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Hydrochemistry Operations Manual

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Abstract

This manual describes the methods employed by the Hydrochemistry group at CSIRO Marine Research Australia to analyse dissolved oxygen (using automated titration), salinity (using a Guildline salinometer) and nutrients (nitrate + nitrite, orthophosphate and silicate using an Alpkem Autoanalyser) in marine waters. The work is usually done while at sea on the research vessels *RV Franklin* and *RV Southern Surveyor*. The manual details sampling techniques, instrumentation, reagents, analysis methods, record keeping, quality assurance/quality control and data processing.

Parts of this manual have been reproduced (with permission) from Eriksen, R. (1997), *A Practical Manual for the Determination of Salinity, Dissolved Oxygen and Nutrients in Seawater*, First Edition, Research Report No.11, Cooperative Research Centre for the Antarctic and Southern Ocean Environment (Antarctic CRC).

1 Introduction

Rebecca Cowley

The Hydrochemistry group at CSIRO Marine Research routinely provides technical services aboard the research vessels *Franklin* and *Southern Surveyor*. Analysis of dissolved oxygen, salinity and nutrients (silicate, nitrate+nitrite and orthophosphate) in marine water samples are analysed on board.

The aim of this manual is to ensure the methods used produce high quality data, of a standard recognised by the WOCE Hydrographic Programme Office. It is intended that the manual be used for all routine analyses on CSIRO Marine Research voyages and other voyages using the services of the Hydrochemistry group are used. The methods are described in great detail, but training from experienced personnel is nonetheless essential to obtain quality results.

The Hydrochemistry Group has been in operation for some 15 years, during which time the analytical methods have changed considerably.

Recent changes include:

- The purchase of a new style of salinometer, a Guildline Autosal, to replace the YeoKal salinometers (September 1996).
- The purchase of an Alpkem Autoanalyser to replace the Technicon II on board the *RV Franklin* (September 1996). The Technicon system is still in use on the *RV Southern Surveyor*.
- The purchase of an automated Dosimat dissolved oxygen analysis system to replace the manual titrations previously performed (February 1998 for the *RV Southern Surveyor*, and November 1994 for *RV Franklin*).

Along with the updates in the technology new methods have been developed and tested and routine quality assurance and quality control procedures introduced. These developments and procedures, as well as field sampling procedures and protocols, are described.

Future editions of this manual will be published when protocols are updated. It is envisioned that developments will take place in the areas of:

- Data collation and processing while at sea (development of a new software package, 'NewHydro').
- Nutrient data collection from the Technicon system (replacement of the current software, 'Dapa').
- Nutrient analysis techniques (general methods as well as the possible development of an ammonia method and underway nutrient analyses).

2 Field sampling protocols

Gary Critchley and Ruth Eriksen

Contents

2.1 Introduction

The sampling methods reported here are adapted from the Antarctic CRC Operations Manual (Eriksen, 1997) with the layout of the *RV Franklin* and *RV Southern Surveyor* laboratories in mind.

The sampling protocols are set out as a step-by-step guide to taking high quality, representative field samples for analyses conducted in the hydrographic or "wet" lab. Before beginning routine sampling, the hydrologist should instruct sample collectors until they are familiar and comfortable with sampling techniques. The Chief Scientist is responsible for ensuring that all samples analysed on board are collected using good technique, with the right equipment. Seawater samples are collected from nominated depths using either 1.7, 2.5, 5 or 10 L Niskin™ style bottles, fitted to a 12 or 24 bottle CTD rosette package.

At the beginning of each voyage, it is important that a test cast is performed, where the CTD is deployed fully loaded with the Niskin bottles to be used on the voyage. The bottles are all fired at the salinity minimum, and salinity samples are collected from each bottle as described in Section 2.4. It is not necessary to collect any other samples. The salinity samples from this cast are tested as soon as possible, and compared to the CTD trace. Any outliers will indicate leaking Niskin bottles which may require repair or replacement.

2.2 Water-sampling apparatus

CSIRO Marine Research uses Niskin-style bottles to capture discrete water samples from the water column. These bottles are mounted on CTD sampling rosettes for deep-ocean hydrological sampling from the main research vessels or attached to a weighted wire for sampling from smaller boats.

One-on-one training in the operation of these bottles is required. The following description of use is meant as a guide.

Before deployment the bottles are "set" (the end-caps are in the open position). This enables the bottles to flush fully as they are lowered to the sampling depth. It is essential that the air

bleed valve (upper) is screwed firmly down and that the sample spigot (lower) is pulled out fully to the "double-click" position. Deep-sea reversing thermometers are mounted in reversing frames at predetermined positions where thermometry is required.

If using a wire for sampling, ensure that messengers are placed on the bottles so that the deeper bottles are triggered. Likewise, when setting the bottles on a rosette, ensure that the ramp of the rosette position is facing ready to fire rosette position 1 (deepest).

The depths of sampling commonly used for a 24 bottle rosette CTD system are given in Table 2.1. These depths are based on WOCE protocol.

Table 2.1 WOCE sampling depths for a 24-bottle CTD rosette

Rosette position	Depth
24	0
23	50
22	100
21	150
20	200
19	300
18	400
17	500
16	600
15	700
14	800
13	900
12	1000
11	1250
10	1500
9	1750
8	2000
7	2250
6	2500
5	2750
4	3000
3	3500
2	4000
1	bottom

When the bottles are triggered the sample that is taken is a discrete sample from that depth. Should the bottle leak, this will be found in the analysis by comparing the CTD and hydrochemistry salinity data.

Some investigators require that latex is not used as the internal closure band. In these cases silicon tubing is used as the closure material. As the silicon tubing is not as elastic as the normal latex, lanyard mono-filament loops must be fitted to the ends of a loop of silicon tubing. Attach the loops of monofilament to the closure groove on the end-caps.

The following sections give a detailed description of how to collect quality samples for dissolved oxygen, salinity and nutrient analyses.

2.3 Dissolved oxygen sampling

- **Dissolved gas samples should always be taken first, unless otherwise specified.**
- **Check that the pickling reagents 1 & 2 and sample bottles have been brought into the CTD room before sampling starts.**
- **A letter identifies each crate of sample bottles, and the bottles within each crate are numbered 1 to 24. For example, C01 to C24.**
- **Fill out the crate ID on the CTD log sheet and check that the sample bottles are in numerical order in the crate.**

1. Dissolved gas samples are taken as soon as each cast is completed, and the rosette has been secured to the deck. Samples are drawn deep to shallow, as the deepwater samples are the farthest from equilibrium with surface atmospheric conditions (they have been in the bottle the longest and undergone the greatest changes in temperature and pressure when they arrive on deck).
2. Before each sample is collected, each Niskin must be checked for leaks and potential contamination. Open the spigot (lower tap) and watch carefully for any water flow. If the Niskin leaks, then air is entering the Niskin, and the sample within is most likely to have been contaminated with water from a shallower depth. Samplers should record this information on the CTD sheet for later reference by the analyst and data processor.
3. Each oxygen sample bottle is numbered to coincide with the rosette position from which the sample is drawn. Select the sample bottle number corresponding to the Niskin bottle number, eg, C01 should be used to collect sample from bottle number 1. If the casts do not use 24 bottles, use the procedure outlined in Figure 2.1.
4. Starting from the deepest sample (ie Rosette position 1), attach a 20 cm length of silicon tubing over the spigot of the Niskin. Start the water flow by opening the air bleed on the Niskin. Allow a small volume to run to waste, and use this to clear the tubing of bubbles. Air bubbles may stick to the sides of the tubing. Squeezing, flicking or manipulating the tubing should remove them.
5. Stop the flow of water by pinching the tubing close to the spigot. Hold the 150 mL sample bottle at an angle of approximately 45 degrees. Lower the tubing into the bottom of the bottle, and slowly release the tubing so that water flows gently into the bottle. Take care to minimise turbulence, to avoid introducing atmospheric oxygen into the sample.
6. When the bottle is about a third full, pinch the tubing closed and discard the water. Repeat the rinse twice. Keeping the tubing in contact with the bottom of the bottle at all times, slowly release the tubing until the water flows freely and fills the bottle. Allow at least twice the volume of the bottle to overflow.
7. Slowly pinch off the tubing to reduce the flow rate, and remove the tubing from the bottle. There should still be enough water flowing to ensure that the bottle is full to the brim when the tubing is completely removed.
8. Without capping the bottle, quickly proceed to the pickling reagents.
9. Immediately add 1.0 mL of reagent 1 (3 mol/L Manganese chloride) by placing the tip of the reagent dispenser below the surface of the sample and pumping the dispenser once. The tip should extend below the neck of the oxygen flask so that the floc does

not form in the excess seawater in the neck of the flask. This water has been in contact with the atmosphere, and could result in sample contamination. Add 1.0 mL of reagent 2 (4 mol/L sodium iodide/8 mol/L sodium hydroxide) in the same manner. The reagents are very much denser than seawater and will sink when added, displacing that portion of seawater that has been directly in contact with the atmosphere.

10. The sample should now be stoppered so that no air bubbles are trapped in the bottle. Try dropping the stopper from a height of about 2 cm above the bottle. Inserting the stopper slowly is more likely to trap a bubble. It is imperative that no bubbles are trapped in the bottle, as they will completely invalidate a sample. If you suspect a sample for any reason, throw it away, rinse the bottle thoroughly and repeat the procedure. Note this down on the CTD sheet for reference by the analyst.
11. Shake the bottle thoroughly at this stage to completely form the floc and disperse it evenly throughout the bottle. The shaking is critical, and should involve a snapping motion of the wrist, where the bottle is completely inverted rather than just shaking. Invert the bottle in this manner at least 20 times before placing the bottle in the box. The samples are light sensitive at this stage, and the lid should be left down at all times.
12. Return the pickling reagents and samples to the lab when sampling is completed.
13. The sample bottles should be shaken a second time in the lab to ensure thorough mixing of the contents.

Good sampling technique is the single most important factor in producing accurate oxygen data.

2.4 Salinity sampling

- **Salinity samples are drawn after the dissolved gases have been sampled.**
- **Each crate of sample bottles is identified by a letter, and the bottles within numbered 1 to 24. For example, C01-C24.**
- **For shallow stations, two or more stations may be collected in one crate. The CTD sheet should be referred to regularly to check that the correct bottle is being used for sampling.**
- **Fill out the salinity crate ID on the CTD sheet and check that the sample bottles are in numerical order in the crate.**
- **Bottles are stored with previously analysed seawater in them to prevent salt crystallisation, which can contaminate the next sample.**

1. Samples are taken deep to shallow, as the deepwater samples have undergone the greatest change in temperature and pressure when they arrive on deck.
2. Ensure that any dissolved gas samples have already been collected before continuing.
3. Select a sample bottle according to the numbering on the CTD sheet. On a 24 bottle cast the sample bottle numbers will match the Niskin bottle position numbers. If the casts do not use 24 bottles, use the procedure outlined in Figure 2.1.

4. Empty out the old sample from the bottle, rinsing off any crystallised salt on the thread of the bottle and cap.
5. Don't touch the end of the spigot with your fingers and don't bring the mouth of the sample bottle in contact with the Niskin spigot. Open the spigot, fill the bottle approximately one-quarter full, fit the cap loosely, and shake to rinse out the inside of the bottle. Pour the contents to waste over the cap to ensure that any traces of old sample are removed. Rinse the spigot as well, as it may be contaminated with surface water or rain, which will affect the integrity of the sample.
6. Repeat the rinsing procedure a further two times and then fill the bottle to the shoulder, leaving a gap of about 3 cm from the top of the bottle to provide for the expansion of cold samples.
7. Cap the bottle tightly, replace it in the crate and proceed to the next Niskin.
8. When using less than 24 bottles per cast, after sampling a station, invert the next empty bottle in the crate to indicate which bottle is to be used for the beginning of the next station.

Figure 2.1 Example of sampling a 12-bottle station, followed by an 8-bottle station.

Begin sampling from the deepest sample in both cases. In the example, sample bottle 13 will be the first sample taken from rosette position 1, followed by 14, 15 and so on. Sampling of the next station requires sample bottle 5 to be filled from rosette position 1, followed by bottle 6 and so on. Italic numbers represent the sample bottle number (bottles in the crate), bold numbers represent the niskin rosette position number, and the arrows indicate the order of sampling. The 12-bottle cast is sampled first.

Try to avoid splitting stations over more than 1 crate for ease of analysis.

24 **12** shallow	*23* **11** ←	*22* **10** ←	*21* **9** ←	*20* **8** ←	*19* **7** ←
18 **6** ←	*17* **5** ←	*16* **4** ←	*15* **3** ←	*14* **2** ←	*13* **1** deepest
12 **8** shallow	*11* **7** ←	*10* **6** ←	*9* **5** ←	*8* **4** ←	*7* **3** ←
6 **2** ←	*5* **1** deepest	*4* spare	*3* spare	*2* spare	*1* spare

2.5 Nutrient sampling

- **Nutrient samples are drawn after salinity samples have been collected.**
- **Nutrient tubes are numbered with a four-digit code. The first two numbers denote the station number, and the second two denote the rosette position eg, Station 5, rosette position 9 is coded as 0509. On a voyage with a heavy sample load the numbering may have to be increased to a 5-digit code to accommodate station numbers in the hundreds.**
- **Check that the nutrient tubes have been numbered correctly and that all tubes have caps.**
- **Check that the nutrient tube numbers have been entered on the CTD sheet.**
- **Nutrient samples are drawn in duplicate.**
- **Tubes are used as provided by the manufacturer, and are for single use only.**

1. Samples are taken deep to shallow, as the deepwater samples have undergone the greatest change in temperature and pressure when they arrive on deck.
2. Do not touch the end of the spigot, or the inside of the tubes or the cap with your fingers. Match the pair of nutrient tubes to the correct rosette position, and rinse the tubes by filling them half full of seawater. Loosely cap the tubes and rinse the inside of the tubes and caps by shaking.
3. Repeat the rinsing a further two times, and then fill the tubes to the 10 mL mark. **Do not fill beyond this mark**, as freezing causes expansion and separation into brine/freshwater layers, leading to losses in nutrient concentrations.
4. **Tightly** cap the tubes and put them in the polystyrene tray.
5. When nutrient sampling is finished, the samples should be placed immediately in the refrigerator or freezer as required by the hydrochemist.

2.6 References

Eriksen, R. (1997), *A Practical Manual for the Determination of Salinity, Dissolved Oxygen and Nutrients in Seawater*, Research Report No.11, Cooperative Research Centre for the Antarctic and Southern Ocean Environment (Antarctic CRC), GPO Box 252c, Hobart, Tas. 7001, Australia.

3 Dissolved oxygen analysis

Ruth Eriksen and Gary Critchley

Contents

3.1 Method review

The analytical method used by CSIRO Marine Research to determine dissolved oxygen in seawater meets the standards expected of participants in the WOCE Hydrographic Program (WOCE, 1991). The manual titration method described in CSIRO Report 51 (Major et al., 1972) has been replaced with an automated method with an amperometric endpoint detection system. An extensive comparison of the new automated method and the manual method was undertaken before the automated method was adopted (Eriksen and Terhell, in prep.). The reported accuracy of the new method is about ±0.02 mL/L with a standard deviation of replicate samples of ±0.005 mL/L (Knapp et al., 1990).

WOCE guidelines state that, with scrupulous technique, the reproducibility of the Winkler technique using Carpenters modifications is ±0.010 mL/L (WOCE Operations Manual, 1991).

The automated method has the following advantages over the manual system previously used:

- It utilises the Carpenter reagent chemistry specified by WOCE.
- It measures reagent blanks, to correct for reducing impurities.
- The system can be calibrated every second day, instead of daily.
- The standardisation method is such that any loss of volatile iodine incurred in the process of collecting an aliquot of sample is taken into account.
- There is a reduction in operator error, as the computer controls the entire titration process.
- The computer acquires data directly, so data processing time is reduced.
- The system is extremely seaworthy and can be used reliably in rough weather, when manual titrations can be exceptionally difficult.
- The method is used by a number of organisations participating in the WOCE program.
- The precision and accuracy of the analyses are significantly greater than the manual Winkler titration.

The automated dissolved oxygen system we use is based on the method developed by Knapp et al. (1990) of the Woods Hole Oceanographic Institution. Instrumentation consists of a Metrohm 665 Dosimat connected to an IBM-compatible 486 computer. The titration process is controlled by WHOI software modified by David Terhell[3] ("DODO"). A dual-plate platinum sheet electrode is used to measure the decrease in voltage as the titration proceeds. A Metrabyte Dascon-1 analogue/digital I/O board digitises the electrode voltage, and the rate of change of electrode voltage determines the speed and increment of dosing of the thiosulfate titrant. A calculated endpoint is used to determine the thiosulfate titre, and hence the concentration of dissolved oxygen in the sample. Titres are corrected for a blank measurement.

[3] CSIRO Marine Research, GPO Box 1538, Hobart, Tas. 7001, Australia. Telephone: +61 3 6232 5222, Fax: +61 3 6232 5000.

The automated method described here is extremely seaworthy, and requires very little maintenance while in use. A detailed description of the method and its specifications can be found in Knapp et al. (1990). The method presented here is a summary of the analytical procedure, and contains sufficient detail for routine laboratory analysis.

3.2 Sampling equipment

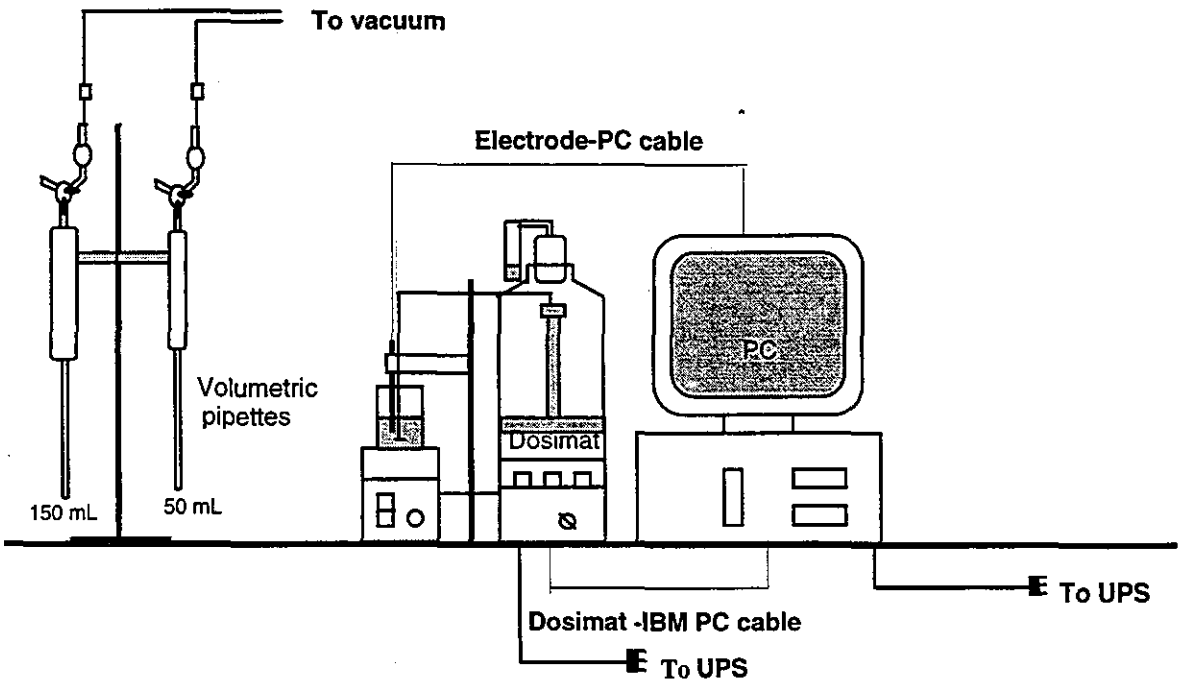
Samples are collected in 150 mL Kimax clear glass bottles with ground glass, tapered-tip stoppers. A rubber leash attaches the stoppers to the bottles. Samples are drawn using a 20 cm length of silicon tubing that can be slipped tightly over the Niskin spigot. Samples are "pickled" using the reagents specified in the WOCE Operations Manual (1991). Sample bottles are stored in the dark in plastic boxes, to prevent photochemical reactions.

