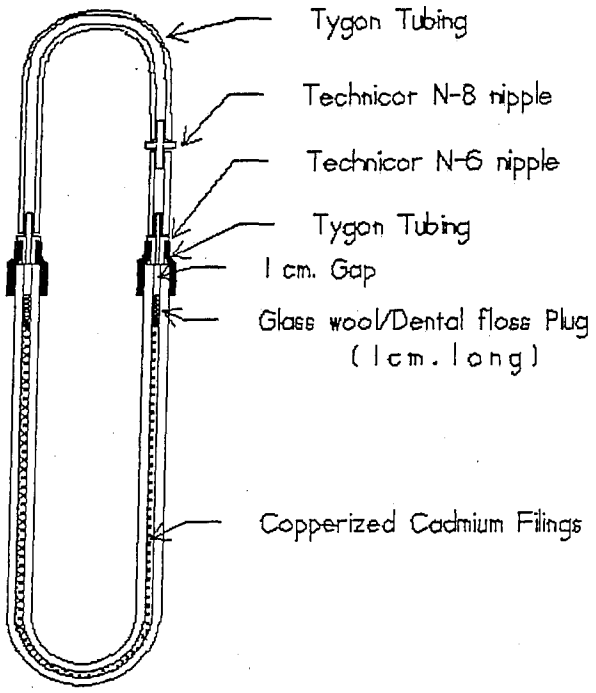

Figure 2. Nitrate Cadmium Column

5. To both ends of the U-tube, insert glass wool or dental floss, sleeve with Tygon tubing and insert N-5 nipples. Cap the U-tube with tubing which has an N-8 nipple placed 2 cm from one end. Fill the tubing and nipples with ammonium chloride solution, avoiding air bubbles.

6. Store the columns in a wide mouth plastic bottle containing half strength ammonium chloride solution.

7. Prior to sample analysis remove the glass wool/dental floss plug from the **inlet** side of the column and replace with copper wool.

8. Always ensure that the ammonium chloride solution is on line before hooking up the cadmium column. Running out of colour reagent, sample or rinse water will not introduce air into the cadmium column, but if the ammonium chloride supply is interrupted, air will enter the column and it must be repacked and reconditioned before use.

9. A new column needs to be conditioned with a 20 to 30 µM nitrate standard to stabilize its reduction efficiency. Place the sampling probe directly into a conditioning standard and pump it through a fully operational nitrate system for 20 - 30 minutes until the signal has stabilized.

10. The efficiency of the column in reducing nitrate is usually 99%. A properly prepared and treated column will last for several hundred samples. To check the efficiency of the column prepare a 20 µM nitrate and 20 µM nitrite standard. Run 4 each of these standards, alternating nitrate and nitrite, through the nitrate channel. Measure the peaks and calculate the percentage efficiency based on the difference (100 % efficiency is zero difference). As reductor efficiency declines with use, a source of analytical error is introduced in any waters that contain nitrite (Garside, 1993).

11. Cd in columns will settle with time and use. Top off the column with copperized cadmium when settling exceeds 1 cm. Recondition the column with 20 to 30 µM nitrate standard before using.

12. When the peak heights of nitrate standards start to decrease (the C2 factor of the standard curve changes, see section 7), replace the column.

5.1.3. Nitrate Interferences and Notes

1. When present in sufficient concentration, metal ions may produce a positive error (Federal Water Pollution Control Administration Methods for Chemical Analysis of Water and Wastes, November, 1969).

2. Samples containing hydrogen sulfide interfere with the copper-cadmium column and should not be run. If the sample smells of sulfide, assume the NO_3 concentration is zero since bacteria utilize NO_3 before starting sulphate reduction.

3. Samples preserved with $HgCl_2$ should not be analyzed unless greatly diluted.

4. Either centrifuge and decant or filter turbid samples. When filtering samples it may be necessary to clean the filters first with distilled water (Marvin et al., 1972).

5. Never run DMQ through the column in place of the ammonium chloride reagent.

6. Human skin is a significant source of nitrate contamination (Kérouel and Aminot, 1987) as are nitric acid vapours produced by some digestion procedures and smoking.

7. Contamination can be introduced to wash waters by the presence of nitrate and nitrite from reagents (e.g. ammonium chloride) and should be determined by using freshly produced DMQ. All stored water absorbs nitrogenous compounds from the atmosphere and the reduced forms are susceptible to oxidation to nitrite and nitrate (ICES Coop. Res. Rep. No. 213, Dec. 1995). Always replace the NaCl and DMQ wash bottles with fresh NaCl and DMQ at the start of each day's analysis. Likewise, ensure there is enough reagent to complete the run - avoid topping up reagents mid-run.

8. High phosphate may decrease the efficiency of the Cd-Cu (Olson, 1980).

9. If the colour reagent becomes unhooked, a white precipitate may develop on the glass coils. This can easily be removed using a syringe filled with 10 % HCl to flush the line. Rinse thoroughly with DMQ afterwards.

In order to determine nitrate, nitrite must be subtracted from the total (nitrate + nitrite).

5.2. NITRITE

Nitrite has only been routinely analyzed for WOCE surveys. Nitrite is analyzed by omitting the cadmium column and its debubbler from the nitrate procedure and greatly boosting the STD CAL of the colorimeter to increase sensitivity. Sample is pumped at 0.32 mL/min..

Connecting a nitrite channel doubles the demand on all nitrate reagents.

5.3. SILICATE

Dissolved Si (silicate) is analyzed by the method described in Technicon Industrial Methods. No. 186-72W and is in essence that of Armstrong et al. (1967). Silicate is reduced to a molybdenum blue complex by ascorbic acid. Oxalic acid is added to remove phosphate interference.

The schematic for the silicate analysis is shown in Figure 3. For the AA-II a 15 mm flowcell (199-B006-01A), 660 nm interference filters, and Technicon S-10 phototubes are used in the colorimeter. This method is non-linear at high silicate concentrations (above 120 µM).

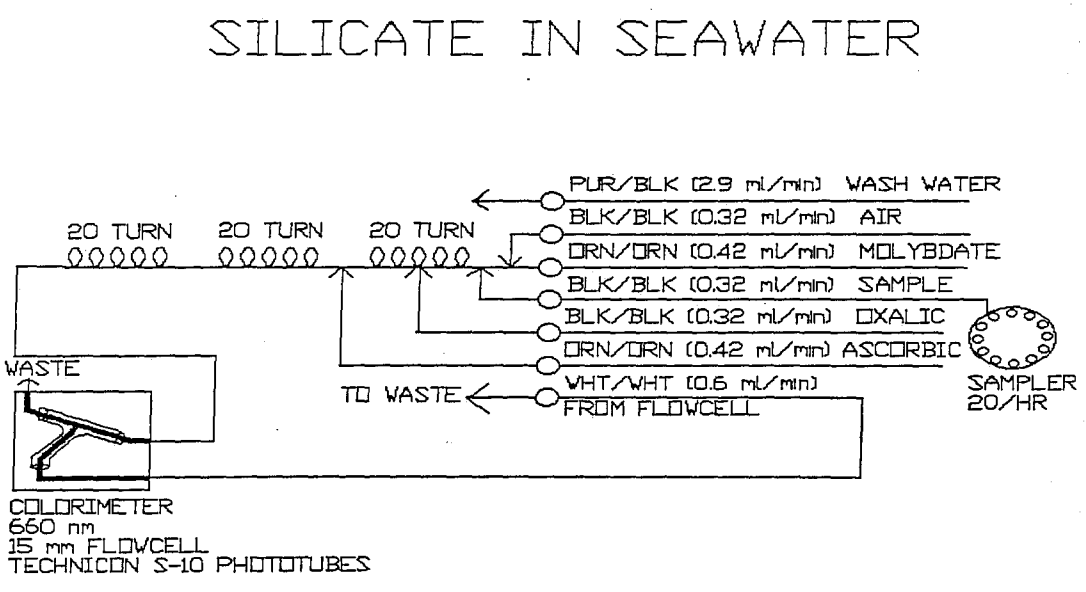

Figure 3. Schematic diagram for AA-II silicate method.

5.3.1. Silicate Reagents

Ammonium Molybdate: Need 250 mL/10 hr

Dissolve 10. 0 g $(NH_4)_6\ Mo_7O_{24}{\cdot}4H_2O$ in 1 L of 0.1 M H_2SO_4 (2.8 mL conc. H_2SO_4 per litre). Store in an amber plastic bottle and check periodically for precipitate on bottle walls. Stable 1 month or less (if standard peak heights decrease, or baseline is noisy, suspect this reagent). Storage in a refrigerator may increase its useful life.

Oxalic Acid: Need 200 mL/10 hr

Dissolve 70 g $H_2C_2O_4 \cdot 2H_2O$ in 900 mL DMQ and dilute to 1 L. Stable about 1 month. Avoid breathing powdered salt - wear a dust mask when weighing oxalic acid.