For detailed sampling procedures please refer to Section 2.3.

3.3 Instrumentation set-up and installation

Figure 3.1 shows the recommended layout of dissolved oxygen analysis equipment in the hydrographic laboratory.

Figure 3.1 Schematic diagram of layout of dissolved oxygen analysis equipment.



To prevent possible loss of data, the PC and Dosimat should be connected to the Uninterruptable Power Supply system.

The dual platinum electrode cable is connected to the PC via the customised circuit board designed by David Edwards[4] (see Figure 3.2). This board, which allows fine-tuning of the

[4] Dave Edwards formerly of CSIRO Marine Research, now contactable at Erico Lightning Technologies, Technopark, Goodwood, Tasmania 7010. Phone +61 3 6237 2300.

digitiser, protrudes from the back of the computer about 3 to 4 cm. The electrode is stored in a beaker of deionised water until required.

The pipettes used for collecting aliquots of sample and standard are attached to a pneumatic ram, which can be raised and lowered into bottles or beakers as required. The laboratory air supply can be used to operate the ram. The pressure regulator should be set to 200 kPa.

The 150 mL and 50 mL volumetric pipettes are attached to a stand on the ram and positioned so that the pipettes will not touch the bottom of the beaker or bottles when the ram is lowered. The beaker holder has been designed to reduce the loss of volatile iodine when taking an aliquot, the sample being drawn up from the bottom of the bottle. The two pipettes are connected to a Masterflex™ pump by lengths of silicon vacuum-rated tubing. Nalgene™ quick-fit connectors fitted close to the pipettes makes connecting and removing the tubing simple.

3.4 Editing the header file

Before any data are collected with the automated system, the header file **oxyhd.dat** should be edited to ensure that the concentration of dissolved oxygen is calculated correctly. The oxyhd.dat file contains the constants required for the calculation of dissolved oxygen in µmol/L. Details such as voyage, analyst, number of Niskins on the rosette, etc are also recorded.

The oxyhd.dat file is found in the NEWWINK directory. To edit this file, type "Edit oxyhd.dat" before proceeding with any analyses, and make the necessary changes. Record all changes in a voyage logbook, along with any other significant comments regarding the use of the dissolved oxygen system (eg, pipette changes). When editing, ensure that there are no blank lines (eg, from hitting the 'enter' key by mistake), or the program may go into an operating loop. A sample header file is shown below.

The fields marked *(*)* were used in the original WHOI version of the program, but are not used in the version adapted by David Terhell, so they do not require editing. In the original method, each sample bottle was calibrated, the bottles were then grouped into crates (labelled A to K below) and each crate was given an average volume based on the bottle volumes included in that crate. We found that there was no significant difference to the calculations when an average volume of all the bottles was used in place of a crate average (David Terhell, *pers. com*). The average bottle volume currently used by CSIRO Marine Research is 147.0 mL.

**********OXYGEN CRUISE DATA**********

Cruise ID	"S9407"
# of rosette bottles	24
Volume of 50 mL bur	49.885
Volume of 150 mL D.W bur	150.600
Volume of biiodate used	15.000
Normality of biiodate used	0.0100
Volume of added reagents	3.000

Volume displaced by reagents	2.000
Oxygen in reagents	0.0017
Dosimat cylinder ID	"S1" *(eg, Surveyor 1)*
Cylinder co-efficient	
Operators name	"VL"
Voltage factor	0.0004986 *(for example)*
Case ID and Volumes Below	
"A"	147.45*(*)*
"B"	147.75*(*)*
"C"	147.65*(*)*
"D"	147.65*(*)*
"E"	148.04*(*)*
"F"	147.73*(*)*
"G"	146.50*(*)*
"H"	146.88*(*)*
"J"	147.57*(*)*
"K"	147.29*(*)*

The quantities shown here are for 150 mL sampling bottles. If 300 mL sample bottles are being used then the following changes should be made:

- volume displaced by reagents, 4.000
- oxygen in reagents, 0.0034
- all case volumes should read "300", or their calibrated volume (if known).

Exit and save the oxyhd.dat file.

3.5 Determining the voltage factor

The voltage factor is used to convert digitiser values to voltages during titrations, and should be determined at the beginning of **every** voyage, or whenever a new dual platinum electrode is used. The voltage factor may also need to be checked if the end voltage of sample titrations exceeds the recommended range. The final electrode voltage, which is typically between 0.012 and 0.024 mV (Knapp et al., 1990) is recorded in the appropriate data file for each station. The voltage factor is derived empirically on the following procedure:

1. Load the program READVOLT, which is found in the NEWWINK directory. This is done at the command prompt - c:\newwink - by typing 'readvolt' then <CR>.
2. Collect 50 mL of MilliQ water in a beaker and add, in the following order (mixing well between additions) 1 mL of 10N H_2SO_4, 1 mL of NaI/NaOH reagent, and 1 mL of $MnCl_2$ reagent. Then add 5 mL of 0.01N biiodate standard, giving a solution with approximately 5 mL/L (equivalent oxygen) dissolved iodine.
3. Immerse the electrode in the solution, and adjust the displayed voltage on the PC A/D board to give maximum value on the digitiser (approximately 4000). Initially, you may

have to coarsely adjust the Metrabyte™ pot on channel zero (refer to Metrabyte manual). Adjusting the pot on the Edwards board will enable fine-tuning of the digitiser value (Figure 3.2 shows a diagram of the Edwards board).

4. Note down this value and also the voltage across the electrode. The electrode voltage is determined by bridging a multi-meter across the two terminals on the Edwards board.

5. The voltage factor is then calculated as follows:

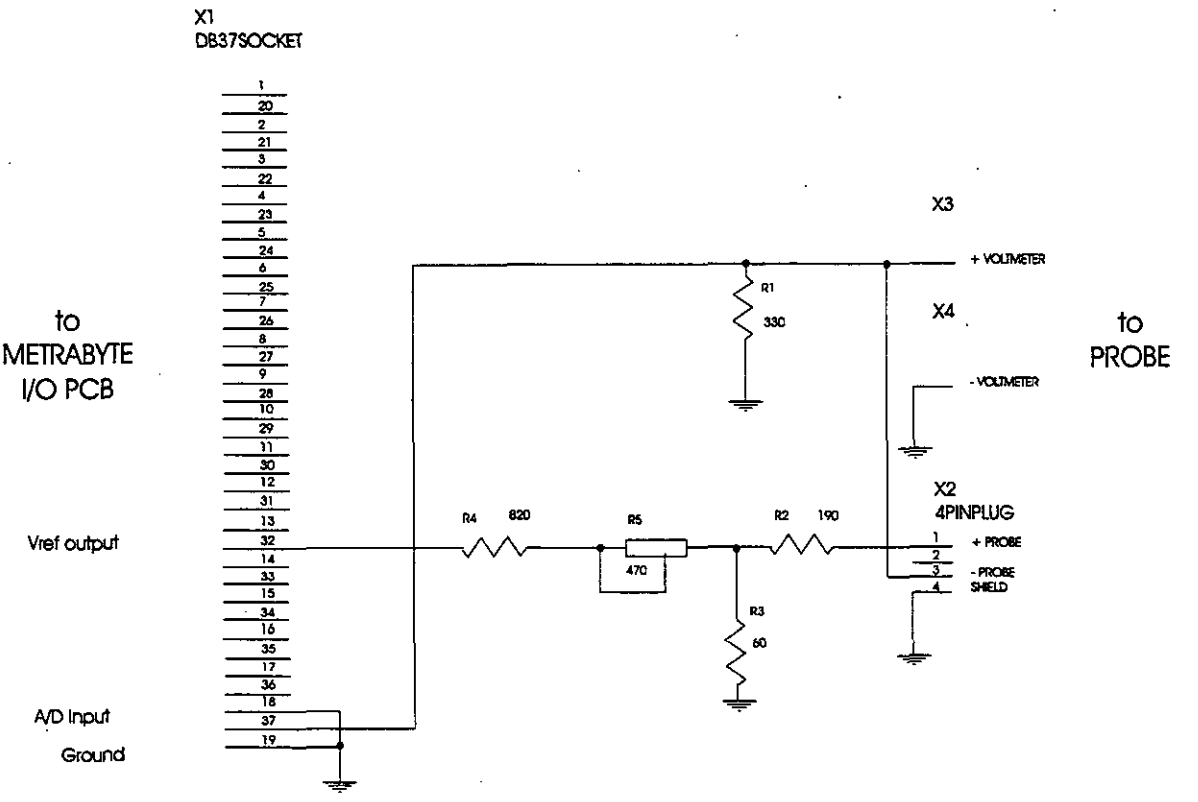
$$V_f = V_e / (10 * D)$$

Where: V_f = voltage factor
V_e = voltage across the electrode (mV)
D = digitiser value

6. The voltage factor is then entered into the oxyhd.dat file as shown in Section 3.4.

The computing preparation for the procedure is now completed. The next step is to begin the chemistry. Details of how to use DODO for analysis are given in Section 3.7.

Figure 3.2 Diagram of the Edwards board.



3.6 Reagent preparation

3.6.1 Glassware

All glassware used in the analysis of dissolved oxygen should be kept clean for the best results. A wash with hot tap water, followed by a deionised water rinse is sufficient.

3.6.2 Safety considerations

The chemical reagents used for the analysis of dissolved oxygen are concentrated, and potentially dangerous. A laboratory coat or protective clothing should be worn when acidifying and processing samples, or when reagents are being prepared. Thick gloves and safety glasses should also be worn when handling 10N H_2SO_4. Safety glasses should be made available to samplers working in the CTD room, and eye wash bottles containing a sterilised saline solution should be clearly visible in both the laboratory and the wet lab.

In the event that the reagents are splashed on skin or clothing, rinse with copious amounts of fresh water, and change clothes if necessary. If reagents are splashed in the eyes, flush with copious amounts of sterile saline solution or freshwater.

3.6.3 Reagent list

The following reagent concentrations are those specified by Carpenter (1965). All chemicals are analytical grade (AR), unless otherwise specified.

Distilled water may be substituted if deionised water is not available. Reagents should be prepared and filtered in dedicated glassware to prevent manganese contamination. All reagents should be prepared before the voyage, and should be stored in amber PVC or 'breaksafe' amber glass bottles to prevent photochemical reactions. Each reagent batch should be labelled with chemical name and concentration, date of preparation, initials of analyst, and the lot number and manufacturer of the chemical. When each reagent batch is used, these details are transferred to the laboratory logbook, so that any possible problems arising from differences in batch or source can be easily traced.

The biiodate standard must be prepared in calibrated A grade volumetric ware at the Hobart laboratories. Balances used for weighing critical masses such as standards must be checked before use with the highest quality calibration masses. All relevant details, including the exact mass of standard salt weighed, the lot number and manufacturer, should be recorded in the calibrations log book at Hobart.

The sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) can be prepared at sea, as it is standardised regularly. Plastic Whirlpaks™ (6 oz) are a convenient size for pre-weighing. The preservative, sodium borate (NaB_4O_7), can be preweighed into 12 mL polypropylene nutrient tubes.

Manganese chloride (3 mol/L) – Pickling reagent 1

Dissolve 600 g of AR grade $MnCl_2.4H_2O$ in 500 mL of deionised water. This solution needs thorough stirring to ensure that all the salt dissolves. Filter the solution **twice** through 'Whatman™ GF/F' filters, and make up to 1 L. Transfer to a 1 L amber bottle with 'Optifix' dispenser attached. Set the dispenser to 1.0 ± 0.05 mL.

Sodium iodide (4 mol/L)/Sodium hydroxide (8 mol/L) – Pickling reagent 2

Dissolve 600 g of AR grade NaI in 500 mL of deionised water. The solution should be clear. If the solution is yellow/brown, it should be discarded and a new batch of reagent used. Slowly add 320 g of AR grade NaOH pearls, and 2 g of sodium azide (NaN_3) while stirring continuously. The sodium azide acts as a preservative and should always be included. When the solution has cooled to room temperature, double-filter with Whatman GF/F filters. Make up to one litre. Transfer to a 1 L amber bottle with Optifix dispenser attached, and set dispenser to 1.0 ± 0.05 mL.

Sulfuric acid (5 mol/L)

Slowly add 280 mL of concentrated AR grade H_2SO_4 to 600 mL of deionised water. Allow the mixture to cool to room temperature and make up to 1 L. This reagent is best prepared in bulk before a voyage. Transfer to a 1 L amber bottle with Optifix dispenser attached, and set dispenser to 1.0 ± 0.05 mL.

Sodium thiosulfate (0.01 mol/L)

Dissolve 5.0 g of $Na_2S_2O_3.5H_2O$ and 0.25 g of sodium borate ($Na_2B_4O_7.10H_2O$) in 2 L of deionised water and make up to the mark. The sodium borate acts as a preservative and the solution should be kept in an amber glass bottle that is tightly sealed to prevent evaporation. Mix thoroughly before use. Sodium thiosulfate that has aged for at least 24 hours has been found to give more reproducible results during standardisation than freshly prepared thiosulfate solutions.

Potassium biiodate standard (0.0100 N or 0.000833 mol/L)

This biiodate solution, which is prepared at the Hobart laboratories, is used as the secondary and working standards for dissolved oxygen analysis.

Dry a quantity of AR grade $KH(IO_3)_2$ in a vacuum desiccator for 48 hours beforehand. $KH(IO_3)_2$ should not be oven dried, as it decomposes at temperatures above ~100 °C (Friederich et al., 1991). Accurately weigh 0.3250g of $KH(IO_3)_2$ using a calibrated balance. Transfer to an A-grade 1 L flask and make up to the mark with deionised water. Mix thoroughly before use. This solution is stable indefinitely.

Vitex indicator

This is not used in the amperometric analysis of dissolved oxygen, but may be used at sea if there is an electrode failure and the end-point of the titration has to be determined manually.

Dissolve two heaped teaspoons of Vitex powder in a small amount of distilled water (about 20 mL). Mix thoroughly and leave to stand for half an hour (or until fully dissolved) before use.

Vitex indicator can be used initially to determine the validity of the electrode endpoint, but should not be used routinely, as the response of the electrode and the slope of the titration curve are affected (Knapp et al., 1990).

At this stage of the procedure, the computing preparation and reagent steps have been dealt with. The analysis procedure requires the operation of DODO.

3.7 Using DODO

Note: the Dosimat unit must be "on", to load DODO.

The original NEWWINK software written by M. Stalcup and G. Knapp of WHOI has been modified by David Terhell of CSIRO Marine Research to incorporate slight differences in sample and data processing on CSIRO Marine Research oceanographic voyages. The modified version of the program, called "DODO", is found under the NEWWINK directory. The computer **must have** Quick Basic and the Crescent Pro Library loaded on it to run the software suite.

3.7.1 Data files

Three types of files are created when dissolved oxygen data are acquired by the DODO program. Data is stored as a NEWWINK\vyyccsss.wnk, where v denotes vessel (S for *Southern Surveyor*, F for *Franklin*, etc), yy denotes year, cc denotes voyage/cruise number, and sss denotes station number eg, S9404001.wnk. A separate data file will be created for each station that is processed. Each file consists of 24 records, based on an entry of 24 against "number of rosette bottles" in the oxyhd.dat file. After the 24th sample is processed (or you exit the program after less than 24 samples), the program defaults to the main menu.

Data files can be reloaded and edited at a later date if required (see Section 3.7.3). The .wnk file contains the frame number, the start and end electrode voltage, volume of thiosulfate added, and the calculated dissolved oxygen concentration in µmol/L. It also contains the values of the constants used for calculation purposes from the header file, ie, biiodate normality, glassware calibrations, standardisation details etc.

All calibration details for standardisation and blank determinations are stored in a locked file NEWWINK\OXYCAL.~~~. This file is updated each time standardisation or blank measurements are made. The file NEWWINK\OXYHIST.DAT contains a history of all standard, blank, and sample titrations performed during the voyage.

3.7.2 Getting started

1. In DOS, at the C:\> prompt, type 'cd newwink' and press Enter to change to the working directory.
2. Next type 'qb DODO /l pro', and press Enter.
3. To start the dissolved oxygen program, select 'Start' from the Run menu in Quick Basic.
4. You will be prompted "Do you wish to use the printer?", enter 'N'.

The user can also elect whether to use the Dosimat to dispense the 15 mL of biiodate required for the standardisation procedure. Enter the appropriate response, depending on whether a standardisation is required or a sample is to be analysed.

The main menu allows you to select the type of titration (standard, blank, or sample) and to edit, save and print previously acquired data. The options are:

1. **Automated oxygen titration**
2. **Automated standardisation**
3. **Automated blank measurement**
4. **Enter oxygen data by hand**
5. **Edit oxygen data**
6. **Print oxygen data**
7. **Send oxygen data to disk**
8. **Read oxygen data from disk**
9. **End program**

When you exit your selected option, the program will default to this main menu.

3.7.3 Manually editing a file

Data files can be edited manually as soon as the station has been completed, and the data saved to disk. To do this:

1. Select Option 8 (Read oxygen data from disk) to load the station you wish to modify. You will be prompted to enter this station number.
2. Select Option 5 (Edit oxygen data), and a summary of frame number, begin and end point voltages, thiosulfate titre and oxygen in mL/L will appear. Select the frame number you wish to modify, and enter the new titration value.

The new oxygen concentration will be calculated automatically. When you have completed your editing, quit the option and select Option 7 (Send oxygen data to disk) from the main menu. You will be prompted, "File already exists. Overwrite?". Enter 'Y' to update the file with the current changes.

3.7.4 Manually entering oxygen data

If a data file is lost for some reason, or you wish to calculate oxygen values from the thiosulfate titres, or calculate sample concentrations for new standardisation values, the data can be entered manually using Option 4 (Enter oxygen data by hand). You will be prompted for details of the standardisation and blank determinations, case ID and station number. You will be presented with a blank spreadsheet with the following parameters:

Frame No.
Begin voltage
End voltage
Titre
Oxygen
Quality index

Enter the thiosulfate titre for each sample (frame). Press <Space> if there is no corresponding data for that frame. When you have entered all the data, exit from the option by typing "E".

Save the data by selecting Option 7 (Send oxygen data to disk).

3.8 Standardisation

3.8.1 Quality assurance

The full standardisation procedure is described in Section 3.8.2.

The stability of the reagents used for the automated dissolved oxygen analysis allows us to standardise the system with a working standard every second day rather than every analytical session.

For quality assurance of the automated dissolved oxygen analysis system, we use three potassium biiodate ($KH(IO_3)_2$) standards:

- The commercially produced CSK 0.0100N $KH(IO_3)_2$ standard is the primary standard.

- The secondary standard is a large batch of 0.0100N $KH(IO_3)_2$ (2.5L) made up by CSIRO Marine Research staff and checked against the expensive CSK standard. This secondary standard is stable indefinitely.
- The third standard, also to be made up by CSIRO Marine Research hydrochemistry staff, is the working standard. This working standard is used during routine sample analysis and is stable indefinitely. Methods of preparing the secondary and working standards are described in Section 3.6.2.

To provide a comparison between the CSK and the cheaper, yet stable, secondary standard, the CSK and secondary standards should be titrated **about every 6 months**:

1. The CSK biiodate solution should be titrated against the thiosulfate until four concordant results to ±0.003 mL are achieved. Record the values in the dissolved oxygen log book on the vessel. To preserve sufficient quantities of the CSK standard, the biiodate draw-up tube can be placed directly into the CSK bottle, purged, and the biiodate system used as a dispenser. This eliminates rinsing and the possibility of evaporation and/or spillage while transferring the standard to another bottle.
2. Titrate the secondary standard in the same manner until there are four concordant results to ±0.003 mL. Record these values in the dissolved oxygen log book.
3. **At the start of each voyage**, check the working standard by thiosulfate titration against the secondary standard – which will already have been checked against the Primary CSK standard. This use of a secondary standard allows us to track the performance of each working standard batch in relation to the Primary CSK standard.
4. Compare the results of the titrations. The results should be better than ±1 ‰ (±0.02 mL) (Knapp et al., 1990). If the titres should differ by greater than this, a full comparative titration series of the three standards should be carried out. If either the secondary or working standard is in error, this procedure should reveal which it is.

3.8.2 Standardisation procedure

Mix the sodium thiosulfate and potassium iodate thoroughly before beginning the standardisation. If a new batch of thiosulfate is to be standardised, flush and prime the exchange unit thoroughly by dispensing 5 x 10 mL to waste. **Since the Dosimat cannot be controlled by the operator while the DODO program is active, the flushing routine should be performed before the program is loaded.**

Standardisation with the working biiodate solution should be performed every second day, or whenever a new batch of the thiosulfate titrant is prepared. The standardisation procedure determines the concentration of the sodium thiosulfate by titration against potassium biiodate with an exact normality of 0.0100 (0.000833 mol/L). Standard and blank determinations use deionised water.

The vacuum on the pipettes should be set so that they fill reasonably quickly, but with minimal turbulence. The 150 mL pipette should take around 30 seconds to fill, and the 50 mL pipette should take around 10-15 seconds.

1. Select Option 2 (Automated standardisation) from the main menu.
2. Dispense 150 mL of deionised water with a clean, calibrated 150 mL pipette (Halu™ Glass). Collect the volume in a clean, dry, manganese-free 300 mL dissolved oxygen bottle.

3. Add 1.0 mL of 5 mol/L H_2SO_4 to the bottle and mix thoroughly. Then add 1.0 mL of sodium iodide/sodium hydroxide reagent, and mix thoroughly. The solution should be perfectly clear at this stage of preparation. *If the solution is coloured or a yellow-brown precipitate appears, the sample has been contaminated with manganese, and should be discarded.* Carefully clean and dry the bottle, or select a new bottle and repeat the procedure.
4. If the sample is colourless, add 1.0 mL of manganese chloride reagent and mix thoroughly. Add a stirrer bar and place the bottle on the magnetic stirrer attached to the Dosimat.
5. Add exactly 15.000 mL of 0.0100 N potassium biiodate standard solution with the 20 mL exchange unit, while stirring thoroughly. The tip of the Dosimat dispenser must be immersed in the beaker to prevent spraying. All dispensers are fitted with anti-diffusive tips, which improve the precision of titrations (Culberson et al., 1991). Gently tap the tip of the dispenser to remove any attached drops.
6. Allowing at least 30 mL to overflow to waste (as this volume is deficient in iodine [Strickland and Parsons, 1972]), collect a 50 mL aliquot of this standard solution with a calibrated, clean, 50 mL volumetric vacuum pipette. Dispense the 50 mL aliquot into a 100 mL beaker containing a small stirrer bar. As the pipette drains, touch the tip of the pipette to the side of the beaker, and hold it there for 5 seconds after the pipette has emptied. The volumetric pipettes have been calibrated to deliver a volume in this manner.
7. Place the beaker on the titration stand, and lower the electrode so that the dual platinum sheets are immersed. The electrode is fragile, and care should be taken not to touch or bend the platinum sheets. Position the electrode and anti-diffusive tip so that they are in the outer part of the vortex, where mixing is greatest. Turn the stirrer on so that a vortex approximately 1 cm deep is formed, and trigger the start of the titration by pressing the space bar. The titration is completely automated from this point, and takes about 1.5 minutes to complete. A new standard solution can be prepared while the titration is proceeding.
8. Rinse the anti-diffusive tip of the thiosulfate dispenser and the electrode with deionised water before commencing the next titration.

Repeat the standardisation procedure until you have 4 successive titrations concordant to within ±0.003 mL. Record the results on the log sheet, and calculate the average titre.

3.9 Blank determinations

Reagent blanks should be measured immediately after the standard solutions have been titrated.

1. Select Option 3 (Automated blank measurement) from the main menu.
2. Add approximately 50 mL of deionised water to a clean 150 mL low form beaker. Add 1.0 mL of 5 mol/L H_2SO_4 and stir thoroughly before adding 1.0 mL of the sodium iodide-sodium hydroxide reagent. Mix well and observe the colour of the solution. *If the solution is not completely colourless, it has been contaminated and should be discarded.* Wash the beaker carefully and try again.

3. Next add 1.0 mL of the manganese chloride reagent and stir. Add a magnetic stirrer and deliver 1.000 mL of standard biiodate solution using the Dosimat. Press <space> to start the titration.
4. When the endpoint has been reached, add another 1.000 mL of the biiodate standard to the beaker using the Dosimat, and repeat the titration.
5. The blank is calculated as the difference between the two titres, and may be positive or negative. Rinse the antidiffusive tip of the thiosulfate dispenser and the electrode with deionised water before commencing the next titration.

The blank determination should be continued until you have 3 successive determinations to within 0.003 mL. Record the results on the log sheet, and calculate the average titre.

3.10 Sample titrations

The crate of samples should be acidified prior to analysis by carefully removing the cap, inserting the tip of the dispenser 1 cm below the surface and adding 1.0 mL of 5 mol/L H_2SO_4. Avoid trapping air bubbles in the sample, recap and mix vigorously. Repeat for each sample. Leave the samples to stand for about 30 minutes after acidification if the precipitate does not dissolve completely. If there is still precipitate remaining, add more 5 mol/L H_2SO_4 dropwise until no floc remains.

It is common for bubbles to appear in the sample after acidification. This is due to carbon dioxide and nitrogen being released from the solution, as the pH of the sample and the solubility of the gases change.

The final pH of the acidified sample should be close to 2.0. Optimum range is 2.0 to 2.5 (Friederich et al., 1991). If the pH is much greater than this, then the $Mn(OH)_2$ floc will not dissolve completely, giving artificially low results and, possibly, unwanted side reactions. If the pH is too low, then the loss of volatile iodine to the atmosphere is increased, again resulting in artificially low results.

1. To perform a sample titration, select Option 1 (Automated oxygen titration) from the main menu. You will be prompted to enter the station number of the samples you are about to analyse, to create the file name for the data. The station number can be up to 4 digits – letters are not acceptable.
2. Enter the average thiosulfate titre from the four concordant results obtained during the standardisation procedure, the average blank titre, and the crate ID for the station. Enter the sample number before beginning analysis of the sample.
3. Begin the analysis at sample 24 from the crate, and analyse in reverse order (ie, from 24 to 1, or shallow to deep). Mix each sample thoroughly just prior to collecting an aliquot for titration. The 50 mL aliquot should be collected in an identical manner to the standard solution. Allow at least 30 mL of sample to overflow, and ensure that the pipette tip is placed near the base of the sample bottle. In this way, a volume of sample that has not been in contact with the atmosphere can be reproducibly collected. Recap the sample bottle in case another aliquot is required.
4. Dispense the sample into a clean 100 mL low-form beaker with stirrer bar, with minimum turbulence and no splashes. Touch the tip of the pipette to the side of the beaker for 5 seconds after the pipette has drained.

5. Place the beaker on the magnetic stirrer, position the electrode and switch the stirrer on. Press <Space> to begin the titration. The titration is completely automated from this point and the next sample can be prepared while the analysis is being performed.
6. Record the result on the dissolved oxygen results sheet.
7. Rinse the antidiffusive tip of the thiosulfate dispenser and the electrode with deionised water before starting the next titration.

If for some reason there is a delay in starting the next titration, the sample should be held in the 50 mL pipette until required. If the sample is left open to the atmosphere in a beaker, appreciable losses of volatile iodine will occur.

3.10.1 Duplicate titrations

For quality control purposes, it is important that duplicate titrations are done frequently during an analytical session. At least two duplicate titrations should be done for every 24-bottle station, and these should be from a range of depths and concentrations. A repeat titration may also be needed if the analyst suspects a measurement to be incorrect.

The "Replace last value" option can be used for both duplicates and repeats.

- If you wish to do a QC duplicate for sample no 12, titrate the duplicate as frame no 13 (frame number is equivalent to bottle/sample number), and record the titration value. Then use the "Replace last value" option when sample 13 is actually titrated. The data file will now show sample 13 against frame 13, and the value for the duplicate of sample 12 will be overwritten.
- If you suspect a result, and want to repeat the analysis, use the "Replace last value" option before titrating the duplicate. The suspect value will be overwritten with the new value. Make a note of all entries and titres on the log sheet, so that the file can be edited manually at a later stage if necessary.

3.11 Maintenance and storage

The system is very robust, and requires minimal regular maintenance. The following points summarise the more common problems that may be encountered.

3.11.1 Dual platinum electrode

The dual platinum electrode should be kept scrupulously clean at all times. Avoid touching the electrode tips with your fingers, and store the electrode in deionised water between analytical sessions. At the end of the voyage, store the electrode in a beaker of deionised water sealed with parafilm. For extended storage times the electrode can be stored dry in the plastic case provided.

Recording the change in end-point voltage with time can easily monitor electrode drift. The end-point voltage varied from 0.020 mV to 0.030 mV over the duration of an *Aurora Australis* two-month voyage, which is equivalent to about 0.0017 mL/L, or 0.076 µmol/L dissolved oxygen.

A spare electrode should be carried on each voyage. If the spare electrode is required, the Edwards board should be adjusted as described in Section 3.5. Calculate the voltage factor, and edit the oxyhd.dat file accordingly.

3.11.2 Optifix reagent dispensers

The Optifix dispensers used to deliver each of the pickling reagents should be checked by dispensing 1.0 mL ten times into a 10 mL measuring cylinder. Adjust the dispenser as necessary. The dispensers should deliver 1.0 mL ± 0.05 mL. The Optifix dispensers tend to become stiff with time, and difficult to operate freely. Every few days, the dispensers should be flushed to remove the build up of chemicals that clog them. Flush the dispensers with deionised water until the plunger flows smoothly and can be operated easily with one hand. Pump out the deionised water before returning the dispenser to the bottle. Prime the dispenser as usual.

3.11.3 Volumetric pipettes

The volumetric pipettes should be stored filled with deionised water between analytical sessions. For longer-term storage, fill the pipettes with 0.1 % Triton X-100 solution and cover the tips of the pipettes with parafilm. The Triton X-100 solution will remove any grease that collects inside the pipettes over time. Ensure there are some beakers sitting under the pipettes in case of leaks.

3.12 Importing dissolved oxygen data into HYDRO

The HYDRO Program is CSIRO Marine Research software written specifically for computing and storing processed salinity, dissolved oxygen and nutrients results. A basic knowledge of Excel and Windows is required.

The DODO program produces a *.wnk file for every station that is processed. These can be imported directly into HYDRO, where they will be merged with other station data such as Niskin bottle number, depth, salinity, temperature.

1. Copy the *.wnk data files you wish to transfer to a 3.5" floppy disk. Create a backup version, and update frequently.
2. Before transferring oxygen data acquired on the automated system, check that the "Bottle Log" in HYDRO is filled out correctly for those stations you wish to process.
3. To transfer oxygen data in HYDRO, select "Dissolved Oxygen" from the HYDRO menu. You will be asked "Do you wish to transfer data from the automated dissolved oxygen system?" Select "OK".
4. You will be prompted to insert a floppy disk containing the relevant *.wnk files into Drive A. HYDRO will open the *.wnk files, transfer the data and re-save the file with a *.win extension.

A cruise file containing details of the stations already transferred will be created so that you do not have to reprocess those stations each time you update HYDRO. At the end of a voyage, the data for the whole voyage may be written to a *.csv (comma separated values) file by selecting "Make CSV file" from the main menu in HYDRO.

3.13 Plotting dissolved oxygen data

It is possible to have a preliminary look at the profiles of the dissolved oxygen bottle data by using the "Plot Data" option in HYDRO. Enter the parameters you wish to plot against the

X-axis and Y-axis prompts (ie, oxygen vs depth), and select the stations you wish to view. This facility is extremely useful for identifying outliers, and should be used when checking the data for typing errors and analytical errors.

Dissolved oxygen data can be edited by following the procedures described in Section 3.7.

3.14 References

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4 Salinity analysis

Val Latham

Contents

4.1 Introduction

Since September 1996, CSIRO Marine Research has used the Guildline Autosal Laboratory Salinometer 8400B to measure salinity ashore and aboard the research vessels *RV Franklin* and *RV Southern Surveyor*. The Autosal can be used to measure salinity values from 0.005 to 42 Equivalent Practical Salinity Units. Manufacturer specifications include a guaranteed salinity accuracy of ±0.002 salinity units.

By practising a refined measuring technique recommended by Guildline, we are able to achieve salinity accuracy of ±0.001 salinity units. This accuracy complies with the standard set by the World Ocean Circulation Experiment Office (1). Laboratory ambient temperature must be strictly controlled, samples must be carefully handled and the refined instrument operating technique must be used.

4.2 Method review

Salinity was originally measured as mass of salt per unit mass of seawater. In 1902, Forch, Knudsen and Sorenson defined salinity as "the total amount of solid material in grams contained in one kilogram of seawater when all the carbonate has been converted to oxide, all the bromine and iodine replaced by chlorine and all the organic material oxidised" (2).

Leading on from this work, the International Council for the Exploration of the Sea (ICES) defined "chlorinity". Based on a premise of the constancy of ionic ratios in seawater, chlorinity was measured by titrating seawater with silver nitrate. Salinity was then calculated from chlorinity by the formula:

$$S\,{}^{0}/_{00} = 1.80655\ Cl\,{}^{0}/_{00} + 0.03$$

Where $S\,{}^{0}/_{00}$ = the mass of total dissolved salts in 1000 g of sample
$Cl\,{}^{0}/_{00}$ = the mass of chloride in 1000 g of sample

In the 1950's, the measurement of electrical conductivity started to replace the chlorinity titration as a means of estimating salinity. Salinometers were developed and new relationships between salinity, chlorinity, conductivity ratio and temperature were established. Salinity measurements of natural seawater over a wide range of salinities were made and in 1966 the "International Oceanographic Tables for computing salinity from conductivity and chlorinity" were published. The tables had limitations and in 1978, the Practical Salinity Scale definition broke the existing chlorinity-salinity tie in favour of a salinity-conductivity ratio

relationship. The practical salinity, symbol S, of a sample of seawater, is defined in terms of the K_{15} ratio of the electrical conductivity of the seawater sample at a temperature of 15 °C and one standard atmosphere of pressure, to that of a potassium chloride (KCl) solution, in which the mass fraction of KCl is $32.4356 * 10^{-3}$, at the same temperature and presssure. A K_{15} value exactly equal to 1 corresponds to a practical salinity exactly equal to 35. The practical salinity is defined by the following equation:

$$\boxed{S = a_0 + a_1 Rt^{1/2} + a_2 Rt + a_3 Rt^{3/2} + a_4 Rt^2 + a_5 Rt^{5/2} + \Delta S}$$

$$\Delta S = (t-15)/((1 + k(t-15))(b_0 + b_1 R_t^{1/2} + b_2 R_t + b_3 R_t^{3/2} + b_4 R_t^2 + b_5 R_t^{5/2})$$

Where:

$a_0 = 0.0080$	$b_0 = 0.0005$
$a_1 = -0.1692$	$b_1 = -0.0056$
$a_2 = 25.3851$	$b_2 = -0.0066$
$a_3 = 14.0941$	$b_3 = -0.0375$
$a_4 = -7.0261$	$b_4 = 0.0636$
$a_5 = 2.7081$	$b_5 = -0.0144$
$\Sigma a_i = 35.0000$	$\Sigma b_i = 0.0000$

t = the bath temperature in °C
k = 0.0162
R_t = the conductivity ratio, which is half the Guildline instrument reading at temperature, t.

The equation is valid for a practical salinity from 2 to 42. In all cases the temperatures are measured according to the International Practical Temperature Scale (1968).

For routine conductivity ratio measurements, a working standard is prepared commercially from seawater calibrated against a defined KCl solution. The standard is the International Association for the Physical Sciences of the Ocean (IAPSO) Standard Seawater, produced and calibrated by the IAPSO Standard Seawater Service.

Salinity is now a dimensionless quantity. The Practical Salinity Scale eliminated the chlorinity units of parts per thousand (ppt or $^0/_{00}$). Sometimes salinity is referred to as practical salinity units (psu) referring to the formula it has been calculated from. Salinity should be reported with no units, such as in the following examples: *"The sample had a salinity of 35"* or *"the salinity was 35 on the Practical Salinity Scale 1978"* (3).

4.3 Sample collection and storage

The samples are collected after dissolved gas sampling and stored in amber glass, 250 mL bottles with bakelite caps with Teflon liners. The bottles are stored in wire crates that hold 24 bottles. Within a crate, the bottles are labeled alpha-numerically, numbers running from 1 to 24 with the same letter on each bottle within the crate.

Section 2.4 describes the collection procedure used for drawing high-quality salinity samples.

After sampling is finished or the crate is full, take the crate into the laboratory to equilibrate to room temperature. The sample temperatures may range from 0-30 °C so allow at least 6 hours' equilibration time before measuring.

Samples may be stored for up to one month, but analysis soon after collection is recommended. Accuracy of ±0.001 in salinity cannot be guaranteed for samples stored longer than a month (4).