Ascorbic Acid: Need 250 mL/10 hr

Prepare acetone/DMQ/Ultrawet™ (replaces Levor IV) solution by diluting 50 mL acetone and 0.5 mL Ultrawet™ to 1 litre DMQ. Add ascorbic acid:

4.4 g to 250 mL or 8.8 g to 500 mL

and stir to dissolve. Solution is stable for 2 to 3 days if refrigerated.

5.3.2. Silicate Interferences and Notes

1. All labware used to prepare reagents for silicate analysis must be plastic as glass is a source of contamination.

2. This chemistry is temperature sensitive, thus lab temperature must be recorded every hour or two during a sample run. If the ambient temperature changes during a run, the silicate response may change by several per cent per degree Celsius (letter from A. Mantyla to Lou Gordon dated 16 April, 1991). Ideally there should be less than a 2 degree difference in temperature during a sample run.

3. Tannin, large amounts of iron, and sulfide interfere.

4. Filter samples containing high levels of particulate matter.

5.4. PHOSPHATE

Soluble orthophosphate is determined by Technicon Industrial Methods No. 155-71 modified by Brynjolfson (1973). The original procedure was that of Murphy and Riley (1962). Ammonium molybdate is added to the sample to form a phosphomolybdenum blue complex. Heating the sample enhances the rate of colour development.

The schematic for the phosphate analysis is shown in Figure 4. For the AA-II™ a 50 mm flowcell (199-B007-01), 800 nm interference filters, 37.5 C heating bath, and Technicon™ wide range S-1 phototubes (CE-25V) are used. The phosphate method is linear up to 8.0 µM (the highest standard concentration used for Saanich Inlet cruises).

PHOSPHATE IN SEAWATER

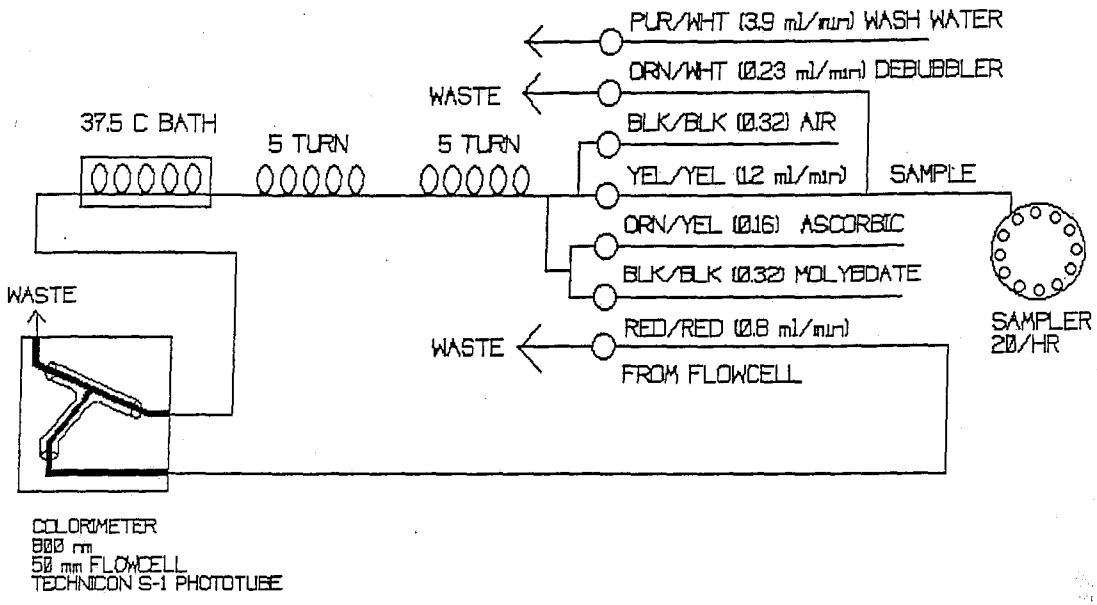

Figure 4. Schematic diagram for AA-II phosphate method.

5.4.1. Phosphate Reagents

Ammonium Molybdate: Need 200 mL/10 hr

Dissolve 10.0 g $(NH_4)_6\ Mo_7O_{24}{\cdot}4H_2O$ in about 1 L DMQ. Slowly add 120 mL H_2SO_4 while mixing. Allow this mixture to cool (use a cool water bath to hasten cooling). Dissolve 0.6 g $K(SbO)C_4H_4O_6{\cdot}1/2\ H_2O$ in 100 mL DMQ and add this to the cooled molybdate/acid mixture. Dilute to 2 L and store in amber glass bottle. Stable several months.

Ascorbic Acid: Need 100 mL/10 hr

Prepare acetone/DMQ/Ultrawet™ solution that contains 167 mL acetone and 4 mL Ultrawet™ diluted to 2 L with DMQ. Add ascorbic acid at 0.50 g per 150 mL and shake to dissolve. Prepare fresh every day.

5.4.2. Phosphate Interferences and Notes

1. A seawater turbidity blank can be estimated by sampling seawater while running DMQ through all reagent and wash lines. On North Pacific cruises where salinity is in the range 30 to

34.7 psu choose a mid depth sample and check this blank against the 3.2 % NaCl wash. There should be little difference, in which case turbidity interferences have been accounted for in using the salt NaCl solution for a baseline.

2. Turbidity blanks for individual samples should be determined when salinity is less than 27 ‰ or particle loads are high. This applies especially to coastal areas and near ice sampling.

3. To obtain a phosphate free blank, treat 3.2 % NaCl solution with 1 mL of 70 % $FeCl_3 \cdot 6H_2O$ plus 10 mL of a 4 % solution of Na_2CO_3 per litre of seawater (Alvarez-Salgado et al., 1992). This solution is left to settle for several days (or heated to boiling) and allowed to precipitate. The supernatant, free of precipitate, is phosphate free and can be compared to the NaCl solution used to set the analytical baseline. We have also compared 0.00 µM phosphate standards from Sagami to the 3.2 % NaCl baseline and found no phosphate present.

5.5. AMMONIA

Ammonia is only occasionally analyzed at IOS. The technique follows that of Koroleff (1970) as automated by Slawyk and MacIsaac (1972). It is based on the Berthelot-reaction where, at alkaline pH, ammonia, phenol and hypochlorite react to form indophenol blue.

The schematic for the ammonia analysis is shown in Figure 5. For the AA-II™ a 50 mm flowcell(199-B007-01), 600 nm interference filters, 80° C heating bath, and Technicon™ S-10 phototubes are used.

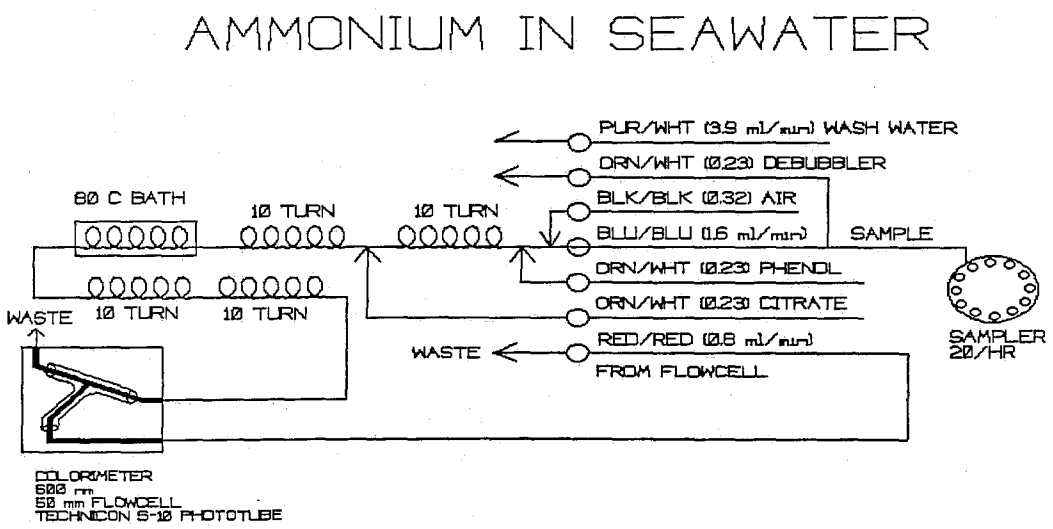

Figure 5. Schematic diagram for AA-II ammonia method.

5.5.1. Ammonia Reagents

Phenol/nitroprusside: Need 150 mL/10 hr

Dissolve 35.0 g phenol and 0.400 g sodium nitroprusside dihydrate in 1 L DMQ. Store refrigerated in amber glass bottle.

*** USE EXTREME CAUTION WHEN HANDLING PHENOL***

Citrate/Na hydroxide: Need 150 mL/10 hr

Dissolve 280 g sodium citrate and 15.0 g sodium hydroxide in 830 mL DMQ. Add 35 mL commercial bleach (freshly purchased and preferably Javex, which contains 6 % Na hypochlorite). Store refrigerated in amber glass bottle.