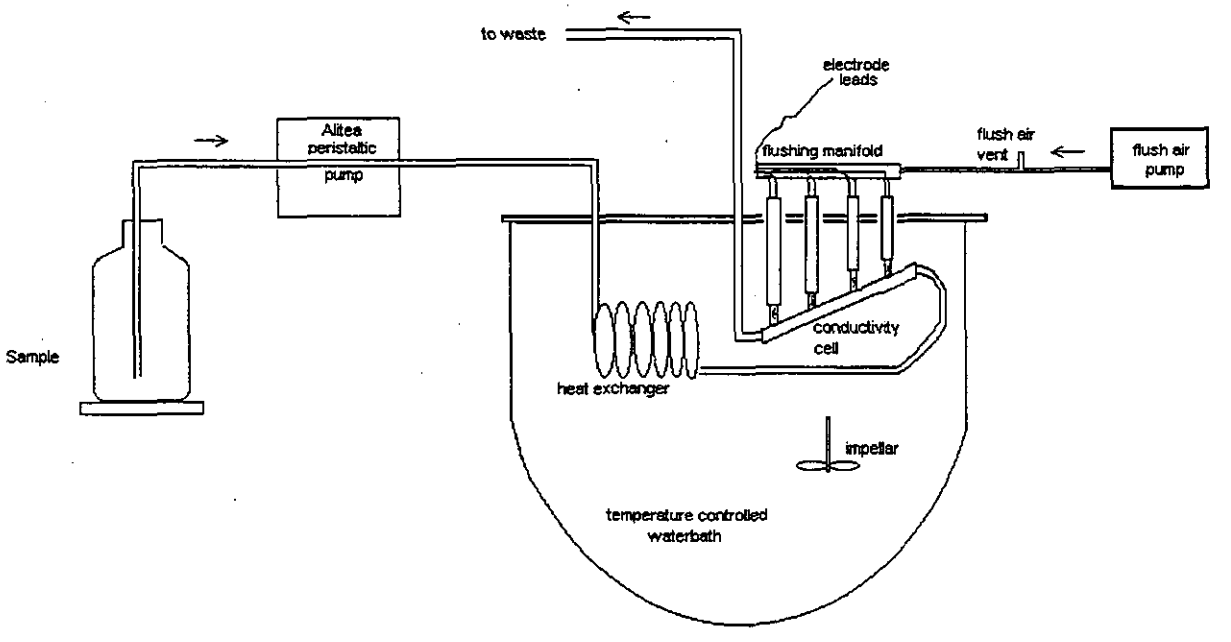
4.4 Introducing the Guildline Autosal salinometer

Guildline provide an excellent Technical Manual with detailed explanations of operation and maintenance procedures plus circuit diagrams. Pages 25-27 of the Guildline manual give instructions for high standards of operation, which, if followed meticulously, will achieve WOCE standard salinity accuracy of ±0.001. Operators should be familiar with the operating manual before attempting to use the Guildline. In addition, training from experienced personnel is essential.

Flow pathway of the salinity sample

The sample is drawn from the bottle by a peristaltic pump, which pushes it through a heat exchanger coil in the water bath and into the cell and then out to waste. Figure 4.1 shows a flow diagram of the sample pathway.

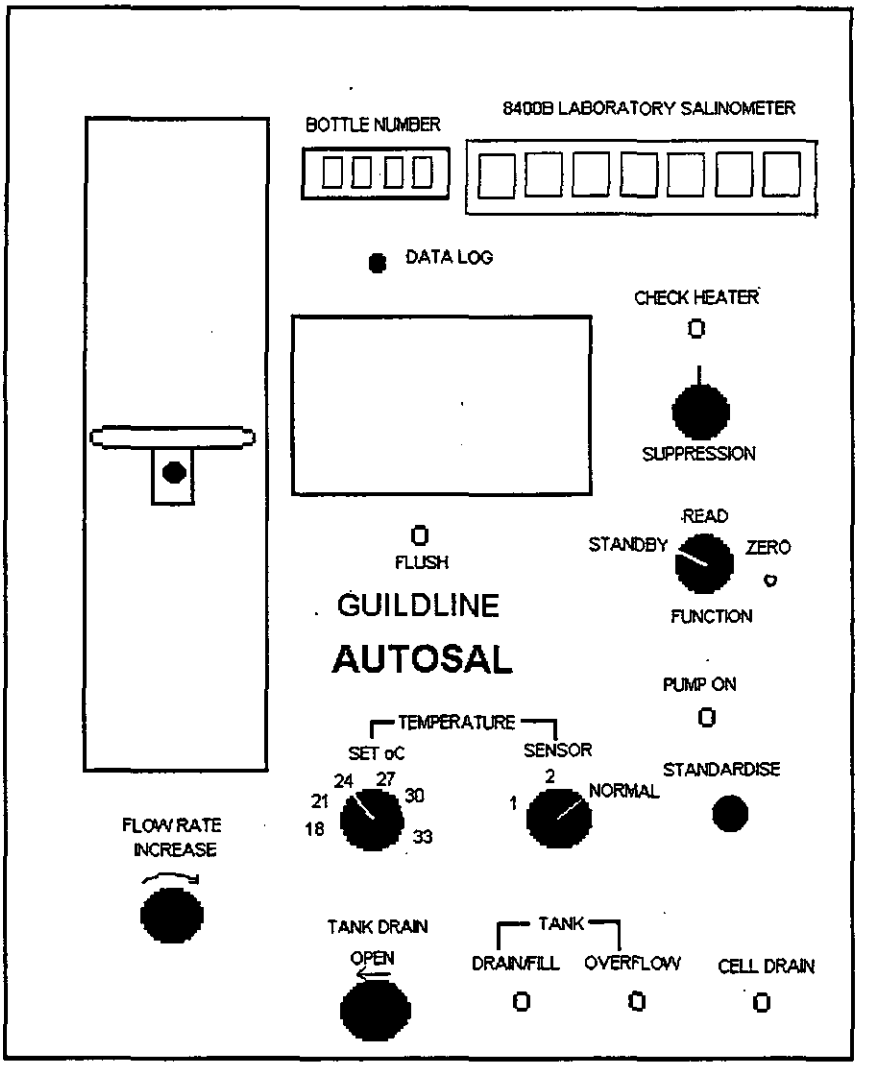
Figure 4.1 Flow diagram of the sample pathway for the Guildline Autosal



The Front Panel

In the following sections, the words in **BOLD CAPITAL LETTERS** refer to labels on the Guildline front panel (see Figure 4.2).

Figure 4.2 Front panel of the Guildline Autosal



4.4.1 Filling the water bath

The water bath is filled with MilliQ or pure water to reduce algal growth. To fill the water bath, place a carboy of MilliQ water on top of the salinometer. Connect a hose from the tap of the carboy to the **DRAIN/FILL** spigot on the salinometer. Open the **TANK DRAIN** valve and the carboy tap and gravity-feed the MilliQ into the water bath. Fill tank until water starts to discharge from the **TANK OVERFLOW** spigot. Close the **TANK DRAIN** valve and the carboy tap. Remove the hose and carboy.

4.4.2 Turning on the Guildline Autosal

The on/off power switch at the back of the instrument. On both CSIRO Marine Research vessels, the power comes through a Critec uninterruptable power supply unit. Turn the Critec unit on before the Autosal.

Once the power is turned on, the heater lamps in the bath should come on. The light is visible through the window on the front panel of the Autosal. The red light above **CHECK HEATER** should flash on and off. If the light is red continuously, one of the heater lamps has blown and should be replaced immediately. The lamps have a frosted coating on the

glass and it may be difficult to determine which one is not working. Ask electronics to check the lamp voltage with the multimeter.

4.4.3 Bath temperature control

The water bath temperature operating range is from 18 °C to 33 °C in 3 °C steps. The water bath temperature should be about 1-2 °C above room temperature (eg, room temperature at 20 °C and the water bath set at 21 °C).

The water bath is accurate to within ±0.02 °C of the temperature selected. During a day of ideal operating conditions, the Autosal water bath is capable of maintaining temperature to ±0.001 °C. The bath is heated by two lamps and cooled by a thermoelectric device based on the Peltier effect. The bath will heat more easily than cool.

Allow about 24 hours for the water bath temperature to equilibrate before starting analysis.

4.4.4 Laboratory temperature considerations

The samples, the Standard Seawater and the Autosal water bath temperatures need to be the same, ideally within 1 °C, and to remain stable. Therefore, the temperature around the salinity workspace needs to be constant.

Temperature sometimes varies between the floor and the bench, from corner to corner of the laboratory and during a 24 hour period. The ship's hull and deck can absorb the heat from the sun and the ocean and affect the laboratory temperature. The air conditioning may not be able to provide a constant temperature to the whole room.

A temperature logger is used to check the temperature around the workspace. It is usual practice to store the samples and standards on the bench near the Autosal.

Using a temperature logger

A temperature logger is a useful tool for monitoring the temperature around the salinity workspace. To get some idea of the daily temperature cycle, log temperature data for at least a 24 hour period before starting salinity analyses. It is useful to log temperature and download the profiles daily during salinity analysis.

Launching the *HOBO* temperature logger

1. Using the supplied cable, connect the HOBO to the COM1 or COM2 port of the computer.
2. Open the Windows **File Manager**, then the **HOBO** program.
3. Double click **Logbook.exe**.
4. Choose **Logger, Launch.**
5. The following message will come up on the screen
   Trying to connect..... Hobo found
6. The settings should be displayed as follows:

   Duration: 1-2 days
   Measure: Temp °C
   ☐ **Wrap around when full and over-right** *Leave this box empty*

7. Click on **START**. The HOBO will be launched and the red light on the HOBO logger will begin flashing.
8. The HOBO can now be unplugged and placed where the temperature needs to be logged.

To download the *HOBO* data

1. Using the supplied cable, connect the HOBO to the COM1 or COM2 port of the computer.
2. Open the Windows **File Manager**, then the **HOBO** program.
3. Double click **Logbook.exe**.
4. Choose **Logger, Readout**.
5. The program will ask for a file name and give a default name of **test.dtf**. Rename this to include the voyage code eg, **FR0898a.dtf**.
6. Save to **C:\HOBO**.
7. A graph and the logging status will be displayed on the screen. Print a hardcopy of the graph and store with the salinity logsheets.

4.4.5 Function check

In the following sections, the words in **BOLD CAPITAL LETTERS** refer to labels on the Guildline front panel (see Figure 4.2).

After the water bath has been filled and equilibrated to temperature for 24 hours, perform a function check to ensure that the Guildline Autosal is operating correctly. It should then be checked daily during use to maintain correct performance.

1. Switch **PUMP** on and ensure air flows from the **FLUSH** air vent. Turn off the pump.
2. Check the impeller is stirring. It can be viewed through the front window.
3. Check the **TEMPERATURE SET** switch. Turn to a higher setting; both lamps should come on for heating. Now turn to a lower setting; both lamps should go off.
4. The red light above **CHECK HEATER** will be continuously red if a heater lamp has blown. Under normal operating conditions the red light will flash on and off.
5. Use the **TEMPERATURE SENSOR** dial to check the two thermistors that control the bath temperature. Set the dial to thermistor **1**. After 4-5 minutes the lamps should begin to cycle in the same way as on the **NORMAL** setting. Repeat the test for thermistor **2**. If a the lamps do not flash normally, then the thermistor is probably drifting. This means that the bath temperature is no longer within specifications. A drifting thermistor usually also affects the Standardise value, which will drift also. The salinometer can be operated provided it is standardised and does not drift during operation. Ideally the Guildline should be replaced with a spare. The Guildline should be sent to Hobart where the bath temperature can be checked with a thermometer accurate to ±0.010 °C. The thermistor can then be replaced if necessary by the Electronics Group.

For further details see Section 5.2.3-5 of the Guildline operating manual.

4.4.6 The Guildline Autosal conductivity ratio display

On the top right hand corner of the Autosal front panel is the digital conductivity ratio display. The **FUNCTION** knob is used to select display options. The options are **STANDBY**, **READ** and **ZERO**.

In **STANDBY** mode the display will show the temperature of the water bath followed by the standby number eg, 21+6204, where 21 is the water bath temperature and 6204 is a constant and is a guide to the stability of the electronics. The **STANDBY** value will change when the **STANDARDISE** value is changed or the water bath temperature changes. If the last digit of the **STANDBY** value changes by more than ±1 unit then it will be necessary to restandardise the instrument.

In **READ** mode the display is showing the conductivity ratio eg, 2.0+0000. This value is twice the K_{15} value. For example, an IAPSO salinity standard with a known conductivity ratio of $K_{15} = 0.99997$ would read 1.9+9994 on the Autosal digital display. A variation of 0.0+0001 on the digital conductivity display of the Guildline Autosal is equivalent to 0.0002 salinity units.

In **ZERO** mode the display is showing the instrument electronics zero eg, 0.0+0010. This value should remain stable. It should be adjusted when the instrument is serviced.

4.4.7 Operating the sample pump

The Alitea pump is off when in speed zero position. Normal operating speed is 2.6, the flow rate is 30 mL/min. This speed allows a sample already equilibrated to room temperature enough time in the water bath to equilibrate to bath temperature. The pump may be left running while samples are read, provided there is enough sample. Maximum pump speed is 3.6, the flow rate is about 43 mL/min. Do not run the pump above speed 3.6 as the pressure created could break seals in the flow line.

More information on the pump specifications is given in Section 4.8.

4.4.8 Treatment of the conductivity cell

The conductivity cell is visible through the window on the front panel of the Autosal. The cell is made of glass and contains four platinum electrodes. It should be stored full of MilliQ when the instrument is not in use. If the cell has been stored dry or empty, the walls may require "wetting" by filling the cell with MilliQ and soaking for up to 6 hours.

Never leave the cell filled with salt water for more than one hour. Fill and flush at least 10 times with MilliQ to remove all traces of salt.

Filling and emptying the cell

1. The Autosal **PUMP** is used to empty the cell by placing a fingertip over the **FLUSH** valve
2. Turn on the Alitea pump (speed 2.6), place the inlet tubing into a beaker of MilliQ water and the cell will fill.
3. Observe the cell for trapped bubbles or blockages.
4. To clear bubbles, empty the cell and lines by removing the inlet line from the MilliQ and emptying the cell. Refill.

5. If bubbles are persistently sticking to the cell walls, rinsing with a solution of Decon detergent made up in 90 % methanol or ethanol may help and will not damage the cell. Never rinse with dilute acids or alkalis as they may damage the glass and electrodes. As a last resort, ultrasonicate the cell for ten minutes. If using cotton buds or tissues to remove algae take care not to damage the electrodes. Refer to the Guildline manual for further details.
6. If there is a blockage in one of the lines connected to the electrodes, open the front panel and open up the tubing connected to the cell. Sometimes salt crystals form in one of the four teflon tubing lines that supply air to the cell. Flush the blocked line with compressed air or water and a syringe.

4.5 Standardisation and salinity measurement

4.5.1 Setting up the data logger

The salinity data can be logged directly from the Autosal to the computer program HyperTerminal. From HyperTerminal it can be transferred to the Hydro program.

To collect data from the Autosal

1. Open the program Hyperterminal and then **guildline.ht**.
2. **Guildline.ht** is the HyperTerminal setup file and is set up for com1. If you are using another com port, change the setting in Hyperterminal by selecting **File, Properties**.
3. Select **Transfer, Capture text** and type in the text file name – this must have the format **c:\hydro\vyycc\vyyccrrr.slt** (eg, c:\hydro\f9902\f9902001.slt).
4. Each time you press the red button **DATA LOG** on the Autosal, the numbers will be displayed and also written to the text file. As a safety precaution you should occasionally print the numbers as displayed from Hyperterminal. You can use **Transfer, Capture text, Stop** or **Pause** when you are resting.
5. You must use the following numbering rules for sample numbers (**BOTTLE NUMBER**) on the Autosal:
   - the first digit must always be 0 for a sample
   - the 2nd digit corresponds to the crate letter (eg, 1 = A, 2 = B, 3 = C etc)
   - the last 2 digits for a sample are the last 2 digits on the bottle (so C08 will be 0308 on the Autosal)
   - to record a standard reading, change the sample number on the Autosal to 998 before pressing the red button
   - to record a standby reading, change the sample number on the Autosal to 999 before pressing the red button
   - to record a zero reading, change the sample number on the Autosal to 000 before pressing the red button
6. The HYDRO salinity log cannot store more than 36 sample results, therfore, the file vyyccrrr.slt should contain no more than 36 sample results per run.

7. Once you have finished a run, select **Transfer, Capture text, Stop** to close the text file.

8. When you want to transfer the data from the text file into the HYDRO data file, use HYDRO to bring up the salinity sheet as normal (refer to Section 4.6). The salinity sheet will have a **Transfer Data** button that, when pressed, transfers all the data from the text file onto the salinity sheet..
   Note that only new salinity sheets will have the button. When you press the button, the program opens the .slt sheet for that run (the salinity sheet number must correspond to the run number). For each bottle number on the salinity sheet, the program tries to find a corresponding conductivity ratio in the text file. If it can't find one (ie, there was no analysis for that sample) it warns you. If the .slt file is found, the program transfers the data to the salinity sheet.

4.5.2 Standardisation of the Guildline Autosal

Standard seawater

Once the definition of salinity became referenced to conductivity instead of chlorinity, it was decided a standard seawater should be produced from one source. That source was the International Association for the Physical Sciences of the Ocean (IAPSO). The Standard Seawater (IAPSO SSW) was produced in Copenhagen by IAPSO until 1975, but is now produced and calibrated by the Institute of Oceanographic Sciences (IOS), England.

The SSW is packaged in glass vials labelled with the date of production, the Batch number and the K_{15} value.

IAPSO SSW is available in salinity values ranging from 10 to 35. The CSIRO Marine Research Hydrochemistry group uses SSW of 35 for routine standardisation.

IAPSO SSW can deteriorate with time, so use vials that are no more than 18 months old (5, 6).

Standardisation procedure

The IAPSO SSW is used to calibrate the Autosal at the start of each analysis session and should be equilibrated to bath temperature before use. Laboratory temperature variations are discussed in Section 4.4.4.

1. Turn on the Autosal **PUMP**. Flush MilliQ from the cell.

2. Set the Alitea pump to speed **2.6** and place the inlet tubing into a bottle containing water of salinity approximately 35. An already opened IAPSO standard or an old salinity sample will do. Do not use a new vial of SSW, as these vials are expensive.

3. Fill the conductivity cell and check for trapped bubbles, as these will affect the measurements. Fill and flush the cell with the old SSW or sample about 10 times to remove all traces of MilliQ.

4. With the cell completely full, read the digital conductivity display. Turn the **FUNCTION** knob to **READ** and observe the signal for about 5 seconds. If the digital display flashes, adjust the **SUPPRESSION** knob until a positive (+) stable value is obtained. If the signal still flashes, there may be an air bubble in the cell. If the signal wanders, return the **FUNCTION** knob to **STANDBY** and then flush the cell. *Do not flush the cell in* **READ** *mode, as this will overload the electrodes and cause a small temporary read offset.* If the signal is stable, leave the pump running and go to the next step.

5. Wrap a new vial of IAPSO SSW in a Teriwipe (paper towel) to reduce any heat transfer from your hands. Gently invert twice to mix contents.
6. Open the vial by breaking off the glass tip from one end of the vial. Do this by scoring the glass with a file and gently tapping the tip. Place the tip that is on the other end of the vial into the hole on the Autosal bottle shelf. It may need one hand to steady it.
7. Dry the inlet tube of the pump with a tissue and pump any previous sample from the tube before placing it into the opening in the vial. Pump the IAPSO SSW into the cell, fill and flush twice.
8. Fill the cell for a third time, then turn the **STANDBY** knob to **READ**. Adjust the **SUPPRESSION** knob to get a stable reading. If the conductivity ratio display is stable to ±0.00002, mentally record the average number. Turn the **FUNCTION** knob back to **STANDBY**, flush and refill the cell then take another reading.
9. Repeat the measurement procedure until at least two successive readings agree to within ±0.00002. The conductivity ratio display should be exactly *twice* the K_{15} ratio on the SSW vial label. If not, release the lock on the **STANDARDISE** knob and turn the knob until the conductivity ratio display is correct.
10. Check that the standardise value on the Autosal agrees with the standardise value entered into the Salinometer Logbook when the Autosal was last used at this water bath temperature. If the standardise value has changed by more than 1.00 units it may have a faulty thermistor or the IAPSO SSW may be contaminated.
11. To check the IAPSO SSW, open a second vial of IAPSO SSW and take more measurements. If the readings for the two vials do not agree, check your technique and the date on the vials and perhaps try a third vial. There should be agreement between at least two of the vials.
12. If the **STANDARDISE** values in the logbook show a gradual drift in one direction then one of the thermistors may be drifting. Eventually the standardise value will drift off scale, so the thermistors shoud be replaced (3). Refer to Section 4.4.5.
13. Record a standby, zero and standard reading using the red **DATA LOGGER** button on the Autosal and the HyperTerminal datalogger. Refer to Section 4.5.1.
14. The following details of the standardisation are recorded on the Salinometer Log Sheet. Some of the details are also recorded in the Salinometer Logbook to provide an ongoing history of the instrument's performance.

    Ship:
    Date:
    Analyst:
    Voyage:
    Lab temp:
    Standard Seawater Batch No:
    Guildline No:
    Rs set: - *Enter the value for the* **STANDARDISE** *dial*
    SBY (start): *Enter the* **STANDBY** *display value at the beginning of the analysis for that sheet*
    Zero (start): *Enter the* **ZERO** *value*
    SBY (end): *Enter the* **STANDBY** *display value at the end of the analysis for that sheet*
    Zero (end): *Enter the* **ZERO** *value at the end of the analysis for that sheet*

In ideal conditions the values entered for SBY, Zero and Rs set should remain constant during a run and from day to day.

The **STANDBY** value will change when the **STANDARDISE** value is changed. It may also change if there is sufficient change in the laboratory temperature. If the last digit of the **STANDBY** value changes by more than ±1 unit then it will be necessary to restandardise the instrument. If in doubt, it is wise to run a new IAPSO SSW to confirm whether the instrument has drifted and by how much. Record any drift from the start of a run to the finish. It is possible to compensate for drift when calculating salinity with the HYDRO program.

4.5.3 Measuring samples

When the Autosal has been standardised, and the information is recorded on the log sheets, the samples can be measured. The samples should have been equilibrated to room temperature for at least 6 hours before analysis begins. Large temperature differences between the samples and the bath can result in degassing of the seawater in the cell. If the sample temperature is markedly different from the water bath temperature the bath may have trouble maintaining temperature. This will be indicated by:

- The irregular manner in which the heating lamps turn on and off – it should be rhythmical.
- Unstable sample readings – readings should be stable to within ±0.00002.

If the water bath does not appear to be coping adequately, then it may be necessary to try one or all of the following options:

1. Check that the room temperature and bath temperature are within 1 °C of each other
2. Wait until the samples have equilibrated to room temperature
3. Stop the pump during each reading
4. Slow the pump down

Analysis steps are as follows:

1. The samples are measured as a station group, starting with the shallowest and finishing with the deepest. Record the station number and the sample bottle numbers in the order that they will be measured on the sheet (usually from 24 to 1).
2. Select the first sample from the crate, check the bottle number and gently invert the sample two or three times for complete mixing without agitation. Vigorous shaking can produce bubbles which will interfere with the measurement if they enter the cell.
3. Turn the Autosal **PUMP** on. Set the Alitea pump to speed **2.6**, dry the pump inlet tubing with a tissue or Teriwipe and allow some air to enter the pump tubing. This is to prevent the new sample becoming contaminated.
4. Use the **FLUSH** valve to pump the previous sample to waste then place the inlet tubing into the sample bottle. Fill and flush the cell. Fill the cell again and check that the four platinum electrodes are completely immersed in sample and there are no bubbles stuck in the cell.
5. Turn the **FUNCTION** knob from **STANDBY** to **READ**. Adjust the conductivity display with the **SUPRESSION** knob until a stable positive reading is obtained. If the reading is wandering, flush and refill the cell.

6. Continue to flush and fill the cell until two consecutive measurements agree to ±0.00002 digits. Each filling uses about 15 mL of sample, so if the sample level is getting low, turn the pump off while taking the sample reading.
7. To electronically record the reading, make sure the sample bottle number and crate code (A=01, B=02 etc) are entered correctly on the Autosal **BOTTLE NUMBER** display then press the red button labelled **DATA LOG.**
8. On the hardcopy logsheet in the column with the heading "Ratio Measured", record the conductivity ratio on the salinometer log sheet next to the sample bottle number.
9. Turn the pump off, remove the sample bottle, recap it and place back in the crate. Repeat the procedure with the next sample.
10. When the salinity of consecutive samples varies by less than ±3 in salinity then three or four cell fills should be sufficient to obtain a stable reading. For larger differences, such as changing from a salinity of 10 to a salinity of 35, about 10 cell fills will be necessary.
11. Record **STANDBY** and **ZERO** values after each station. If there is a drift in the **STANDBY** value of more than ±1 unit then the standardisation should be checked with another vial of SSW, and the value recorded for use in the data processing.

4.6 Using the HYDRO computing program to calculate salinity

Salinity can be determined directly from the conductivity ratio and the temperature by using the formula listed below. For more details on the formula see Section 4.2.

$$S = a_0 + a_1 Rt^{1/2} + a_2 Rt + a_3 Rt^{3/2} + a_4 Rt^2 + a_5 Rt^{5/2} + \Delta S$$

At CSIRO Marine Research, this formula is used to calculate salinity in a computing program called HYDRO. HYDRO is an "in-house" program used to process all the hydrochemistry data produced on a voyage at sea. HYDRO runs in Microsoft Excel Version 4.0.

If you have used the HyperTerminal logging program, most of the following information will be transferred automatically to HYDRO by clicking the **Transfer Data** button. (Refer to Section 4.5.1). However, to open up the HYDRO salinity log sheet and to enter data manually, use the directions below.

1. Open the HYDRO program by double clicking on **Hydstart.xla.**
2. Check that the **Cruise Name** and **Station Number** details are correct, then click **OK.**
3. A form displaying the message **Welcome to Hydro** appears. At the top is a menu bar. Select **Hydro, Bottle Log.**
4. Check that the **Cruise Name** and **Station Number** details are correct, then click **OK.**
5. The **Bottle Log Sheet** will be displayed. Enter Niskin bottle and salinity bottle numbers from the CTD log sheet or field sheet. If the bottle numbers have already been entered go to step 6.
6. Choose **Hydro, Salinities.**
7. Check that the **Cruise Name** and **Station Number** details are correct, then click **OK** .
8. The next message box asks '**Are you using a GUILDLINE salinometer?**' click **OK.**

9. The next message box asks '**Enter Salinity Analysis Sheet Number**'. Enter the salinity sheet number and click **OK** (salinity sheets are numbered from 1 onwards in order of analysis).

10. The **Salinity Analysis Sheet** should now appear on the screen. Check that the **Sheet No.** and **Cruise** name are correct.

11. Complete details at the top of the form. Use the following as an example of what to enter.

    In the International Seawater box fill in the details from the IAPSO SSW vial, for example

    Batch: P133
    Date: *(from vial)*
    Cond: 1.99972 *(twice the conductivity on the SSW vial)*

    Fill in the following details:
    Stations: 1 to 5
    Instrument No: 62547
    Analyst: VL
    Date Measured: 12/5/98
    International temp: 21.0 *(this is the Autosal water bath temperature)*
    T comp: *leave blank*
    Stdze: *leave blank*

    Sub-standard temp: 21.0 *(this is the Autosal water bath temperature)*

    T comp: *leave blank*
    Stdze: *leave blank*
    Conductivity ratio: 1.99972 *(twice the conductivity on the SSW vial)*

12. Move the cursor to the blue box under **Temp**. Type in the water bath temperature and press *enter*. The cursor will move one box to the right. Type in the Guildline conductivity ratio for the standard, eg, 1.99972 (twice the conductivity ratio on the SSW vial) and press *enter*.

13. The station data (station number, depth and bottle number) should appear under the first blue line. Type in the conductivity ratio for each sample, pressing *enter* after each entry. The water bath temperature for the next sample will enter automatically if *enter* is pressed after typing in the conductivity ratio. To delete a bottle place the cursor on the appropriate line and click **Delete Sample.**

14. To enter another station's data, put the cursor on the first free line and click **Next Station.**

15. When all the conductivity ratios have been entered, move the cursor to the next free line and click **Insert Sub-Std.** Enter the conductivity ratio for the IAPSO SSW at the end of the analysis. This should be the same as the start if the instrument has not drifted. The program will now calculate the salinity values and correct for any drift from the first standard reading to the last one. The corrected salinity will be automatically entered into the far right column labelled **Salinity**.

16. To exit from the salinity sheet, click **Hydro** in the menu and make the next selection.

4.7 Closing down and storing the Guildline Autosal

1. Check that the conductivity cell is filled with MilliQ, turn off the power switch at the back of the Autosal. Turn off the power to the Critec unit at the ship's powerpoint.
2. If the Autosal will not be used for more than one month (eg, at the end of a voyage), drain the tank. To drain the tank, connect a hose to the **DRAIN/FILL** spigot on the salinometer, open the **TANK DRAIN** valve and empty the water into the sink via a hose or bucket.
3. Remove the silicone tubing from the Alitea pump head so that it does not become crimped.

4.8 More on sample pumps

The sample needs to be pumped around the Autosal at about 30 mL/min. This speed will allow the sample sufficient time in the heat exchanger coil for temperature equilibration with the bath, provided the sample temperature is within 1 °C of the bath. Under these conditions, it should not be necessary to turn the pump on and off.

Alitea pump

The Alitea U1-SP peristaltic pump is used because it operates quietly, has variable speed control and does not require the salinity bottle to be pressurised. The pump sits on top of the Guildline salinometer above the sample inlet. The pump outlet tubing is connected to the Teflon tubing leading to the heat exchanger. The pump inlet tubing is placed in the sample bottle, which sits on the sample holder. The tubing used around the pump head is silicone tubing connected with fittings to Tygon R-3603 tubing. Flow rates are given in Table 4.1. The pump should not be run faster than about 43 mL/min as the pressure may break seals in the sample path.

Part numbers and suppliers for this pump are shown in Section 4.12.

Guildline pump

Experience has found the Guildline pump to be hazardous, as sample bottles are pressurized and have exploded on two occasions. To use this pump, place the sample bottle in the holder with its neck pressing against the rubber seal. Passing through the rubber seal are two stainless steel tubes – a straight one connected to an air pressure pump and an L-shaped one connected to the heat exchanger line. The L-shaped tube has about 15 cm of Teflon tubing for sample inlet. Turn on the salinometer pump and adjust the pressure delivered to the bottle by using the **FLOW RATE INCREASE** knob. Full speed is about 31 mL/min.

OSI pump

The OSI peristaltic pump is not entirely suitable, as it is noisy and the slowest pump speed is about 42 mL/min. To use the OSI pump, connect the pump outlet tubing to the L-shaped stainless steel inlet tubing of the heat exchanger. The pump inlet tubing passes through the hole in the bottle platform and the pump is secured under the platform with the plastic nut. Sample bottles can be hand-held or a clip made by the workshop can be used (this clip grips the bottle at the neck and sits in the bottle-platform track). The OSI pump has two speeds,

but only speed 1 is recommended. This speed will deliver about 42 mL/min, while speed 2 will deliver about 45 mL/min.

Table 4.1 Pump flow rates at chosen settings

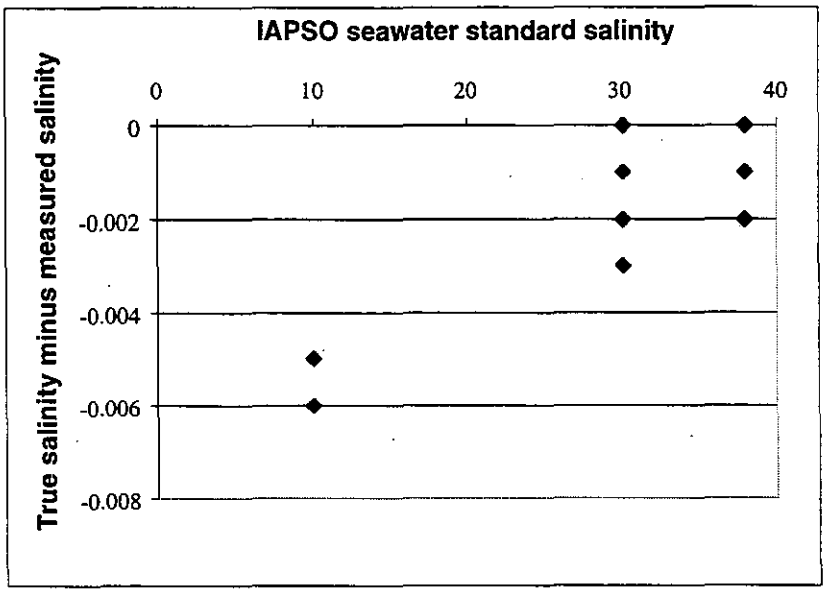
Pump	Type	Setting	Flow rate (mL/min)
Alitea U1- T	peristaltic	3.6	42.5
Alitea U1-T	peristaltic	2.8	33
Alitea U1-T	peristaltic	2.6	30
OSI	peristaltic	1	42
OSI	peristaltic	2	45
Guildline	pressure	Maximum flow	31

4.9 Linearity and salinity range

If the samples to be measured are of low salinity, it is possible to measure them after calibrating the Guildline with an IAPSO standard of salinity value around 35, but be aware that there will be some error associated with the result.

Experiments were conducted on Guildline Serial No: 62547 to determine the accuracy of low-salinity measurements after the Autosal had been standardised with a 35 standard. IAPSO seawater standards of salinity values ranging from 10 to 38 were measured and the accuracy recorded. The error associated with each measurement increased as the salinity was lowered, with a maximum error of -0.006 at a salinity of 10. The trend is plotted in Figure 4.3.

Figure 4.3 Error associated with salinity measurements made with Guildline salinometer, Serial No: 62547, standardised at 35.



4.10 Possible sources of error

1. Differences between batches of IAPSO seawater can alter salinity results for different voyages. That relative SSW batch-to-batch errors occur has been well documented by several studies (5, 6). New batches should be checked against old.
2. Discontinuity between suppression dial settings on the Guildline. This should be checked periodically.
3. Offset in the water bath temperature of more than ±0.02 °C.

4.11 References

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4.11.1 Further reading

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4.12 Spare parts and suppliers list

Item	Part Description	Part Number	Supplier
Alitea pump	Pump head tubing, Silicone, 4.8 mm I.D., 2.4 mm wall	HS-0480-24	A.I. Scientific, Ph: 07 3897 3888
Alitea pump	Connector fittings	1050-6	A.I. Scientific Ph: 07 3897 3888
Alitea pump	Tygon tubing	R-3603	TACS Australia Ph: 02 9955 7388
IAPSO SSW	P-series (salinity 35)		OSI. Osil@oceanscientific.co.uk
Light globes	Showcase bulb T-8 tubular 120V, 40W		John Joynt, Guildline, 102363.3211@CompuServe.COM
Salinity bottles	Amber glass, 150 mL		Plasdene Glass-Pak Pty Ltd, Preston, Vic 3072 Ph: 1800 650 632
Bottle lids	Wheaton Black Plastic Screw Caps with Teflon Liner Size 24-400	Catalogue No: 240418	Edwards Instrument Company, Narellan, NSW
Salinity crates	Stainless steel crates		CSIRO Marine Research workshop

5 Nutrient analysis (Alpkem)

Rebecca Cowley and Ruth Eriksen

Contents

5.1 Method review

The Alpkem Flow Solution (III) Analyser (or autoanalyser) is used for the simultaneous analysis of reactive silicate, nitrate plus nitrite, and dissolved inorganic phosphate (orthophosphate) in seawater. The autoanalyser uses the principles of colorimetric analysis, in a continuous flow system where all manipulations of the samples are automated. Thus, each sample and standard is treated identically, by precision timing and proportioning of reagent additions. An automated sampler introduces seawater samples into the analytical stream at precisely timed intervals. The sample is then split into individual streams according to the number of chemistries to be determined, and each sample segment is then mixed with reagents, which are dosed into the analytical stream by a peristaltic pump. As the sample moves through the system, the sample mixes with the reagents, and a coloured compound whose light absorbance is approximately proportional to the nutrient concentration in the sample is formed (Gordon et al., 1993). Each channel is calibrated with calibrants of known concentration, establishing the relationship between the absorbance at the selected wavelength and the standard concentration.

Normal concentration ranges are 0 to 35 µmol/L, or 0 to 140 µmol/L silicate, 0 to 3 µmol/L orthophosphate, and 0 to 35 µmol/L nitrate + nitrite. Details of chemistries for the individual analyses can be found under Section 5.4.

The system consists of the following modules:

- 1 x 501 XYZ autosampler
- 1 x 509 power distribution module (PDM)
- 1 x 502 bi-directional, variable-speed peristaltic pump
- 1 x 503 cartridge heater, with programmable thermostat units
- 3 x reaction cartridges, one for each analysis
- 3 x 510 monochromator detectors, one for each analysis
- 3 x 550 monochromator detectors, one for each analysis
- 1 x PC with WinFlow Software for recording and analysing data
- 1 x YEW 3-channel chart recorder

The system can be operated in either Flow Injection Analysis mode (FIA) or Segmented Flow Analysis mode (SFA). All analyses on board *RV Franklin* are conducted in the SFA

mode. Data are acquired and processed with the software package WinFlow (Alpkem). Data are backed up by a YEW type 3056 multi channel chart recorder. Results can be processed manually from this hard copy in the case of data loss.

An Excel spreadsheet has been written to process results from WinFlow. A separate program, HYDRO, is used to process salinity and dissolved oxygen data collected on oceanographic cruises. A basic knowledge of Excel and Windows is required to use the spreadsheet and HYDRO.

Sampling protocols used to collect representative nutrient samples are documented in Section 2.5.