5.5.2. Ammonia Interferences and Notes

1. DMQ used to prepare standards and reagents should be freshly produced and collected directly from the DMQ outlet.

2. The air for segmenting the stream should be bubbled through 10 % HCl prior to introduction to the system.

3. Ammonia is very volatile so minimize exposure of samples, reagents, and standards to air. Following sampling, cover completely filled test tubes with parafilm before capping. Wearing gloves when collecting and handling ammonia samples, reagents and standards reduces contamination.

4. Keep samples refrigerated and do not store them or more than a few hours before analysis.

5. Run the colorimeter on Damp 1 or 2 if the baseline appears noisy.

6. Collect the waste from the ammonia line in a separate bottle marked "PHENOL WASTE". Add several pellets of NaOH to minimize the phenol smell and dispose of the wastes down the drain, flushing with lots of water.

7. Avoid analyzing low concentrations of ammonia at the same time that nitrate is being run because of potential contamination from ammonium chloride.

6.0. INSTRUMENT SET-UP

Two Technicon systems are used to analyze nutrients at IOS. The Ocean Science and Productivity Division AutoAnalyzer-II™ is attached to a wooden frame and is usually completely set-up and ready to run. Spare parts (tubing, glass coils, phototubes etc.) are stored in drawers below and the whole unit is easily moved from lab to ship despite its bulk. The Contaminants Chemistry/Ecology AA-II™ is designed for shipping and has been split into sections which fit into aluminum boxes. Each section is latched to the next to form a stable base and the tubing connections have to be made when setting up the instrument. Detailed set up instructions are found in Technicon manuals.

Systems are connected to PCs and/or strip chart recorders to log output from up to 4 colorimeters. The signal to the PCs is digitized in a black box that handles 4 inputs and connects to a computer via a serial port.

6.1. DAILY START UP

Turn colorimeters on and allow lamps 1/2 hour for warm-up. Check the condition of the lamps; if they appear blackened, replace them. Lamps can be left on when being used daily during cruises or long analytical runs to improve instrument stability (baselines especially show less drift). Set the colorimeter display to zero at the end of the day to protect phototubes.

Empty the DMQ and NaCl jugs and fill with fresh solutions at the start of each day as they can become contaminated from volatile nitrogen compounds. Make sure you have enough reagents to complete the day's run without having to refill in the middle of a run. Remove the sulphanilamide (nitrate), ascorbic acid (silicate) and ammonium molybdate solutions (silicate) from the refrigerator, loosen the caps, and allow to warm up to room temperature.

At this time, all reagent tubing lines should be hooked up to DMQ with the main valve to the DMQ bottle turned off. Stretch the tubing manifold onto the holding pins and remove the kimwipes from under the tubing. Attach the pump platen, open the main valve to DMQ and turn on the pump.

Allow DMQ to run through the system for 15 to 30 minutes, and check the system to ensure a good flow pattern. Make sure the silicon air bar tubing isn't pinched, check tubing fittings, look for leaks, check to see that no tubing is caught under the pump platen. Gently tap the tubing to dislodge any tiny bubbles in the lines. Once the DMQ has reached the phosphate (and ammonia) bath, turn it on. The bubble pattern may not be perfectly smooth until all reagents are hooked up because some reagents contain wetting agents to lubricate the flow.

Turn on the computer and start the program when the bubble pattern is uniform and past the flowcells of the colorimeter. Log data on the computer's hard disk, checking that there is adequate disk space (a typical day's run for three nutrients requires 150 KB). The data logging program, GETDATA, has a routine which queries the operator before it overwrites an existing file. Data files can be up to eight letters long and are usually named by the date of analysis (i.e. yymmddA for the first run that day, a subsequent analytical run on the same day being labeled yymmddB etc.) Files are generally stored in the same directory as the program on a hard disk, although they can be logged onto a floppy disk. The program will log up to four channels at a time (nitrate, silicate, phosphate, and either NO_2 or NH_4).

Confirm that each channel is operating correctly by setting first zero then full scale on a colorimeter and checking that the chart recorder and computer respond to these signals. Set the colorimeters to NORMAL sensitivity and adjust the baseline to just above zero (to allow for some drift over the day) by adjusting the reference aperture (coarse adjustments) or baseline adjust (fine adjustments) on the colorimeter. Always keep the sample aperture as open (turned clockwise) as possible. If a baseline is difficult to find, connect a voltmeter to the colorimeter output. The signal should be slightly positive, but could lie anywhere between +13 v and -13 vDC. Any erratic or unresponsive reading becomes the basis for troubleshooting the colorimeter (section 8).

Make up any reagents while DMQ is running through the lines. Make sure there is enough 3.2 % NaCl to prepare the standards and use as wash water for the entire run. Avoid making up a new batch in the middle of a run. Hook up the reagents starting with the ammonium chloride. Loosen the lids of the reagent bottles to allow unhampered flow. By the time all the reagents have been hooked up, the ammonium chloride solution has usually reached the cadmium column and the column can be hooked up. Label the strip chart (if using) where DMQ and reagents are added. There should be only a slight increase in the baseline when the reagents come on-line. If there is a significant increase, check your reagents and re-make any you suspect of being contaminated (phosphoric acid can contaminate glassware very easily).

6.2. CADMIUM COLUMN HOOKUP (NITRATE)

The cadmium column, which must be kept free of bubbles, is placed in line several minutes after the ammonium chloride is connected. Disconnect the pull through tubing (ammonium chloride plus sample) after the five turn coil and debubbler and connect the column (copper wool side first) without introducing any bubbles. If bubbles are pulled through, QUICKLY disconnect the column before they reach the cadmium. Use a 5 mL syringe filled with NH_4Cl to dislodge bubbles trapped in the column.

Always connect the side pushing NH_4Cl from the pump to the column first.

Make sure all connections are tight, especially to the ammonium chloride bottle. Bubbles must not reach the cadmium granules or the column has to be removed, retreated and reconditioned (a royal pain). Always top up the ammonium chloride bottle at the start of the day and check and re-check the connections. The debubbler will remove bubbles introduced by missing the sample or wash water, but will not remove bubbles if you run out of ammonium chloride.

Once the column is on line, hook up the NaCl wash water container and mark on the chart (if using) when it comes through. The baseline for phosphate should increase only slightly when the NaCl solution comes through (equal to about 0.05 µM phosphate).

6.3. COLORIMETER CHECKS

Colorimeters must be optically peaked whenever they have been changed, repaired or shipped for the start of a cruise. See section 9.1. to optically peak the colorimeters.

As the highest concentration standard for a set of analyses is running through the system, the STD CAL control on the colorimeter is used to set the voltage range to 90 to 95% of full range. Record the STD CAL setting in the nutrient log book, on data sheets and chart paper every day.

6.4. WAKE-UP SAMPLES

When the reagents, cadmium column and NaCl are on line, run 4 - 6 "wake-up" samples. These can be old samples or standards from the previous day and are used to condition the instrument. Make sure the peak shape is smooth and that the baseline is stable. An old high standard can be used to check the gain on the colorimeter at this time.

6.5. SAMPLE ANALYSIS

Record all pertinent information about sample identification, instrument operation, standards, etc. on nutrient data sheets (see Appendix 4). Log the lab temperature every 1 to 2 hours during analyses, as lab temperature and salinity of the sample is required to calculate results as umol/kg. Also, silicate colour development varies with lab temperature, so analyses should be carried out in an area where temperature changes are minimal.

Label strip charts (if using) with coloured pens (green - phosphate, red-nitrate, blue - silicate), and include date, cruise, station, standard concentration range, STD CAL, computer filename, and analyst, at the beginning of each run. Label each peak with the sample number in the appropriate colour.

When analyzing frozen samples, note if any of the sample tubes have been overfilled, samples are encrusted with salt and/or caps are loose. If samples have been frozen in an alcohol bath, examine each sample before thawing to determine if there is any alcohol sitting on top of the sample. If so, note this on log sheet, and pour off the alcohol before thawing.

A set of standards is run every 30-40 samples.

The PEAKBASE program accepts only 12 standards to calculate regressions.

When analyzing frozen samples, obtain a copy of the cruise log. Load about 20 samples at a time onto the sample carousel in the order standards, two zeroes (to bring the baseline down), samples from surface to bottom, two zeroes, then standards. Insert a zero to bring the baseline down at the end of a profile to avoid carryover into a sample that has low nutrient concentrations.

The GETDATA program is set up to log data for a maximum of 15 hours.

If you are going to analyze for a long period, break up your runs into shorter files, labeling them A, B, C etc.. Make sure you have enough space in the directory for the nutrient files (an 8 hour run for three nutrients uses about 150 KB).

As a check, run a standard as an unknown with each set of samples, trying to match the concentration with neighbouring sample concentrations so that there is no carryover effect. Check the calculated concentration - it should be within 1% of the expected concentration. If not, check your standard peaks and/or your baseline. When reading sample peaks on the strip chart or computer, check for unexpected duplicates or samples that seem out of sequence. The nutrient analyst is often the first to notice a double trip or mis-trip on a rosette cast.