5.2 Principles and components of segmented flow analysis

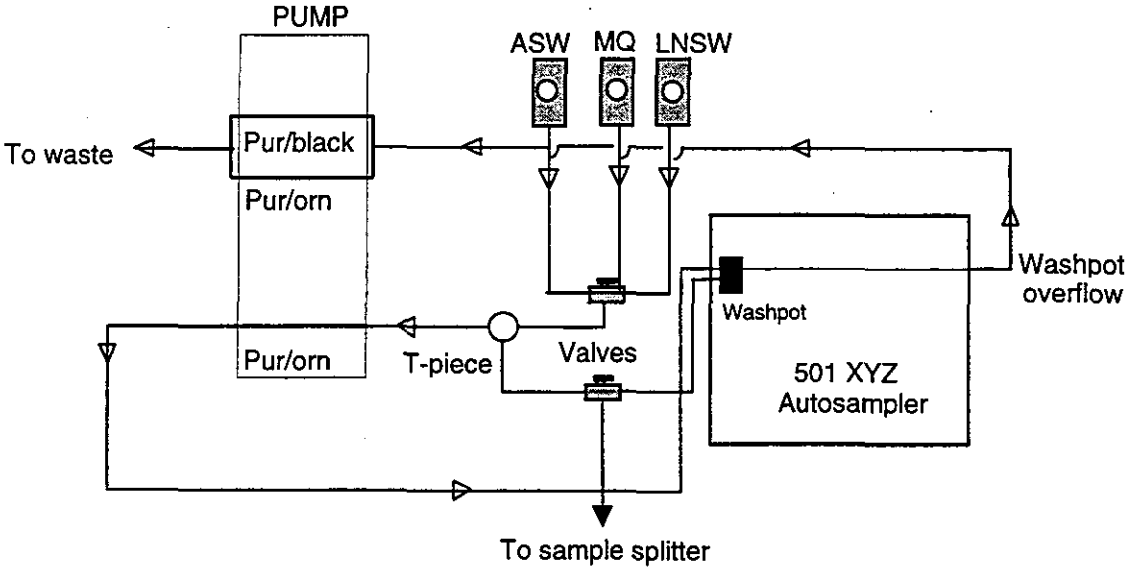
5.2.1 Autosampler

The 501 XYZ Autosampler is capable of holding up to 300 samples in 6 racks specially modified to take the 10 mL polypropylene tubes in which the nutrient samples are collected. The autosampler can be operated in stand-alone mode, or be remotely controlled by WinFlow.

The autosampler is used to introduce the nutrient sample into a carrier or wash solution of artificial seawater (ASW). Each sample "slug" is separated from the carrier solution by an intersample air bubble introduced as the sample probe moves to and from the "home" or washpot position. The control panel allows selection of the sample/wash cycle, selection of the type of sample vial, and the total number of samples to be analysed during the run. For seawater analysis, the autosampler is programmed to draw up the sample for 60 seconds, and then return to the washpot for 30 seconds, when the ASW solution is drawn up. The sample probe then moves to the next tube in the sampling rack, so that a new sample is introduced into the analytical stream every 90 seconds.

The ASW is supplied to the wash pot in excess of the sampler probe demand, so that the probe is always completely immersed and cannot draw up air. The excess wash solution also acts as a rinse for the outside of the probe so that intersample contamination is eliminated. Overflow from the washpot is carried away to waste by tubing connected to the peristaltic pump. A small Hamilton 90° three-way valve allows a choice of MilliQ, LNSW (Low Nutrient Seawater) or ASW as the wash solution, and another two-way valve allows the sampler to be switched out of the system while running baselines. See Figure 5.1 for a schematic diagram of the autosampler configuration.

Figure 5.1 Schematic diagram of autosampler configuration and plumbing for nutrient analysis in seawater



5.2.2 Peristaltic pumps and flow-rated tubing

The autoanalyser has one Ismatec 502 bi-directional, variable speed pump, which essentially controls the whole analysis system. The 502 pump has 24 independent platens, each with a tension lever to individually adjust the compression of the flow-rated tubes. The flow rates are determined by the internal diameter of the pump tubing, and by the pump speed setting. Different internal diameter (ID) pump tubing is easily recognised by the two colour-coded collars, which also serve to hold the tubes in place on the platens. Table 5.1 summarises the colour codes, IDs, and flow rates at various pumps speeds for the complete range of Alpkem pump tubes available. Pump tube lifetime is between 200 and 500 hours of continuous operation, depending on the nature of the reagent and the tension on each tube (Grasshof, 1976). Pump tubing should be replaced at least every two weeks of continuous operation under cruise conditions (ie, instrument operating 24 hours a day) (Eriksen, 1997). Platens should always be released when the system is not in use to extend the lifetime of the pump tubes. Pump tubes should always be stored with deionised water in them. For long-term storage, pump tubes should be left dry.

Table 5.1 Summary of pump tubes' colour codes, ID and flow rate at various pump settings.

The flow rate at a given pump speed setting (S) on the pump is calculated as follows: Flow rate at S % = (S/99) x (Flow rate of pump tube at 99 %) (Adapted from Eriksen, 1997).

Pump tube colour code	ID (in.)	Flow at 40 % (mL/min)	Flow at 1 % (mL/min)	Flow at 99 % (mL/min)	Manifold Nut ID (in.)	Connections Ferrule ID (in.)
orn/grn	0.015	0.10	0.004	0.250	0.100	0.100
orn/yel	0.020	0.18	0.008	0.438	0.100	0.100
orn/wht	0.025	0.25	0.010	0.625	0.100	0.100
blk/blk	0.030	0.32	0.013	0.800	0.100	0.100
orn/orn	0.035	0.41	0.013	1.025	0.100	0.100
wht/wht	0.040	0.56	0.013	1.388	0.100	0.100
red/red	0.043	0.71	0.025	1.750	0.125	0.125
gry/gry	0.051	0.84	0.025	2.088	0.125	0.125
yel/yel	0.056	1.01	0.038	2.500	0.125	0.125
yel/blu	0.060	1.12	0.038	2.775	0.125	0.125
blu/blu	0.065	1.35	0.050	3.350	0.125	0.125
grn/grn	0.073	1.57	0.063	3.888	0.125	0.125
pur/pur	0.081	2.05	0.088	5.075	*	*
pur/blk	0.090	2.45	0.100	6.063	*	*
pur/orn	0.100	2.71	0.113	6.700	*	*
pur/wht	0.110	3.24	0.138	8.025	*	*

5.2.3 The use of surfactants

Segmented flow analysis is dependent on constant and smooth flow rates for precision analysis. Reactions are not required to reach a steady state equilibrium from the time the sample is mixed with reagents to when it reaches the detector (ie, 100 % of the maximum signal is not necessarily obtained), so reproducible conditions are critical (Valcarcel and Luque de Castro, 1987; Gordon et al., 1993). Surfactants are added to each manifold to ensure the maintenance of smooth flow and regular air segmentation, and to minimise peak broadening as a result of frictional turbulence from the walls of the tubing. No single surfactant is compatible with all three chemistries, so two principal surfactant types are used: 'Brij-35' is used for nitrate plus nitrite analysis, and sodium dodecyl (or lauryl) sulfate (SDS) is used for silicate and orthophosphate analysis. Surfactant solutions are used as part of the start-up procedure, to wet or prime the tubing and the flowcells. The surfactants are also added to various reagents so that a constant concentration of wetting agent is present in the system at all times.

Until August 1997 'Dowfax' was used by CSIRO Marine Research as a surfactant for the silicate and orthophosphate channels, as described in the Alpkem methodology. Dowfax

causes a matrix effect in both silicate and orthophosphate chemistries by enhancing seawater peaks, their size depending on the concentration of Dowfax used (Cowley, 1998).

5.2.4 Reaction cartridges and cartridge heaters

The analytical manifold or reaction cartridge is the site where air segmentation, reagents and diluent (if necessary) are introduced or mixed with the sample after it has been split into analytical streams. The reaction cartridges are designed to be easily modified, and all fittings are connected by finger-tight screw-in fittings. Each cartridge is positioned in the 503 Cartridge heater, which has four programmable heater cones to provide thermostatic heat to the reaction cartridges if required. The silicate and orthophosphate chemistries have insulated PEEK mixing coils built into the cartridge. The rate of the reaction can be accelerated by increasing the temperature at which the reaction occurs, via the cartridge heater unit. The configuration of each of the individual analysis cartridges is shown in Section 5.4.

5.2.5 Air segmentation

As its name implies, segmentated flow analysis segments the sample by air or inert gas bubbles. The bubbles are introduced in the same manner as the chemical reagents, that is with flow-rated pump tubing. The bubbles in the analytical stream ensure that each segment (or unit volume of sample) receives a measured amount of the reagent, whose dosage rate is controlled by the ID of the pump tube. The addition of mixing coils to the reaction manifold ensures maximum mixing of the sample segments before it reaches the detector. The mixing is critical, as the sample and reagents have different specific gravities. Mixing is achieved by frictional turbulence from the tube walls, and from turbulence created when the sample segments and reagents are inverted as they pass through the coils (Sakamoto et al., 1990). This results in a homogeneous zone between each pair of bubbles, which ensures the development of chemical equilibrium. The bubbles also minimise carry-over of the colour developed by one sample mixing with the subsequent sample, and clean the transmission and EVA mixing coil tubing as they scrape past the surface of the tubing (Smythe-Wright et al., 1992).

5.2.6 Detection system

The digital 550 detectors supplied with CSIRO Marine Research's system too sensitive to movement to be used at sea. As the WinFlow software only works with the firmware incorporated into the 550 detectors, A to D converters have been inserted into the 550 detector box in place of the regular flow-cell set-up. The older model 510 detectors, which do work at sea (Eriksen, 1997), are then connected to the digital interface.

The Alpkem 510 Monochromator detector is a variable wavelength, flow-through absorbance detector with tungsten or deuterium lamps to give a total wavelength range of 190 to 800 nm. The orthophosphate detector has been modified to allow wavelength selection up to 900 nm (Faithful and Eriksen, 1995). Absorbance of the solution passing through the flowcell is measured by a photodiode adjacent to the cell. Bubbles are actively removed from the analytical stream via a debubbler immediately in front of the flowcell. The debubbler also removes a small proportion of the total volume of solution flowing through the system. The debubblers have been mounted outside the flowcell housing, so that any problems can be easily detected without removing the housing. The external position also provides an easy diagnostic aid for monitoring anomalies in flow and air segmentation (eg,

flowcell blockages) for each channel (Foley, 1994). Note that the debubbler must be perpendicular to function effectively.

The waste effluent from the detector flows to waste through 0.5 mm ID Tygon tubing. The tubing should be at least 30 cm long, and may need to be a coiled 50 cm length, as the back pressure created helps the debubbler to function effectively, and prevents small bubbles entering the flow cell. It also helps to prevent degassing inside the flowcell. The standard settings for each of the detectors are listed with the flow diagrams for the reaction cartridges (Figures 5.3 to 5.5).

The flow cells on the silicate and orthophosphate detectors have a larger aperture than the standard flow cells supplied with the 510 detectors. These flow cells are used to minimise the refractive index effect due to the different refractive indices of ASW or seawater and deionised water. The effect is most pronounced with the molybdate chemistries used for silicate and orthophosphate analysis.

5.2.7 Power distribution modules

There is one 509 power distribution module (PDM) with eight power outlets, to which the analytical modules are connected. The PDM is then connected to the UPS, so that power surges and failures will not affect the instrumentation or the analysis. All peripheral instrumentation, such as the PC, chart recorder and printer are connected to the UPS independently of the PDM. The 502 Ismatec pump can be mounted on top of the power module, and the storage drawer in the PDM provides a convenient place for frequently used tools, chart pens, etc.

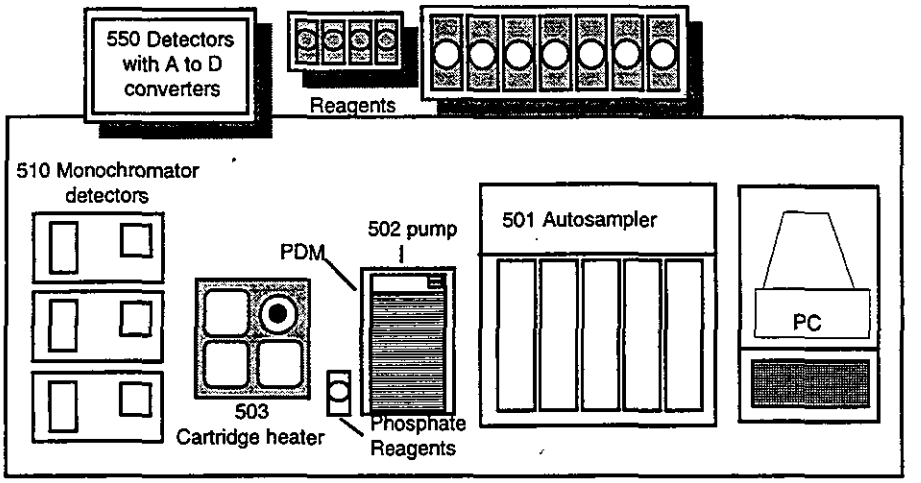
5.2.8 Switching valves

The Hamilton 90° two- and three-way valves incorporated into the system are designed to minimise solution change-over times. There are five valves: three two-way valves for the background/colour solutions of each chemistry, one three-way valve to allow changes between MilliQ/ASW/LNSW, and another two-way valve to allow the autosampler to be switched in and out of the system. These valves enable the operator to run a variety of baselines in order to correct sample results for the blank and instrument offset (see Section 5.9 for an explanation of the calculations involved, and Section 5.8.5 for operating details).

5.3 Instrumentation set-up and installation

There are specially made boxes and restraining fittings for some of the modules of the Alpkem autoanalyser. These are fixed permanently to the base board, but can be easily re-arranged if the described set-up is not satisfactory. The position of each module is shown in Figure 5.2.

Figure 5.2 Suggested layout of the Alpkem autoanalyser in the hydrographic laboratory. Shaded components are fixed to the wall above the bench.



Reaction cartridges and detectors are plumbed with finger-tight screw-in fittings, sleeve-type tubing connectors and various size nipples, which allow for easy connection and disconnection. A colour coding system makes installation and troubleshooting easier for the analyst. The colour codes are:

nitrate + nitrite = blue
reactive silicate = green
orthophosphate = red

The various tubing, valves, reagent containers and waste lines can be easily identified by this coding system.

5.3.1 Temperature considerations

The air conditioning system in the hydrographic laboratory on *RV Franklin* was upgraded in 1998 to provide a constant temperature environment for the analysis of salinity, dissolved oxygen and nutrients. The quality of all analyses depends on the maintenance of a constant temperature, so a number of precautions are recommended to prevent fluctuations in room temperature and hence data quality. The laboratory temperature should be controlled to within ±1 °C of the selected temperature.

While at sea, temperature should be monitored regularly. A portable data logger has been installed for continuous monitoring of laboratory temperature (see Section 4.4.4 for data logger operating instructions). Recording of laboratory temperature is particularly important for nutrient analysis where the scientist wishes to report the results as mass units. The ambient temperature is required for the conversion of volumetric to mass units (WOCE, 1991).

5.4 Chemical methods

5.4.1 Silicate analysis

The determination of dissolved inorganic or reactive silicate in seawater is based on the formation of a yellow silicomolybdic acid complex, when the acidified sample is treated with

a molybdate reagent. Ascorbic acid reduces the silicomolybdic complex to a blue heteropoly silicomolybdic complex, which is measured at 660 nm. The influence of orthophosphate in the sample can be suppressed by adding oxalic acid, by maintaining the appropriate ratio of sulfuric acid to molybdate, and by using an optimal acidity in the final solution (Grasshof, 1976). A background reagent is substituted as required for the ascorbic acid solution; it contains acetone, surfactant and MilliQ water. This solution does not reduce the silicomolybdic complex and is used to obtain a background signal for low-level analysis.

Colloidal and soluble silicate can be determined using this chemistry. The isomeric form of the silicomolybdic acid is dependent on pH. At pH 3.5 to 4.5, α-silicomolybdic acid is formed; it is extremely stable but requires a lengthy period for complete formation. β-silicomolybdic acid is formed in the pH range 2.5 to 0.8, and is less stable than the α form (Grasshof, 1976).

There is a demonstrated temperature effect on the sensitivity of this chemistry (Gordon et al., 1993), and as a result the method has been modified to include thermostating of the reaction cartridge to 37 °C. Smythe-Wright et al. (1992) state that there can be up to 3 % change in sensitivity for every 1 °C change in ambient temperature. The tubing carrying the reaction product from the reaction cartridge to the detector is insulated with a layer of Tygon tubing to further reduce the effect of fluctuations in ambient room temperature. Careful maintenance of a constant temperature environment in the hydrograhic laboratory is critical to the performance of this analysis. The flow diagram for reactive silicate analysis is shown in Figure 5.3.

Ammonium molybdate commonly coats the inner surfaces of the tubing. Use 1 mol/L NaOH as a cleaning agent.

Storage problems with samples high in silicate (greater than about 70 µmol/L) have been reported by several authors. Freezing causes polymerisation of silicon, and thawing rates are critical for accurate analysis of silicate concentration (MacDonald et al., 1986). The effect is exaggerated as the storage time increases. Samples are best analysed within 1-2 hours of sampling.

As the chemistry is often non-linear, it is important that the calibration range chosen covers the range of silicate concentrations expected.

Figure 5.3 Flow diagram for reactive silicate cartridge configuration.

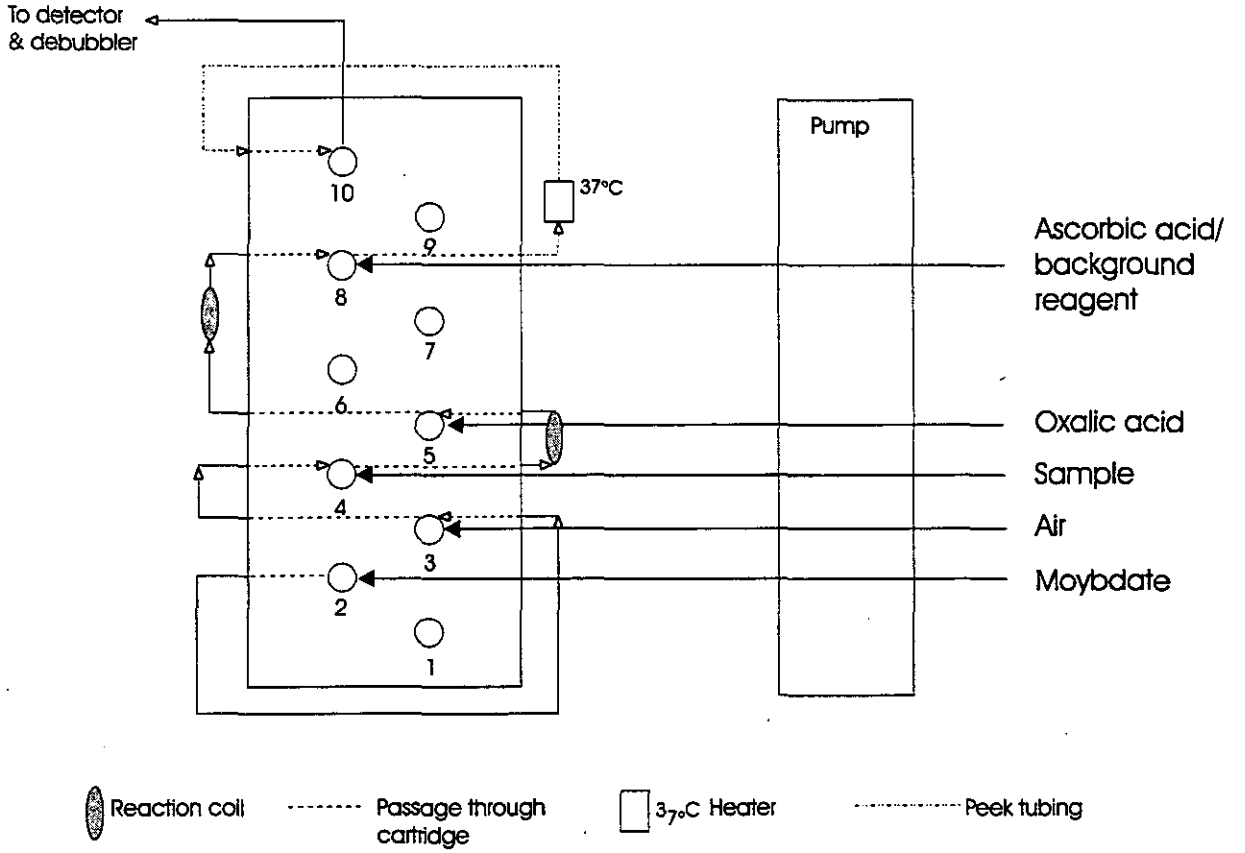


Table 5.2 Pump configuration for silicate analysis

Reagent	Pump tube	Flow rate (40 % pump speed)
Ammonium molybdate	Orange/orange	0.41 mL/min
Air	Orange/white	0.25 mL/min
Sample	White/white	0.56 mL/min
Oxalic acid	Black/black	0.32 mL/min
Ascorbic acid	Orange/orange	0.41 mL/min

Table 5.3 Analysis parameters for silicate analysis

Standard range	0-35 µmol/L or 0-140 µmol/L
Detector wavelength	660 nm
Rise time	3 s
Range	0.05 AUFS
Detection limit of this method	0.1 µmol/L
Field precision of this method	0.88 %[1]
Sample/wash cycle	60/30
Chart recorder range	50 mV
Chart recorder speed	20 cm/h

[1] Determined on *RV Franklin* voyage Fr9902 as the coefficient of variation of 15 repeat samples taken from a single bulk sample.