6.6. SHUT DOWN PROCEDURE

Turn off the sampler after the last standard and wait 20 minutes for the last peak to appear and the baseline to return to normal. Replace the NaCl wash with DMQ and wait 10 minutes before removing the cadmium column. Disconnect the pull through side of the column first to avoid pulling air into the Cd granules, then disconnect the other side, top up the column with NH_4Cl and connect both ends with tygon tubing filled with NH_4Cl. Connect all reagent feeds to DMQ and flush the system for 10 - 15 minutes.

*Stop data collection by pressing F10 on the computer keyboard. *

Turn off the black box, chart recorders and heaters, and turn all colorimeter displays to zero. Turn off the pump, close the DMQ valve (to prevent back flushing into the DMQ bottle), and remove the pump platen. Store the pump platen so that the rubber surface is protected from damage. Place a couple of kimwipes between the tubing and the rollers and loosen the tension on the tubing by removing the pump manifold from the holding pins. Backup the data file by copying it onto a floppy disk. Empty the waste containers down the sink with copious amounts of water.

6.7. QUICK START AND SHUT DOWN

QUICK START

1. Turn on colorimeter lamps.
2. Replace wash water jugs with fresh DMQ and NaCl.
3. Take reagents out of the refrigerator and allow to warm up.
4. Attach pump platen, open the main DMQ valve, and turn on the pump.
5. When the DMQ has reached the heaters, turn them on.
6. When the DMQ has reached the flowcell and has an even bubble pattern, turn on the computer and start the GETDATA program.
7. Prepare reagents and standards as required.
8. Check colorimeter ZERO and FULL SCALE on the computer and recorder (if using), then set to NORMAL.
9. Hook-up reagents, starting with ammonium chloride.
10. By the time all the reagents are hooked up the ammonium chloride should be flowing and the cadmium column can be hooked up.
11. Switch to NaCl wash water.
12. Run 4 - 6 "wake-up" samples to condition the system.
13. Run standards (0, low to high, 0) in duplicate (in triplicate for Arctic cruises).
14. Run 30 - 40 samples between standard sets.
15. Finish the run with a set of standards.

SHUT DOWN

1. Turn off sampler and wait 20 minutes for last sample to pass through the flowcell and baseline to return to zero.
2. Switch to DMQ wash water.
3. Disconnect cadmium column.
4. Turn off heaters.
5. Disconnect reagents.
6. Rinse with DMQ for 10 minutes.
7. Hit F10 to stop data logging on the computer and set lamp display to zero. Transfer data to disc.
8. Turn off the pump, close the DMQ valve, remove pump platen, place kimwipes between tubing and rollers before loosening tension on tubing.
9. Empty waste containers.

7.0. DATA COLLECTION AND PROCESSING

Nutrient data is logged and results calculated using the programs GETDATA.EXE (version 8/15/93) and PEAKBASE.EXE (version 7/21/92) respectively which were written by David Jones at the Department of Oceanography, University of B.C. GETDATA, which queries the operator before writing over an existing file, replaces an earlier version called LOG.EXE. GETDATA.EXE generates a data file of extension .AAZ. PEAKBASE displays a computed baseline whereas the most recent version (PEAKDATA.EXE-version 2/16/94) does not. PEAKBASE generates a data file of extension .ANZ and this data file can be imported into a spreadsheet. The program RECALC.EXE (version 8/17/93) is used to read in an edited .ANZ file and recalculate the regressions and concentrations. A description of the programs and example of an .ANZ file is found in Appendix 3.

Duplicates are analyzed for 10 - 15 % of the fresh samples and usually more often for frozen samples.

Since silicate and nitrate are not linear over their concentration ranges, standards are fit to a second order polynomial equation: $Y = C1*X^2 + C2*X + C3$. Assuming each sample set is bracketed by a set of standards, the usual procedure for calculating the concentrations (Appendix 3 for details) of a set of samples using PEAKBASE includes:

1. Calculate the regression factors for each set of standards (bringing the baseline to zero using either the uniform or differential adjustment).
2. Bring the sample set down to zero and calculate the concentrations using the two bracketing regression factors.

The data is thus compensated for any system gain changes. Keep track of the regression factors C1, C2, and C3 (see example in Appendix 5). There should be little change over the course of a day's run and from day to day.

8.0. TROUBLE SHOOTING AND HINTS

The Auto Analyzer II™ lives up to its name only rarely and requires constant attention to achieve best results. Monitor the system for leaks, plugged reagent lines, bubbles etc. to avoid re-running samples. Most trouble shooting can be done from the computer screen or strip charts.

If the baseline is noisy - especially the silicate channel - check the ammonium molybdate reagent - it can build up a precipitate that plugs the transmission lines or gets stuck in the flowcell. Replace the reagent at the first sign of a precipitate. Storing the reagent in the refrigerator may alleviate this problem.

Bubbles often get stuck in the phosphate flowcell. Pinch the tygon tubing at the exit end of the flowcell to draw another bubble through and dislodge it.

Use a tubing cutter or razor blade to cut tubing - scissors will crimp the tubing and possibly affect the flow.

Occasionally clean the system with 10 % HCl or phosphate free detergents such as RBS (Phosphate Free) or FL70. Disconnect all the reagents, pump the cleaning solution through all lines, including the sampling probes, for 15 to 30 minutes. Rinse for one hour with DMQ to flush the lines. Cleaning solutions can be left in overnight but don't leave the sample probes in 10 % HCl and don't run the pump on high speed with acid in the lines.

Occasionally the flowcell accumulates precipitate and causes a noisy signal. Stronger acid may be required to remove the precipitate. Carefully remove the flowcell and examine the path length using a flashlight.

Replacing a colorimeter lamp will eliminate noise caused by a defective bulb.

Because the program doesn't save data during a power failure, log data onto a laptop computer equipped with a working battery.

Rinse sampling tray with hot water to remove salt crystals that can jam the tray and test tubes.

Don't use long pieces of tubing to connect to the sampler or wash water intake. These will get stuck between the sample tray and the sampler and strip the gears in the sampler, or catch in the gears under the sampler.

If analyzing ammonia samples, waste water from the analysis must be diverted into a sealed bottle marked "phenol" to avoid the unpleasant odour. Add several granules of NaOH to neutralize the phenol smell and dispose of the wastes down the sink with plenty of water.

Don't turn off any lamps in the middle of a run as this can interfere with other channels.

Don't forget to hit F10 to stop the data logging program before turning off the computer or an entire run may be lost.

On Line P Cruises, nutrient samples are collected in duplicate plastic test tubes when samples are being analyzed at sea. All three nutrients (silicate, nitrate and phosphate) are analyzed from the one test tube. Analyze one set immediately and store the other in the refrigerator (in case it is necessary to re-run a set). In the case of equipment failure, freeze the samples and

begin collecting phosphate samples in glass tubes. For Ships of Opportunity and Arctic work, samples are collected in duplicate plastic and glass tubes because plastic gives low phosphate results when stored for more than one day. Nitrate samples can also be stored in glass but not silicate samples.

After analysis, remove labels from the test tubes and rinse tubes and caps (separating plastic and glass caps) in hot water. Store in plastic bags for return to IOS for acid cleaning.

Minimize the number of connectors used in a chemistry, and match tubing and fitting IDs to reduce smearing of sample peaks.

Check that the standard response is similar to the response of the previous day. Take the time to verify the operation of the system - if necessary put samples in the refrigerator. Questionable operation rarely provides quality data. If in doubt, verify operation before running samples.

9.0. INSTRUMENT MAINTENANCE

A complete Technicon maintenance manual is kept with each AutoAnalyzer™. The following are procedures that may be more frequently needed.

9.1. OPTICALLY PEAKING COLORIMETERS:

The colorimeter has two identical optical channels. Each channel is comprised of two lenses (one fixed and one movable), an adjustable aperture, an interference filter assembly, a flowcell assembly and a phototube housing assembly. A single source lamp assembly provides the light for both optical channels. Light that enters the flowcell travels through the liquid and is projected onto the light sensitive surface of the phototube to develop an electrical signal.

The colorimeters must be optically peaked whenever parts have been replaced or the instrument has been shipped for a cruise. Optically peaking is used to obtain the most efficient use of the optical system. Check the focusing lenses occasionally - they have been known to break off.

Optically peaking is done when the colorimeter is hooked up to a chart recorder or voltmeter to read the output. The colorimeter should be in full system operation (i.e. reagents, DMQ, and NaCl hooked up) for 15 minutes (warm-up time) before making any adjustments.

If using a voltmeter, set it to DC V and plug the leads going from the colorimeter to the black box into the +(red) and -(black) terminals on the voltmeter. If using the Ecology AA you will have to replace the lead from the colorimeter to the black box with one that has voltmeter fittings. **NOTE:** Not all colorimeters are wired the same (e.g. Ecology's nitrate colorimeter) and using the voltmeter leads may not work. In this case you must optically peak the colorimeter using the chart recorder or computer.

When the Display Rotary Switch is in position FULL SCALE, a signal is introduced which produces a 5.0-volt output. When turned to position ZERO some circuits are grounded and others opened so that the output is zero (grounded).

Procedure:

1. Turn the colorimeter to "Normal" sensitivity. ("Normal" gives an immediate response. The Damp 1 and Damp 2 settings average 2 and 10 second responses respectively.)
2. Unlock the STD CAL dial and turn to 1.0. Lock the dial.
3. Turn the sample aperture ("A") control fully clockwise (open), and turn the reference aperture ("B") control to obtain 1/2 scale signal on the system display (approx. 4 volts). It may be necessary to partially close the sample aperture.
4. At this time check to see if both filters are matched by switching the sample and reference filter around (set the Display Rotary Switch to ZERO to protect the phototubes). The output should be about the same (i.e. 40 - 60 % full scale). The interference filters can be cleaned with "Windex" and a soft cloth. Push the filter out of the holder to clean it and hold it up to a bright light to check for delamination of the surface.
5. Adjust the sample flowcell peaking screw inside the colorimeter to give a minimum recorder signal. Adjust the reference flowcell screw to give a maximum recorder signal.
6. Unscrew the two knurled head screws holding the light shield in place and lift the light shield straight up.
7. Gently loosen the screw on the movable lens of the sample channel (the one furthest from the lamp) until it can be moved along the track of the sample channel to give a minimum recorder signal. Repeat for the reference channel lens to give a maximum signal.
8. Recheck step 5 and replace the light when setting baselines shield.

If the colorimeter BASELINE control will not adjust the voltage output to the desired level while setting, make a coarse adjust with the reference rather than the sample aperture.

If the output signal strength from a colorimeter changes when its cover is lifted, stray light is reaching the phototubes. Use black tape to cover the joins in the phototube housing.

9.2. PUMP PLATEN PRESSURE, CLEANING, AND OILING

See Technicon pump reference manual to check the pump platen pressure and oiling schedule. Clean the pump platen every week with alcohol to keep grease and oil off the tubing rollers, pump tubing and the platen. Record any maintenance in the AA log book.

9.3. TUBING REPLACEMENT

Replace the pump tubing after ~100 hours (usually after a 3 - 4 week cruise) or before a major run of several weeks. Condition the tubing for 1 - 2 hours by pumping DMQ through all the lines. Replace the transmission, silicone air bar, or any other tubing when it looks dirty or crimped. Ammonium Molybdate reagent (silicate) often develops a white precipitate with age and will clog tubing. The pull through tubing from the cadmium column to the pump can

become plugged with fine cadmium or glass wool which seriously affects both flow rate and peak shape.

9.4. CLEANING

Clean all tubing whenever a major autoanalyzer overhaul occurs. Connect all the lines to 10 % HCl, FL70 or phosphate free RBS, pump through to waste and let cleaning solutions sit in the lines overnight. Never turn the pump on high speed with acid in the lines, as the connections may break and spray acid. Wipe off the sampling probes every week with alcohol to remove oil.

10.0. REFERENCES

Alvarez-Salgado, X.A., F. Fraga and F. F. Pérez. 1992. Determination of nutrient salts by automatic methods both in seawater and brackish water: the phosphate blank. Marine Chemistry. 39, 311-319.

Aminot, A, and K. Kirkwood. 1995. Report on the Results of the Fifth ICES Intercomparison Exercise for Nutrients in Sea Water. ICES Coop. Res. Rep. No. 213. 79 pp.

Armstrong, F. A. J., C. R. Stearns, and J. D. H. Strickland. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon™ AutoAnalyzer™ and associated equipment. Deep-Sea Res. 14(3): 381-389.

Brynjolfson, S. J. 1973. A modification of the Technicon™ methodology for the determination of orthophosphate in seawater. Paper presented at Water Resources Services, Vancouver, B.C.

Collos, Y., Y. Kedong, and P. J. Harrison. 1992. A note of caution on reduction conditions when using the cadmium-copper for nitrate determinations in aquatic environments of varying salinities. Mar. Chem. 38, 325-329.

Garside, C. 1993. Nitrate reductor efficiency as an error source in seawater analysis. Marine Chemistry, 44, 25-30.

Kérouel, R., and A. Aminot. 1987. Procédure optimisée hors-contaminations pour l'analyse des éléments nutritifs dissous dans l'eau de mer. Mar. Environ. Res. 22: 19-32.

Koroleff, F and Grasshoff, K. 1983. Determination of Nitrate. In: K. Grasshoff, M. Ehrhardt and K. Kremling (Editors), Methods of seawater analysis. 2nd.rev.ed. Verlag Chemie, Weinhein. 419 pp.

Macdonald, R. W., F. A. McLaughlin and C. S. Wong. 1986. The storage of reactive silicate samples by freezing. Limnol. Oceanogr. 31(5):1139-1142.

Marvin, K. T., R. R. Proctor, and R. A. Neal. 1972. Some Effects of Filtration on the Determination of Nutrients in Fresh and Salt Water. Limnol. Oceanogr. 17: 777-784.

Murphy, J. and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta: 27, 31-36.

Olson, R. J., 1980. Phosphate interference in the cadmium reduction analysis of nitrate. Limnol. Oceangr. 25(4):758-760.

Technicon AutoAnalyzer II™ Industrial Method No. 154-71W/B, Released February 1973, Revised January 1978, Ammonia in water and seawater. Technicon Industrial Systems, Tarrytown, N.Y.

Technicon AutoAnalyzer II ™ Industrial Method No. 155-71W, Released January 1973, Orthphosphate in water and seawater. Technicon Industrial Systems, Tarrytown, N.Y.

Technicon AutoAnalyzer II ™ Industrial Method No. 158-71W/B, Released December 1972, Revised August 1979, Nitrate and nitrite in water and seawater. Technicon Industrial Systems, Tarrytown, N.Y.

Technicon AutoAnalyzer II ™ Industrial Method No. 186-72W, Released March 1973, Silicates in water and seawater. Technicon Industrial Systems, Tarrytown, N.Y.

APPENDIX 1. REAGENT AND STANDARD LABELS

Note: Labels appearing in Appendix 1 (pages 29 - 33) are designed to be photocopied, cut and pasted to reagent and standard bottles.

NITRATE:

Ammonium Chloride (NO3) 100 g
Supplier: Lot:
Date: By:
Dissolve in DMQ and bring to 10 L
Adjust to pH 8.5 with NH4OH
(requires 15 - 20 mL conc.)

Ammonium Chloride (NO3) 100 g
Supplier: Lot:
Date: By:
Dissolve in DMQ and bring to 10 L
Adjust to pH 8.5 with NH4OH
(requires 15 - 20 mL conc.)

Ammonium Chloride (NO3) 100 g
Supplier: Lot:
Date: By:
Dissolve in DMQ and bring to 10 L
Adjust to pH 8.5 with NH4OH
(requires 15 - 20 mL conc.)

Ammonium Chloride (NO3) 100 g
Supplier: Lot:
Date: By:
Dissolve in DMQ and bring to 10 L
Adjust to pH 8.5 with NH4OH
(requires 15 - 20 mL conc.)

N-1-N (NO3) 1.0 g
Supplier: Lot:
Date: By:
Add to Sulfa. and H3PO4 sol'n.
to prepare N-1-N reagent.

N-1-N (NO3) 1.0 g
Supplier: Lot:
Date: By:
Add to Sulfa. and H3PO4 sol'n.
to prepare N-1-N reagent..

N-1-N (NO3) 1.0 g
Supplier: Lot:
Date: By:
Add to Sulfa. and H3PO4 sol'n.
to prepare N-1-N reagent.

N-1-N (NO3) 1.0 g
Supplier: Lot:
Date: By:
Add to Sulfa. and H3PO4 sol'n.
to prepare N-1-N reagent..

Sulfanilamide (NO3) 20.0 g
Supplier: Lot:
Date: By:
To 1500 mL DMQ add 200 mL conc.
H3PO4 , 20 g Sulfanilamide and
dissolve. Add 1 g N-1-N, dissolve and
bring to 2 L. Add 1 mL Brij-35.
Store in reefer. Stable 2 mos.

Sulfanilamide (NO3) 20.0 g
Supplier: Lot:
Date: By:
To 1500 mL DMQ add 200 mL conc.
H3PO4 , 20 g Sulfanilamide and
dissolve. Add 1 g N-1-N, dissolve and
bring to 2 L. Add 1 mL Brij-35.
Store in reefer. Stable 2 mos.

Sulfanilamide (NO3) 20.0 g
Supplier: Lot:
Date: By:
To 1500 mL DMQ add 200 mL conc.
H3PO4 , 20 g Sulfanilamide and
dissolve. Add 1 g N-1-N, dissolve and
bring to 2 L. Add 1 mL Brij-35.
Store in reefer. Stable 2 mos.