5.4.2 Orthophosphate analysis

Analysis of orthophosphate is based on the formation of a phosphoantimonylmolybdenum blue species. Ascorbic acid is added as a reductant to form a mixture of heteropoly acids (α and β forms), which is a highly coloured blue compound analysed at 880 nm. Silicate interference is depressed by adjusting the final reaction pH to less than 1, and ensuring that the sulfuric acid to molybdate ratio is 2 to 2.5 mol/L H_2SO_4:1 % molybdate (Grasshof, 1976). The concentration of H_2SO_4 in the final solution should be around 0.1 mol/L (Grasshof, 1976). The reaction manifold is thermostated to 37 °C.

Two colour-producing reagents are prepared as required. The first contains sulfuric acid, ammonium molybdate and antimony potassium tartrate, the second ascorbic acid. A background reagent which is substituted as required for the ascorbic acid solution, contains surfactant and MilliQ water. This solution does not respond to orthophosphate; it is used to obtain a background signal for low-level orthophosphate analysis.

The blue phosphomolybdic complex has a tendency to be adsorbed onto plastic surfaces, and is desorbed when solutions low in this complex are passed through the system. This manifests itself as tailing or carryover. The system can be flushed daily with 1 mol/L NaOH if the effect is severe.

The selected detection wavelength is 880 nm, to improve the sensitivity of low-level determinations. The flow diagram for orthophosphate analysis is shown in Figure 5.4.

Figure 5.4 Flow diagram of orthophosphate cartridge configuration

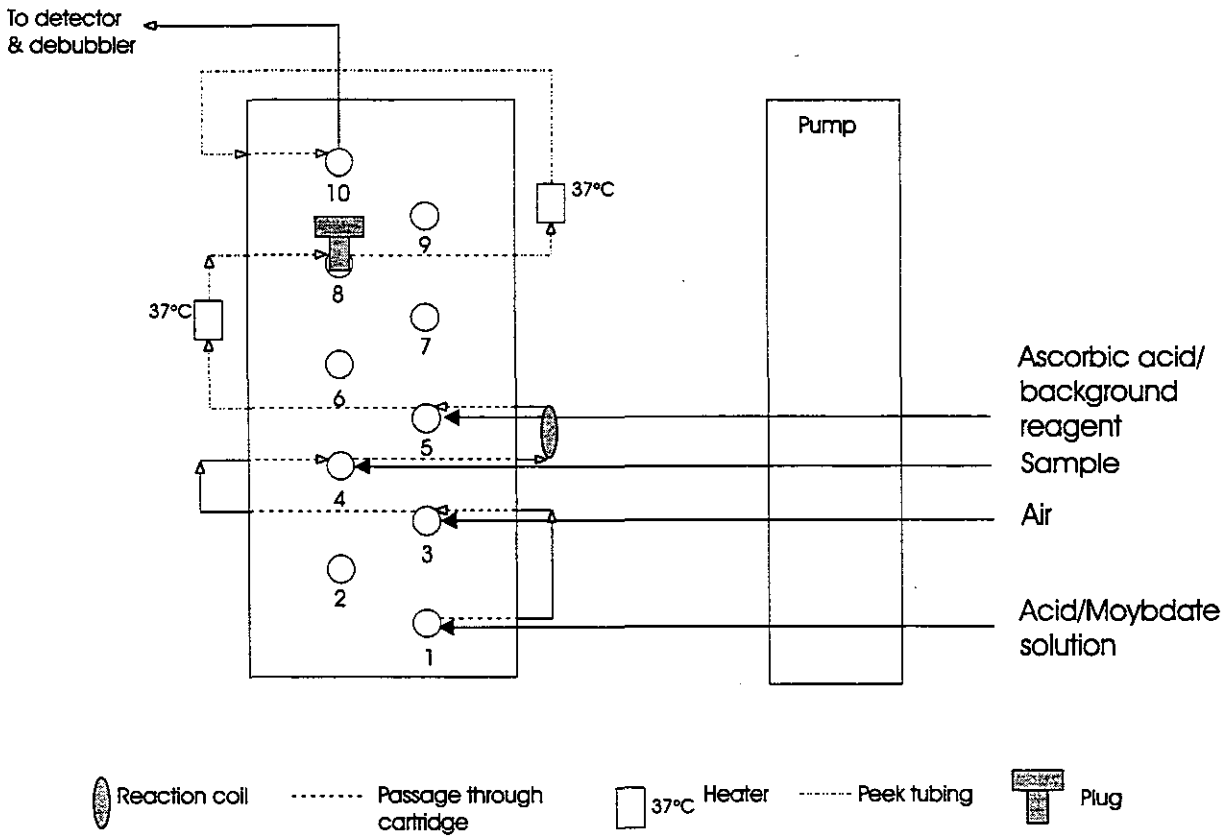


Table 5.4 Pump configuration for orthophosphate analysis

Reagent	Pump tube	Flow rate (40 % pump speed)
Acid molybdate solution	Red/red	0.71 mL/min
Air	Black/black	0.32 mL/min
Sample	Red/red	0.71 mL/min
Ascorbic acid	Orange/green	0.10 mL/min

Table 5.5 Analysis parameters for orthophosphate analysis

Standard range	0-3.0 µmol/L
Detector wavelength	880 nm
Rise time	3 s
Range	0.02 AUFS
Detection limit of this method	0.012 µmol/L
Field precision of this method	0.50 %[1]
Sample/wash cycle	60/30
Chart recorder range	50 mV
Chart recorder speed	20 cm/h

[1] Determined on *RV Franklin* voyage Fr9902 as the coefficient of variation of 15 repeat samples taken from a single bulk sample.

5.4.3 Nitrate plus nitrite analysis

Nitrate analysis is based on the quantitative reduction of nitrate to nitrite, and the subsequent formation of an azo dye. The reduction is achieved by passing the sample through a copperized Open Tubular Cadmium Reactor (OTCR) incorporated into the reaction manifold. Imidazole buffer is used to adjust the pH of the samples to 7.8, optimising the reduction to nitrite and preventing further reduction to hydroxylamine and ammonia (Grasshof, 1976). The reduction efficiency of the OTCR is monitored continuously by passing nitrate and nitrite solutions of the same nominal concentration through the column and calculating the ratio of nitrate peak height to nitrite peak height. Efficiencies of 0.95 (95 %) or more are considered optimal. Nitrogen is used as a segmentation gas, as air will degrade the performance of the OTCR. Helium can be used as a substitute, and some institutions have reported better performance using this gas (Garside, 1993).

The effluent from the OTCR undergoes diazotization with sulphanilamide, and subsequent coupling with N-1-napthylethylenediamine dihydrochloride. The resulting azo dye is analysed at 540 nm. It constitutes any nitrite originally present in the sample, plus nitrate that has been quantitatively reduced to nitrite. To report nitrate only, the column should be removed from the manifold and a separate nitrite determination performed. Nitrate can be calculated by the difference in these two quantities. The flow diagram for nitrate plus nitrite analysis is shown in Figure 5.5.

A background reagent which is substituted as required for the NEDD solution, contains surfactant and MilliQ water. This solution does not form an azo dye, and is used to obtain a background signal for low-level analysis.

Figure 5.5 Flow diagram of nitrate plus nitrite cartridge configuration.

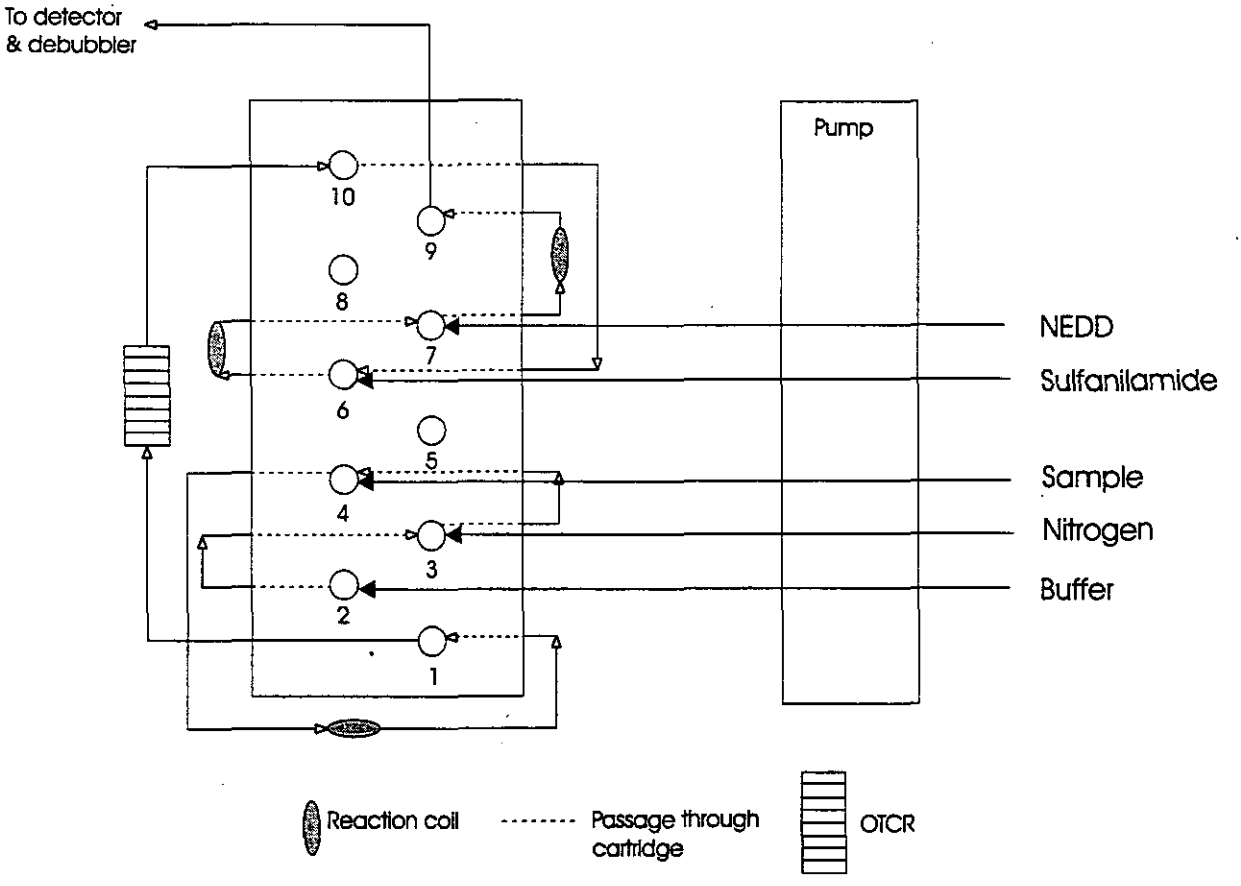


Table 5.6 Pump configuration for nitrate+nitrite analysis

Reagent	Pump tube	Flow rate (40 % pump speed)
NEDD	Orange/yellow	0.18 mL/min
Sulphanilamide	Orange/yellow	0.18 mL/min
Sample	Black/black	0.32 mL/min
Nitrogen	Orange/white	0.25 mL/min
Imidazole Buffer	Black/black	0.32 mL/min

Table 5.7 Analysis parameters for nitrate+nitrite analysis

Standard range	0-35 µmol/L
Detector wavelength	540 nm
Rise time	3 s
Range	0.5 AUFS
Detection limit of this method	0.1 µmol/L
Field precision of this method	0.24 %[1]
Sample/wash cycle	60/30
Chart recorder range	50 mV
Chart recorder speed	20 cm/h

[1] Determined on *RV Franklin* voyage Fr9902 as the coefficient of variation of 15 repeat samples taken from a single bulk sample.

5.5 Preparation of stock nutrient standards

Standards for nutrient analysis should be prepared with the utmost care and attention to detail. Full details must be kept of all aspects of standard preparation, including calibration of glassware and analytical balances.

Nutrient stock standards are prepared on land from dry, analytical grade, crystalline salts. Intermediate and working calibrants are prepared regularly by dilution while at sea. Stock standards should be renewed approximately every six months, using calibrated glassware. Protocols for calibration of volumetric glassware, along with corrections for density of water and air buoyancy are detailed in the WOCE Operations Manual (1991). Balances to prepare critical solutions such as stock nutrient standards should first be checked with the highest quality calibration weights, and details recorded. A sample of each of the old stock standards should be kept for comparison with the new batch, as a QC check. Gordon et al. (1993) recommends that batches of working calibrants prepared from stock solutions should agree to within 0.2 % for silicate, 0.3 % for nitrate+nitrite and 0.4 % for orthophosphate.

Working calibrants are prepared as required by dilution with LNSW (natural seawater low in nutrient, filtered through a 0.45 µm HA filter). Calibrants are decanted into 10 mL polypropylene nutrient tubes and frozen until required.

A working calibrants log maintained at sea should contain the date of preparation, batch of stock from which working calibrants were prepared, initials of analyst and any pertinent comments for each set of calibrants prepared.

5.5.1 Silicate stock standard

We have experienced some difficulty locating a high-quality source (analytical reagent grade) of sodium hexaflourosilicate (Na_2SiF_6), but have found that sodium hexaflourosilicate supplied by the Aldrich Chemical Company (Product no. 25,017-1) is suitable for routine work. For the most accurate results, the exact composition of the salt should be determined by comparison with a fusion of pure silicon dioxide (Gordon et al., 1993)

Dry about 3 g of Na_2SiF_6 (FW = 188.06) at 105 °C for at least two hours in a petri dish or weighing dish. Cool and store in a vacuum desiccator over active desiccant until required. Weigh out 2.6328 g and note the exact mass weighed and record the details in the standards log. This will give a stock standard concentration of 14000 µmol/L SiO_4.

Dilute to about 900 mL in a calibrated, one litre A-grade volumetric flask. Mix thoroughly, as the salt is slow to dissolve completely (this can be done with a magnetic stirrer, *after* the solution has been made up to the mark). Immediately transfer the dissolved solution to an acid cleaned Nalgene polythene one litre bottle. Cap tightly and store in the dark at 4 °C when not in use. Exposure to glassware should be kept to a minimum.

5.5.2 Orthophosphate stock standard

Dry about 2 g of potassium dihydrogen phosphate (FW = 136.09) for at least two hours in a petri dish or weighing dish at 105 °C. Cool and store in a vacuum desiccator over active desiccant until required. Weigh out 1.6331 g of KH_2PO_4, note the exact mass weighed and record the details in the standards log. This will give a stock standard concentration of 12000 µmol/L PO_4.

Dilute to about 900 mL in a calibrated, one litre A-grade volumetric flask. Make up to the mark, and transfer to an acid-cleaned pyrex one litre bottle. Cap tightly and store in the dark at 4 °C when not in use.

Orthophosphate intermediate standard

Using only A-grade calibrated glassware, pipette 10 mL of the 12000 µmol/L phosphate stock standard into a 100 mL volumetric flask. Make up to the mark with deionised water. This will give a standard concentration of 1200 µmol/L PO_4.

Store this solution in a 100 mL pyrex bottle, at 4 °C in the dark. Discard the old stock and rinse with a little of the new stock standard before filling. Replenish this solution every two to three days. Record the date on which the standard was made on the bottle.

5.5.3 Nitrate stock standard

Dry about 2 g of potassium nitrate (FW = 101.10) for at least two hours in a petri dish or weighing dish at 105 °C. Cool and store in a vacuum desiccator over active desiccant until required. Weigh out 1.4154 g, note the exact mass weighed and record the details in the standards log. This will give a stock standard concentration of 14000 µM NO_3.

Dilute to about 900 mL in a calibrated, one litre A-grade volumetric flask. Make up to the mark, and transfer to an acid-cleaned Nalgene polythene one litre bottle. Cap tightly and store in the dark at 4 °C when not in use.

5.5.4 Nitrite stock standard

Dry about 1.5 g of sodium nitrite (FW = 68.99) for at least two hours in a petri dish or weighing dish at 105 °C. Cool and store in a vacuum desiccator over active desiccant until required.

Weigh out 0.9659 g of $NaNO_2$, note the exact mass weighed and record the details in the standards log. This will give a stock standard concentration of 14000 µM NO_2.

Dilute to about 900 mL in a calibrated, one litre A-grade volumetric flask. Make up to the mark, and transfer to an acid-cleaned Nalgene polythene one litre bottle. Cap tightly and store in the dark at 4 °C when not in use.

5.6 Preparation of working calibrants, column checks and SRMs

Working calibrants, nitrite column checks and standard reference materials (SRMs) are prepared from the stock solutions on a regular basis, decanted into nutrient tubes and frozen for later use. This method of storage is convenient for busy voyages where preparaing fresh calibrants daily is not possible, and allows 8 to 10 runs to be completed before a new batch is required. These calibrants etc, should be used within a week of freezing.

5.6.1 Calibrants and nitrite column checks

A nitrite standard equivalent in concentration to the top nitrate standard (35 µmol/L) is prepared in order to check the reduction efficiency of the OTCR with every batch of calibrants and samples. The performance of the column can then be determined by calculating the change in reduction efficiency over the duration of the run (Garside, 1993).

Working calibrants and nitrite column efficiency checks are prepared with an Eppendorf Multipette, with a 2.5 mL syringe. For the most precise work, these 2.5 mL syringes should

be used in conjunction with the yellow tips provided. To prevent contamination, avoid touching the end of the syringe tip.

Working calibrants and nitrite column efficiency checks are made up in LNSW collected from surface waters and filtered through 0.45 µm HA filters.