Sulfanilamide (NO3) 20.0 g
Supplier: Lot:
Date: By:
To 1500 mL DMQ add 200 mL conc.
H3PO4 , 20 g Sulfanilamide and
dissolve. Add 1 g N-1-N, dissolve and
bring to 2 L. Add 1 mL Brij-35.
Store in reefer. Stable 2 mos.

Sulfanilamide (NO3) 20.0 g
Supplier: Lot:
Date: By:
To 1500 mL DMQ add 200 mL conc.
H3PO4 , 20 g Sulfanilamide and
dissolve. Add 1 g N-1-N, dissolve and
bring to 2 L. Add 1 mL Brij-35.
Store in reefer. Stable 2 mos.

Sulfanilamide (NO3) 20.0 g
Supplier: Lot:
Date: By:
To 1500 mL DMQ add 200 mL conc.
H3PO4 , 20 g Sulfanilamide and
dissolve. Add 1 g N-1-N, dissolve and
bring to 2 L. Add 1 mL Brij-35
Store in reefer. Stable 2 mos.

APPENDIX 1. (CONTINUED)

SILICATE:

Ammonium Molybdate (SIL) 10 g
Supplier: Lot:
Date: By:
Dissolve in 1 L 0.1 M H2SO4.
Stable 1 month of less. Discard if white precipitate forms.

Ammonium Molybdate (SIL) 10 g
Supplier: Lot:
Date: By:
Dissolve in 1 L 0.1 M H2SO4.
Stable 1 month of less. Discard if white precipitate forms.

Oxalic Acid (SIL) 35 g
Supplier: Lot:
Date: By:
Dissolve in 400 mL DMQ and bring to 500 mL. Stable 1 month.

Oxalic Acid (SIL) 35 g
Supplier: Lot:
Date: By:
Dissolve in 400 mL DMQ and bring to 500 mL. Stable 1 month.

Oxalic Acid (SIL) 35 g
Supplier: Lot:
Date: By:
Dissolve in 400 mL DMQ and bring to 500 mL. Stable 1 month.

Oxalic Acid (SIL) 35 g
Supplier: Lot:
Date: By:
Dissolve in 400 mL DMQ and bring to 500 mL. Stable 1 month.

Ascorbic Acid (SIL) 4.4 g
Supplier: Lot:
Date: By:
Dissolve in 250 mL SIL acetone/levor mix.
Store in reefer for 2 days.

Ascorbic Acid (SIL) 4.4 g
Supplier: Lot:
Date: By:
Dissolve in 250 mL SIL acetone/levor mix.
Store in reefer for 2 days.

Ascorbic Acid (SIL) 4.4 g
Supplier: Lot:
Date: By:
Dissolve in 250 mL SIL acetone/levor mix.
Store in reefer for 2 days.

Ammonium Molybdate (SIL) 10 g
Supplier: Lot:
Date: By:
Dissolve in 1 L 0.1 M H2SO4.
Stable 1 month of less. Discard if white precipitate forms.

Ammonium Molybdate (SIL) 10 g
Supplier: Lot:
Date: By:
Dissolve in 1 L 0.1 M H2SO4.
Stable 1 month of less. Discard if white precipitate forms.

Oxalic Acid (SIL) 35 g
Supplier: Lot:
Date: By:
Dissolve in 400 mL DMQ and bring to 500 mL. Stable 1 month.

Oxalic Acid (SIL) 35 g
Supplier: Lot:
Date: By:
Dissolve in 400 mL DMQ and bring to 500 mL. Stable 1 month.

Oxalic Acid (SIL) 35 g
Supplier: Lot:
Date: By:
Dissolve in 400 mL DMQ and bring to 500 mL. Stable 1 month.

Oxalic Acid (SIL) 35 g
Supplier: Lot:
Date: By:
Dissolve in 400 mL DMQ and bring to 500 mL. Stable 1 month.

Ascorbic Acid (SIL) 4.4 g
Supplier: Lot:
Date: By:
Dissolve in 250 mL SIL acetone/levor mix.
Store in reefer for 2 days.

Ascorbic Acid (SIL) 4.4 g
Supplier: Lot:
Date: By:
Dissolve in 250 mL SIL acetone/levor mix.
Store in reefer for 2 days.

Ascorbic Acid (SIL) 4.4 g
Supplier: Lot:
Date: By:
Dissolve in 250 mL SIL acetone/levor mix.
Store in reefer for 2 days.

APPENDIX 1. (CONTINUED)

PHOSPHATE:

Ammonium Molybdate (PO4) 10 g
Supplier: Lot:
Date: By:
Dissolve in 1 liter DMQ. Slowly add 120 mL H2SO4 while mixing. Cool. Add K(SbO) sol'n and dilute to 2 L.

Ammonium Molybdate (PO4) 10 g
Supplier: Lot:
Date: By:
Dissolve in 1 liter DMQ. Slowly add 120 mL H2SO4 while mixing. Cool. Add K(SbO) sol'n and dilute to 2 L.

Antimony Potassium Tartrate
K(SbO) (PO4) 0.6 g
Supplier: Lot:
Date: By:
Dissolve in 100 mL DMQ and add to PO4 Amm Moly.

Antimony Potassium Tartrate
K(SbO) (PO4) 0.6 g
Supplier: Lot:
Date: By:
Dissolve in 100 mL DMQ and add to PO4 Amm Moly.

Ascorbic Acid (PO4) 0.50 g
Supplier: Lot:
Date: By:
Dissolve in 150 mL PO4 acetone/levor mix.

Ascorbic Acid (PO4) 0.50 g
Supplier: Lot:
Date: By:
Dissolve in 150 mL PO4 acetone/levor mix.

Ascorbic Acid (PO4) 0.50 g
Supplier: Lot:
Date: By:
Dissolve in 150 mL PO4 acetone/levor mix.

Ascorbic Acid (PO4) 0.50 g
Supplier: Lot:
Date: By:
Dissolve in 150 mL PO4 acetone/levor mix.

Ascorbic Acid (PO4) 0.50 g
Supplier: Lot:
Date: By:
Dissolve in 150 mL PO4 acetone/levor mix.

Ammonium Molybdate (PO4) 10 g
Supplier: Lot:
Date: By:
Dissolve in 1 liter DMQ. Slowly add 120 mL H2SO4 while mixing. Cool. Add K(SbO) sol'n and dilute to 2L.

Ammonium Molybdate (PO4) 10 g
Supplier: Lot:
Date: By:
Dissolve in 1 liter DMQ. Slowly add 120 mL H2SO4 while mixing. Cool. Add K(SbO) sol'n and dilute to 2 L.

Antimony Potassium Tartrate
K(SbO) (PO4) 0.6 g
Supplier: Lot:
Date: By:
Dissolve in 100 mL DMQ and add to PO4 Amm. Moly.

Antimony Potassium Tartrate
K(SbO) (PO4) 0.6 g
Supplier: Lot:
Date: By:
Dissolve in 100 mL DMQ and add to PO4 Amm. Moly.

Ascorbic Acid (PO4) 0.50 g
Supplier: Lot:
Date: By:
Dissolve in 150 mL PO4 acetone/levor mix.

Ascorbic Acid (PO4) 0.50 g
Supplier: Lot:
Date: By:
Dissolve in 150 mL PO4 acetone/levor mix.

Ascorbic Acid (PO4) 0.50 g
Supplier: Lot:
Date: By:
Dissolve in 150 mL PO4 acetone/levor mix.

Ascorbic Acid (PO4) 0.50 g
Supplier: Lot:
Date: By:
Dissolve in 150 mL PO4 acetone/levor mix.

Ascorbic Acid (PO4) 0.50 g
Supplier: Lot:
Date: By:
Dissolve in 150 mL PO4 acetone/levor mix.

APPENDIX 1. (CONTINUED)

STANDARD LABELS:

NITRATE STANDARD # 96-
KNO3 0.2527 g

Dilute to 1 L DMQ=2,500 umol/L

NITRATE STANDARD # 96-
KNO3 0.2527 g

Dilute to 1 L DMQ=2,500 umol/L

NITRATE STANDARD # 96-
KNO3 0.2527 g

Dilute to 1 L DMQ=2,500 umol/L

NITRATE STANDARD # 96-
KNO3 0.2527 g

Dilute to 1 L DMQ=2,500 umol/L

SILICATE STANDARD # 96-
Na2SiF6 1.4105 g

Dilute to 1 L DMQ = 7500 umol/L

SILICATE STANDARD # 96-
Na2SiF6 1.4105 g

Dilute to 1 L DMQ = 7500 umol/L

SILICATE STANDARD # 96-
Na2SiF6 1.4105 g

Dilute to 1 L DMQ = 7500 umol/L

SILICATE STANDARD # 96-
Na2SiF6 1.4105 g

Dilute to 1 L DMQ = 7500 umol/L

PHOSPHATE STANDARD # 96-
KH2PO4 0.3402 g

Dilute to 1 L DMQ for primary=2500 umol/L

PHOSPHATE STANDARD # 96-
KH2PO4 0.3402 g

Dilute to 1 L DMQ for primary=2500 umol/L

PHOSPHATE STANDARD # 96-
KH2PO4 0.3402 g

Dilute to 1 L DMQ for primary=2500 umol/L

PHOSPHATE STANDARD # 96-
KH2PO4 0.3402 g

Dilute to 1 L DMQ for primary=2500 umol/L

APPENDIX 2. NUTRIENT SAMPLING AND STORAGE

Note: Appendix 2 (pages 33 and 34) is designed to be photocopied, plasticized and used whenever nutrient samples are collected in the field.

Nutrient samples are routinely collected from field programs for analysis at IOS. Correct sampling and storage procedures are important to ensure high quality data. The following guide is intended to help achieve that goal and keep the nutrient analysts happy!

NUTRIENT SAMPLE TUBES + LABELING

Nutrient samples are collected and frozen at sea for analysis at IOS. Samples should be collected in duplicate polystyrene (for silicate and nitrate analysis) and glass (for phosphate analysis) test tubes. The tubes are acid washed and stored in plastic bags. As the test tubes fit directly into the AA-II sampling tray both glass and plastic tubes must be the same length (125 mm) so samples do not need transferring.

Make sure test tubes are labeled with water and alcohol proof labels and pens. Label the samples as simply as possible, including cruise number, cast/station ID, and sample bottle number/depth. An even simpler label includes just cruise ID and a unique sample number which corresponds to that in the cruise log. A cruise log must be made available to the nutrient analyst.

SAMPLING PROCEDURE

Nutrient samples are drawn from samplers after all gas sampling (CFC's, oxygen, CO_2, etc.) has finished. Avoid touching the spigot, inside of the cap or test tube, with your grubby fingers. Also avoid contaminating samples with raindrops or any other seawater dripping off the sampler. The test tubes (including the caps) are rinsed two to three times with sample and filled to within 2 cm of the top. Do not overfill the test tubes because samples will expand during freezing and may leak through the cap. If the sample is overfilled, expansion during freezing will cause some of the sample to be lost through the cap and the remaining sample will be compromised. Do not under fill the test tubes either. The test tubes fit directly into the sampling tray on the Auto Analyzer™ and the sampling probe will not reach into the sample if the tube is less than 2/3 full.

STORING FROZEN SAMPLES

Samples should be frozen as soon as possible after sampling. Freeze samples in a chest freezer (do not use the frost free freezer above a refrigerator, unless necessary) using wire racks to allow for air circulation. Samples must remain upright until frozen to avoid loss of unfrozen brine and should ideally remain upright during storage. A quicker method of freezing employs an alcohol filled freezing bath. Ensure that samples remain upright and that the cap is above the level of the alcohol during freezing. Before storing frozen samples in plastic bags, wipe excess alcohol from the tubes and re-tighten caps that may have loosened during freezing. Store the plastic and glass test tubes together for each station and mark the outside of the plastic bag with cruise number and station ID.

NUTRIENT SAMPLING TECHNIQUE

COLLECT TWO PLASTIC AND TWO GLASS TEST TUBES FOR EACH NUTRIENT SAMPLE.

1. LABEL THE TEST TUBES WITH WATER AND ALCOHOL PROOF MARKERS AND LABELS.

2. RINSE THE CAPS AND TEST TUBES 2-3 TIMES BEFORE COLLECTING SAMPLE.

3. TEST TUBES ARE FILLED TO 2 CM FROM THE TOP - NO MORE - NO LESS.

4. FREEZE SAMPLES UPRIGHT IN A RACK. IF USING AN ALCOHOL BATH FOR FREEZING, DO NOT SUBMERGE THE CAPS IN ALCOHOL.

5. RETIGHTEN THE CAPS AFTER FREEZING AND WIPE THE ALCOHOL (IF USING) OFF THE TUBES BEFORE STORING THE SAMPLES UPRIGHT IN LABELED PLASTIC BAGS.

AVOID CONTAMINATING SAMPLE WITH RAIN WATER OR WATER DRIPPING OFF SAMPLERS.

AVOID TOUCHING SPIGOT, INSIDE OF CAP OR TEST TUBE WITH YOUR FINGERS.

NO SMOKING ON DECK WHEN SAMPLING.

APPENDIX 3. NUTRIENT PROGRAMS AND SAMPLE .ANZ FILE

The 3 programs that are described below all operate in DOS. Limited use in Windows 95 has been successful.

1. GETDATA

This program replaces an older version called LOG.EXE. The video hardware requirements to display graphics are CGA, but VGA is routinely used. The program logs voltages from up to input 4 channels into an operator named file that has an .AAZ extension.

2. PEAKBASE

The program is used to calculate standard regressions and sample concentrations from the .AAZ file collected by LOG.EXE or GETDATA.EXE. Its output goes into an ASCII file that is named the same as its source .AAZ file but has an .ANZ extension.

NOTE: The format of ..ANZ files are different in PEAKDATA than in PEAK and PEAKBASE. RECALC can be used to generate new .ANZ format files for PEAKDATA if you want to use previously analyzed data files. See the section below on RECALC.EXE.

HARDWARE REQUIREMENTS

The screen must be VGA.
The keyboard should be the extended type with 12 function keys.
A numeric keypad is used for cursor movement (see KEYS below).
The program has been tested on a limited number of machines. NUMLOCK OFF provides the best chance of compatibility between keyboard and program.
File loading is much faster from a hard drive. Copy .AAZ files to the PEAKBASE directory then calculate results.

GRAPHICS DISPLAY

The screen has two graphic windows, the main one displaying the selected channel in a peak height versus time format. A vertical line cursor may be moved through the data backwards and forwards. The smaller window on the right is a magnification of the cursor area. F11 and F12 are used to modify the contents of the left window (details later in this appendix).

An area of text (upper right) is used for display of current cursor time position (in seconds), the current cursor step size and the raw peak value (as selected by the cursor). The concentration (voltage) baseline ZERO is displayed graphically.

At the bottom of the screen, calculated standard regressions are numbered.

STARTING PEAKBASE

Type
PEAKBASE ↵

Follow a series of onscreen commands.

Drive:
The screen prompts for the drive, so enter the drive (c or a, etc.). Please insure that a floppy, if used, is NOT write protected.

INPUT FILENAME (no extension) :
The data files of extension .AAZ are displayed and one should be selected by entering its name.

STEP SIZE = 176 (for example)
DO YOU WISH TO CHANGE STEP SIZE..N ?
The cam step rate (seconds) may be altered here or by pressing F1 later. This information sets the time step the program uses in its search for peaks. The steps are close to 176 for the OSAP instrument and 178 for Ecology's when equipment is operated on 60 Hz. Times have been longer when 50 Hz power has been used since the sampler speed is set by the AC power frequency.

SELECT CHANNEL = (N,S,P,A)
NITRATE, PHOSPHATE, SILICATE or A can be selected (A = nitrite or ammonia, if analyzed).

Find Peak Manually ... y ?
Answering **y** means that the peak is selected by your movement of the cursor. The alternative is Automatic peak finding, where the area around the cursor is searched for the highest value, a process which can introduce errors when peaks are spiky or are not regularly spaced.

WAIT ... DATA LOADING
This is a grim test of patience on a slow PC since large data files are loaded into memory. A fast hard drive, big disk cache and at least a 486 computer is a pleasant combination.

The program will now load any previous analysis of this file so that existing standard regressions may be used again.

EDITING AND CONTROL KEYS

Function keys

F1 CAM STEP - used to change the value of the cam delivery time or the distance between peaks.

F2 SET BASE - the position of the baseline may be adjusted up or down.

F3 REG SEL - the regressions to be used to calculate peak concentration can be selected. This effects the drawing of the concentration ZERO, the horizontal line at the bottom of the data.

F4 SMTH DATA - a toggle for smooth on or off

F5 ENTER STD - enter the concentration of a selected standard

F6 CALC REG - begin processing peak and conc values to quadratic fit. The program allows data to be edited before regressions are calculated.

These keys control where baseline adjustments occur and where regression fits of peaks take place.

F7 DATA START selected data start

F8 DATA END selected data end

F9 START CALC begin calculating concentration from peak

F10 STOP CALC stop automatic movement of cursor

F11 HOR UP magnifies area around cursor to maximum

F12 HOR DOWN decreases magnification by 2

Numeric key pad with* NUM LOCK *off

These move the line cursor

→ (right)

← (left)

These change the cursor step size

↑ up by double

↓ down by half

5 (on Numeric Keypad) changes current step size to cam step size

HOME jumps to start of data file

END jumps to end of data file

CALCULATING STANDARD REGRESSIONS

It is expected that sets of standards are run periodically throughout a sample run. Regressions are calculated from these standards, which are then used to convert sample voltages (peak heights) to concentrations.

Select the start (F7) and end (F8) to a standard group at points where the baseline is well established. Adjust the baseline to zero using F2, routinely using the differential adjustment. Select a standard peak using cursor controls then press F5. The program asks that the concentration be entered. If an error is made, it is possible to edit it later. After a number of standards are entered (>3, but no more than 12), the regression is calculated (F6). Standard peaks and concentrations are displayed and may now be edited. If y is given to the edit ?

prompt, the operator can move among the values with the arrow keys and type in new values (return to enter new value) then use the ESC key to finish editing. The coefficients for a regression are displayed, and the line of fit and the data points are graphed on screen. Hitting the spacebar (or any key) will allow the program to proceed.

All standard groups are typically selected before sample concentrations are computed. On a longer run, it is possible to have 3 or 4 standard regressions, as noted below the baseline on screen.

CALCULATING NUTRIENT CONCENTRATIONS

A single regression may be used for calculating sample concentrations, but more commonly two regressions that bracket a sample group are used to compensate for any changes in sensitivity over an analysis period. Differences between two regressions are partitioned equally over any chosen set of samples.

> **The application and interpolation of calibration regressions occur between the data start (F7) and the data end (F8) that are selected by the operator, and are not governed by the positions of samples in relation to standards.**

The order in which regressions are selected is important. Generally, the order should be as recorded, the first in time should be the first regression chosen. If there is no shift or drift in baseline, select all of the data between standard groups with F7 and F8. Adjust the baseline (F2) then select the first peak on the left with the line cursor, and press F9 to start sample calculations. If this is the first sample group selected from this data file, then the operator will be asked which regression(s) are to be used. This selection then becomes the default for all subsequent calculations. When different regressions are required, they can be entered by using F3.

The default method for peak selection uses the value that the cursor is on. It is possible at the start of the data file input, to change this to automatic peak finding. In this case the area around the cursor is searched for the highest point and this is used. After the best point on a peak is selected, the concentration is "fitted" and displayed. The cursor then automatically jumps forward (defined by the cam step size, F1) and the process begins again. At this point, a beep sounds, signaling that the analysis can be stopped with F10 and the cursor can be moved to a more desired location. The F9 key reactivates the concentration calculation. The smooth function (F4) can be toggled on/off at any time. Data near the cursor will be smoothed and the new data used for the calculation. This process will continue until the end of the data is reached or F10 is used.

The output from the PEAKBASE program is in ASCII text format. Alphabetic characters are enclosed by quotes for easier import into spreadsheets (e.g. Excel text tab delimited), but can be easily viewed in any text editor. Numbers are delimited by tabs also for this reason.

APPENDIX 3. (CONTINUED)

ADDITIONAL DETAILS ON PROGRAM FUNCTIONS

BASELINE OFFSET:
The F2 key opens the baseline compensation routines: uniform or differential.

The uniform (u) compensation assumes that the baseline in a region is elevated the same throughout. The elevated region can be removed by using the offset selection routine. The area is defined by two function keys (F7 and F8), then the offset is subtracted from the trace in that region. This can remove the offset completely. It is available for all peaks.

In the differential (d) correction, used if the baseline is changing drastically, the start baseline (defined by F7), and the end baseline (F8) are subtracted from the trace. The baseline in between is interpolated using these two endpoints. This results in the peak heights being adjusted according to the differential contribution of each baseline position.

SMOOTHING:
If a peak has noise on it, a moving average smooth can be applied. During the calculation of concentration (F9), if smooth is ON, the data peak will be smoothed. This cannot be applied to the standards so use with some caution.

ZOOMING:
The horizontal scale of the screen may be zoomed by two keys (F11 and F12). F11 magnifies to highest power. F12 then halves the magnification each time it is pressed until the full file is displayed.

ESC QUIT:
This key allows you to leave the program; to change channels or if hit by mistake, to continue.
The prompts are:
Continue with current data : answering y puts you back to the current conditions.
Same file : allows you to select a different channel from the current data file.
Do you wish to quit : you can quit or select another data file.

EDITING STANDARDS AND PEAKS:
When the calculate regression key (F6) is hit, the keys are re-assigned to the following actions.
ESC : terminates editing of standard peaks and concentration values.
→ : moves from current standard concentration value to the peak.
← : moves from current standard peak value to the concentration.
↑ : moves from current standard peak or concentration value to the next one up. Also cycles off the top to the bottom.
↓ : moves from current standard peak or concentration value to the next one down. Also cycles off the bottom to the top.

After F6, the concentration (left) and peak (right) values are displayed. The following query appears:

Do you wish to edit ?
To change a peak or concentration value, enter y to above.
A small arrow key points to the current peak or concentration value. To move, use the arrow keys, and the selection indicator will move. Begin typing in the new value, and finish the edit with ↵.

After finishing editing, hit ESC and the standards will be fitted.

3. RECALC.EXE

This program will read in a .ANZ file, use the raw and measured peak values and recalculate the regressions. It will then recalculate sample concentrations. The final step is writing out a new .ANZ data file.

Sample .ANZ format

```
[Regression]
" Y = C1 * X ^ 2 + C2 * X + C3"
"chan" "rnum" "rpos"  "C1"    "C2"    "C3"
"N"      1      1400  -8.441E-07   +1.095E-02   -2.114E-01
"N"      2      600   +2.597E-05   +6.191E-03   +7.531E-02
"A"      1      912   -7.282E-12   +1.000E-01   -1.640E+01
"A"      2      1512  -3.061E-06   +1.039E-01   -1.102E+01
[peaks]
"chan" "rnum" "raw"   "meas""calc"
"N"      1      32     0      +1.381E-01
"N"      1      104    1.04   +9.183E-01
"N"      1      178    1.78   +1.711E+00
"N"      1      312    3.12   +3.123E+00
"N"      1      537    5.37   +5.425E+00
"N"      1      729    7.29   +7.323E+00
"N"      1      895    8.95   +8.913E+00
"N"      2      32     .3     +3.000E-01
"N"      2      104    1      +1.000E+00
"N"      2      178    2      +2.000E+00
"A"      1      164    0      +4.300E-07
"A"      1      728    56.4   +5.640E+01
"A"      1      548    38.4   +3.840E+01
"A"      1      368    20.4   +2.040E+01
"A"      2      368    26.8   +2.680E+01
"A"      2      548    45     +4.500E+01
"A"      2      728    63     +6.300E+01
"A"      2      910    81     +8.099E+01
[concentration]
"chan" "num"  "Concentration"      "Peak" "Time" "Reg#1"      "Reg#2"      "n"
"N"      1      +6.579E+00   653    1136   1      0      .0000
"N"      2      +9.639E-01   107    426    1      2      .1588
"N"      3      +9.682E-01   107    408    1      2      .2080
```

APPENDIX 3. (CONTINUED)

"N"	4	+1.854E+00	180	608	1	2	.4080
"N"	5	+3.964E+00	311	808	1	2	.6080
"N"	6	+9.565E+00	532	984	1	2	.7840
"N"	7	+1.787E+01	721	1184	1	2	.9840

APPENDIX 4. NUTRIENT DATA SHEET

AUTOANALYZER NUTRIENT ANALYSIS PAGE OF

DATE: ANALYST:

CRUISE: FILENAME:

SAMPLES: DEFROST TIME:

STANDARD BATCH:

Gains	Stds	L M H X
	NO3:	
	Si:	
	PO4:	
	NH4:	

Tray	Sample	NO3&NO2		Dissolved Si		React. PO4		NH4/NO2		Comments
		Abs.	Conc.	Abs.	Conc.	Abs.	Conc.	Abs.	Conc.	
1										
2										
3										
4										
5										
6										
7										
8										
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APPENDIX 5. NUTRIENT DATA CONTROL CHART

CRUISE	Nitrate Coefficients			Silicate Coefficients			Phosphate Coefficients			Nitrite/Ammonium					
9631										Coefficients			COMMENTS		
DATE	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3			
170996	2.70E-07	0.02760	-0.0129	3.20E-06	0.05240	0.0708	-1.20E-07	0.00293	0.0006						
	8.20E-07	0.02730	-0.0045	3.70E-06	0.05200	-0.0002	-1.60E-07	0.00295	0.0020						
	9.50E-07	0.02720	-0.0429	6.70E-06	0.05100	0.0255	-1.30E-07	0.00293	0.0039						
180996	6.50E-07	0.02736	-0.0229	2.50E-06	0.05370	-0.0166	-1.20E-07	0.00292	0.0039						
	2.70E-07	0.02745	-0.0437	2.30E-06	0.05273	-0.0937	-1.70E-07	0.00297	0.0016						
	7.90E-07	0.02685	-0.0330	2.20E-06	0.05291	-0.0907	-2.10E-07	0.00300	0.0153						
190996	1.10E-06	0.02690	0.1970	1.20E-06	0.05440	-0.0550	-2.80E-07	0.00315	0.0046						
	6.40E-07	0.02730	-0.0207	1.20E-06	0.05430	0.0138	-2.90E-07	0.00313	0.0027						
200996	5.40E-07	0.02798	0.0064	1.40E-06	0.05356	-0.0413	-1.60E-07	0.00294	0.0033						
	5.90E-08	0.02825	-0.0350	1.50E-06	0.05368	-0.0390	-1.40E-07	0.00293	0.0005						