1. At least half an hour before making up the calibrants, nitrite check and SRMs, give the carboy of LNSW a good mixing and decant enough LNSW for the batch (usually around 1500 to 2000 mL) into a container. Sit it on the bench to reach room temperature. It is also convenient to decant enough of each nutrient stock to labelled nutrient tubes to make up the working calibrants, and allow them to come to room temperature (eg, 10 mL of silicate will be required for 0 to 35 µmol/L range, but 30 mL will be required for the 0 to 140 µmol/L range). NEVER take stock directly from the stock bottles with a pipette, as this risks contaminating the primary stock solutions.
2. Pre-label 10 mL nutrient tubes with 'N' (for nitrite), '0', '1', '2', '3', '4' and '5'. You will need 10 vials each for nitrite and calibrants 0 and 3, 20 vials for calibrants 1, 2 and 4, and 40 to 50 vials for calibrant 5.
3. Take 3 x 100 mL, 3 x 200 mL and 1 x 500 mL A grade volumetric flasks and label the 100 mL flasks 'N', '0' and '3', the 200 mL flasks '1', '2' and '4', and the 500 mL flask '5'.
4. Rinse all flasks three times with LNSW, and fill about ¾ full with LNSW.
5. Using the Eppendorf Multipette, attach a 2.5 mL syringe with tip and after rinsing, add the appropriate amount of the nitrate stock to the flasks (see Table 5.8). Wash down the insides of the flasks with LNSW between additions and mix well.
6. Insert a new 2.5 mL tip, and repeat the procedure with the orthophosphate intermediate standard, then again with the silicate standard, ensuring you choose the correct standard range.
7. Shake well and make all flasks up to the mark with LNSW. Mix again.
8. Immediately transfer the calibrants into labelled nutrient tubes to minimise contamination with silicate from the glassware.

This will give combined calibrants of the following concentrations:

SiO_4: 0, 28, 56, 84, 112, 140 µmol/L; or 0, 7, 14, 21, 28, 35 µmol/L

NO_3: 0, 7, 14, 21, 28, 35 µmol/L

PO_4: 0, 0.6, 1.2, 1.8, 2.4, 3.0 µmol/L

Other calibration ranges are sometimes required, depending on the type of work being undertaken. Often, surface water samples are predominately collected, and the calibration range required is 0 to 7 µmol/L for nitrate+nitrite and silicate, and 0 to 0.6 µmol/L for orthophosphate analysis. Calibrants are stored in the freezer until required for analysis. They should not be kept for longer than one week.

5.6.2 Standard reference material (SRM) preparation

SRMs are prepared at the same time as calibration standards are made. Two 100 mL mixed nutrient standards are made from the commercially available OSI (Ocean Scientific International) Marine Nutrient Standards Kit. Included in the kit are three stock standards (100 µmol/L phosphate, 1000 µmol/L nitrate and 1000 µmol/L silicate) and low-nutrient seawater, which is used to dilute the stocks. If there is no LNSW supplied with the kit, the stock used to make our working calibrants can be used.

The following method can be used to make up a low SRM (1 µmol/L phosphate and 10 µmol/L nitrate and silicate) and a high SRM (3 µmol/L phosphate and 30 µmol/L nitrate and silicate). Adapt the concentrations to suit the calibration range used.

1. Ensure that the stocks and LNSW are at room temperature.
2. Pre-label 10 nutrient tubes for each SRM. Also label 15 tubes for the blanks. (In total there should be 35 tubes labelled.)
3. Rinse two 100 mL A-grade volumetric flasks with the LNSW and fill to ¾ full.
4. Using the Eppendorf Multipette, attach a new 12.5 mL syringe with tip and after rinsing, add 1000 µL (1 mL) of the nitrate SRM stock to one of the flasks (see Table 5.8 for Eppendorf settings). Rinse down the insides of the flasks with LNSW and mix well.
5. For the higher concentration standard, add 3000 µL (3 mL) of nitrate standard to the other flask (see Table 5.8 for Eppendorf settings). Rinse down the insides of the flasks with LNSW and mix well.
6. Insert a new 12.5 mL tip, and repeat the procedure with the phosphate standard. Repeat again with the silicate standard. Mix the flasks well after each addition.
7. Make both flasks up to the mark and decant to the nutrient tubes.
8. Fill the remaining 15 tubes with the LNSW used for dilution of the SRMs. These are to be used for a blank correction.

Freeze the SRMs and SRM blanks with the calibrants. Do not keep for more than one week.

Table 5.8 Eppendorf settings for dispensing selected calibrant and SRM concentrations.

Numbers in italics are optional methods of delivering the volume.

Flask volume (mL)	Working calibrant concentrations (µmol/L)			Volume dispensed (µL)	2.5 mL tip (1 click = 50 µL)		12.5 mL tip (1 click = 250 µL)	
	NO_3 & NO_2 (14000 µmol/L)	SiO_4 (14000 µmol/L)	PO_4 (1200 µmol/L)		Setting	Number of clicks	Setting	Number of clicks
100	0	0	0	0	0	0	0	0
100	7	7	*0.6*	*50*	1	1		
200	7	7	0.6	100	2	1		
100	14	14	1.2	100	2	1		
200	14	14	1.2	200	4	1		
100	21	21	1.8	150	3	1		
100	28	28	2.4	200	4	1		
200	28	28	2.4	400	4	2		
100	35	35	3.0	250	5	1		
500	35	35	3.0	1250	5	5		
100		*56*		*400*	*4*	*2*		
200		56		800	4	4		
100		84		600	4	3		
100		112		800	4	4		
200		112		1600	4	8		
100		140		1000	4	5	*4*	*1*
500		140		5000	*4*	*25*	5	4
SRMs								
100	10	10	1				4	1
100	30	30	*3*				4	3

5.6.3 Notes on the use of volumetric pipettes

The pipette should be rinsed three times with a few millilitres of the appropriate solution before delivering the required volume. Fill the pipette using a rubber bulb above the calibration mark, and remove any liquid adhering to the outside of the pipette with a clean, dry tissue. Slowly drain the solution until the bottom of the meniscus is level with the calibration mark on the neck of the flask. Drain the contents by touching the tip of the pipette to the side of the flask, and hold it there for 5 seconds after the pipette is drained. The pipette should then be rinsed thoroughly with MilliQ afterwards and stored upright to drain.

If the pipette is greasy inside and is not draining fully (liquid is adhering along the length of the pipette), it will require cleaning with a detergent that is free of phosphate and ammonia. The pipette should not be used if it cannot be cleaned properly.

5.6.4 Notes on the use of Eppendorf Multipettes

Experience with these pipettes has shown that highly accurate volumes can be repeatedly delivered if the following procedure is used. Attach the syringe to be used to the Multipette, fill the syringe approximately half full with the solution to be pipetted, and draw up air until the piston is fully withdrawn. Rinse the syringe thoroughly and discard the contents by depressing the filling lever. Repeat twice.

Fill the syringe completely, and check to see if any bubbles are trapped on the walls of the syringe. These can be most effectively removed by introducing a large bubble into the syringe and collecting up the smaller bubbles by manipulating the angle of the Multipette. Remove the bubble by holding the Multipette upside down and depressing the filling lever, until all air is removed.

For the most accurate work, at least three volumes should be dispensed to waste before delivering the final volume. Do not touch the tip of the pipette to the inside of the flask while delivering the required volume; hold the pipette vertically.

New tips should be used each time a set of calibrants or SRMs are made to avoid contamination. Multipettes should be calibrated approximately every six months, using the protocols described in the WOCE Operations Manual (WOCE, 1991).

5.7 Analytical reagents

This section describes the preparation of the analytical reagents required for the analysis of orthophosphate, reactive silicate, and nitrate plus nitrite in seawater. All chemicals are analytical grade reagents (AR), or the highest quality available, unless otherwise specified. All dry chemicals are pre-weighed into 8 oz Whirlpaks in Hobart. Each packet is labelled with the chemical name, mass weighed, date of weighing, and initials of the analyst preparing the chemicals. These details, in addition to the lot number and the manufacturer of the chemical, are recorded in the reagent log book maintained in Hobart. The dates on the whirlpaks are then transferred to the quality control log sheet on the ship when the reagent is made up for nutrient analysis, along with the date of preparation and initials of the analyst. This log can then be used to trace any problems arising from contamination of chemicals, or differences due to batches/sources of chemicals. At the end of a voyage the sheets are returned to Hobart with all other data.

An A-grade volumetric flask or plastic beaker is used to prepare the reagents. Volumetric ware used for the preparation of reagents does not need to be calibrated.

Deionised water for the preparation of reagents is obtained by passing the ship's laboratory water supply through a pretreatment system. Water from this system is then polished through a four-cartridge Waters MilliQ system. The quality of water from the MilliQ system should be checked regularly by measuring the conductivity.

Artificial seawater (35 g/L)

Dissolve 70 g of AR-grade sodium chloride (NaCl) in about 1800 mL of deionised water. Make up to two litres and mix well.

5.7.1 Reactive silicate analysis

Surfactant

15 % SDS (sodium dodecyl sulfate). Add 150 g of SDS to 800 mL of MilliQ and make up to one litre. This solution may require warming to fully dissolve. *(Note that this solution is also used for orthophosphate analysis.)*

Ammonium molybdate solution

Add 20 mL of 5N sulfuric acid (made for orthophosphate stock solutions) to 800 mL of deionised water. Dissolve 10 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) in the solution, and make up to one litre. Add 1 mL of 15 % SDS after the solution has been decanted to a reagent container (0.015 % SDS in final volume). Store at 4 °C.

Ascorbic acid solution

Add 50 mL of acetone to about 800 mL of deionised water. Dissolve 17.6 g of ascorbic acid ($C_6H_8O_6$), and make up to one litre. Store at 4 °C when not in use.

Background solution

Mix 800 mL of MilliQ and 50 mL of acetone and dilute to one litre.

Oxalic acid solution

Add 100 g of oxalic acid ($(COOH)_2.2H_2O$) to about 1800 mL of deionised water. Make up to two litres and mix well.

1 mol/L sodium hydroxide

Dissolve 40 g of sodium hydroxide pearls (NaOH) in about 900 mL of water, and allow to cool. Make up to one litre, and store in a polyethylene bottle. This solution is used to clean the manifold when carryover is a problem. *(Note that this solution is also used in orthophosphate analysis.)*

5.7.2 Orthophosphate analysis

Stock solutions

Surfactant

15 % SDS (sodium dodecyl sulfate). Add 150 g of SDS to 800 mL of MilliQ and make up to one litre. This solution may require warming to fully dissolve. *(Note that this solution is also used for silicate analysis.)*

2.5 mol/L sulfuric acid

Carefully add 140 mL of concentrated sulfuric acid to about 800 mL of deionised water in a one litre beaker. When the solution has cooled, dilute to one litre.

Caution: a great deal of heat is generated when H_2SO_4 is diluted with water.

Stock ammonium molybdate solution

Dissolve 20 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) in about 400 mL of deionised water and dilute to 500 mL. Store the stock solution in a polyethylene bottle, at 4 °C.

Stock antimony potassium tartrate solution

Dissolve 3.0 g of antimony potassium tartrate ($KSbO.C_4H_4O_6$) to about 800 mL of deionised water and make up to one litre. Store the stock solution in a polyethylene bottle at 4 °C.

Reagent 1 stock (400 mL)

Add 200 mL of 5N H_2SO_4, 20 mL of Stock Potassium Antimony Tartrate, 60 mL of Ammonium Molybdate and 120 mL of MilliQ. This solution is stable and should be stored at 4 °C when not in use.

Working solutions

Reagent 1 working solution (1104 mL)

Add 100 mL of reagent 1 stock, 1000 mL of MilliQ and 4 mL of 15 % SDS (0.05 % SDS in final volume). This solution is stable and should be stored at 4 °C when not in use.

Ascorbic acid solution

Dissolve 9 g of ascorbic acid ($C_6H_8O_6$) in about 400 mL of deionised water and make up to 500 mL. After decanting, add 5 mL of 15 % SDS. Store in an amber polyethylene bottle at 4 °C. The solution should be clear and can be used as long as it remains colourless.

Background solution

Mix 100 mL deionised water and 1 mL of 15 % SDS (interchange with reagent 2 to change to background baselines).

1 mol/L sodium hydroxide

Dissolve 40 g of sodium hydroxide (NaOH) pearls in about 900 mL of water, and allow to cool. Make up to one litre, and store in a polyethylene bottle. This solution is used to clean the manifold when carryover is a problem. *(Note that this solution is also used in silicate analysis.)*

5.7.3 Nitrate plus nitrite analysis

2.5 % v/v HCl

Carefully add 25 mL of concentrated hydrochloric acid to about 800 mL of deionised water and make up to one litre.

Imidazole (1,3-diaza-2,4-cyclopentadiene) buffer pH 7.8

Add 4.25 g of Imidazole ($C_3H_4N_2$) to about 950 mL of deionised water. Add 45 mL of 2.5 % HCl to adjust the final pH to 7.8, and make up to one litre. Check the pH of the solution before use. Use an Eppendorf to add 0.25 mL of 30 % w/v Brij-35 after decanting the solution into a reagent container (0.0075 % Brij-35 in final solution). Less Brij-35 can be used if it appears that the cadmium column efficiency is affected (see Section 5.8.3).

N-1-napthylethylenediamine dihydrochloride (NEDD)

Add 0.31 g of NEDD ($C_{12}H_{14}N_2.2HCl$) to about 800 mL of deionised water. Mix thoroughly and make up to one litre. Use an Eppendorf to add 0.25 mL of 30 % w/v Brij-35 after decanting the solution into a reagent container (0.0075 % Brij-35 in final solution). Store at 4 °C.

Background solution

Add 0.25 mL of 30 % w/v Brij-35 to one litre of deionised water. Mix thoroughly. (Interchange with NEDD solution for background baselines).

Sulfanilamide

Add 31 mL of concentrated HCl to about 800 mL of deionised water. Dissolve 3.12 g of sulfanilamide ($NH_2.C_6H_4.SO_2.NH_2$) in this solution and make up to one litre.

Column regeneration reagents

2 % copper sulfate

Dissolve 20 g of copper sulfate ($CuSO_4.5H_2O$) in about 800 mL of deionised water. Make up to one litre, and store in a clean Nalgene polythene bottle.

0.5 mol/L hydrochloric acid solution

Carefully add 21 mL of concentrated HCl to about 400 mL of deionised water, mix well and make up to 500 mL. Store in a clean Nalgene polythene bottle.

Imidazole buffer pH 7.8

Add 4.25 g of Imidazole to about 950 mL of deionised water. Add 45 mL of 2.5 % HCl to adjust the final pH to 7.8, and make up to one litre. Check the pH of the solution before use. *Do not add Brij-35 to this reagent.*

2 mol/L nitric acid solution (for strong acid regeneration of OTCRs)

Carefully add 125 mL of concentrated HNO_3 to about 800 mL of deionised water in a one litre beaker, and mix well. Make up to the mark, and store in a glass bottle. This solution should be made before going to sea and stored away from reagents and solutions used in nutrient analysis. ***Use extreme caution when making this solution.***

5.8 Alpkem operation

5.8.1 Pre start-up procedure

1. Change pump tubes if necessary (see the Alpkem notes book for details of previous pump tube changes and record date of changes).
2. Give the instrument a quick check-over, looking for evidence of leaking nuts from the last voyage. Clean around these nuts with MilliQ, tighten or replace them if necessary, and clean up any other spills you can see. Also check the flow cell connections for evidence of leaks, and clean these up. If you start with a clean instrument, it is easier to spot leaks that might occur during a run.
3. Check that the MilliQ, LNSW and ASW reagent containers have sufficient volume to get you through the day's work.
4. Make up reagents according to reagent list. Check the colour of the ascorbic acid reagents for orthophosphate and silicate. If the ascorbic acid is coloured to your eye, it is too old, and needs replacing. Aged solutions will reduce the sensitivity of the

technique, and you will notice a huge baseline change when changing from background to colour. NEDD should be checked also, but is less likely to cause problems.

5. Check with the voyage leader the most common depth of casts, so that you can select the right calibrant range for the samples. Make up calibrants from natural seawater stocks in the vegie fridge on the ship. Ensure that all stocks and the seawater are at room temperature before making up (see Section 5.6 for details).
6. Make up two concentrations of OSI standards to suit the calibration range (see Section 5.6 for details).
7. Fill in the QC sheets after making up your reagents, calibrants and SRMs, in preparation for the first run.

5.8.2 Starting up the Alpkem

1. Check that all waste lines are connected to the waste drain.
2. Turn on the Alpkem at the power supply unit. Ensure that all detectors are on.
3. Put the platens down and operate the pump at a speed of -40. Don't leave the platens down without the pump running for too long. Tighten the platens to about 45° from horizontal, or until the system is pumping smoothly. Tightening beyond this is unnecessary when the pump tubes are new.
4. Leaving all other tubing in the MilliQ supply, insert the end of the molybdate tube for silicate and the reagent 1 tube for orthophosphate into the 1 mol/L NaOH and allow the system to run for 5 minutes. This will clean the silicate line of molybdate that has plated out, and help to reduce carryover in the orthophosphate line. Do not run NaOH through the system while reagents are on-line, as the change in pH can cause precipitation in some channels. Flush the system with MilliQ for at least 10 minutes before continuing.
5. Attach all the reagent bottles to their respective tubing and ensure that they are sufficiently full and not too old. (Do not put the cadmium column in at this stage.)
6. Allow the system to run for 10 minutes or so. If the bubble patterns are still jerking or if one reagent is not moving into the cartridge, then tighten the appropriate platens. It will take a while for the system to settle, as it takes some time for reagent to get through all the tubing.
7. Check all the switching valves to ensure they are letting reagent through and check the instrument for leaks at joins, nuts and valves. Fittings should be finger tight.
8. Let the instrument run for enough time to ensure smooth bubble patterns, no surging and no leaks. Prime all the switching valves with washes and reagents while you are waiting, so that the lengths of tubing up to the switching valves do not contain air. This should take around half an hour, assuming that nothing goes wrong!
9. If there are problems with bubble patterns, or leaks etc, refer to the troubleshooting guidelines at the end of the manual, or to the document kept on the hydrochemistry lab PC. Fix these problems before continuing.
10. The wash solution valve should be set to "LNSW", and the reagent valves should be switched to "background reagent" for all channels. This will give the lowest absorbing baseline.

11. Set each of the channels on the detectors and chart recorder to "zero". Check that each of the flow cells is clear of bubbles and giving a stable signal. A rapidly changing absorbance reading on the detector is an indication that air bubbles are present in the flow cell. Apply back pressure to the flowcell by pinching off the waste line tubing and holding for a few seconds. Releasing the line quickly should dislodge the bubbles. If this does not appear to work, remove the flow cell from the detector and gently tap it, while holding the outlet higher than the inlet.
12. When the instrument has been pumping on LNSW and background reagents for 10 to 15 minutes and you are satisfied that the detector readings are constant, zero the detectors. It is important that this is done on LNSW and background reagents, as the software does not respond at negative absorbances.

5.8.3 Open tubular cadmium reactors

While the system is being primed with reagents, the Open Tubular Cadmium Reactor (OTCR) should be regenerated for nitrate+nitrite analysis. This should be done after every run for optimal performance. The lifetime of the column will depend primarily on the number and composition of the samples, and on the age and storage conditions of the OTCR.

Reagents for OTCR regeneration

Full details on how to make these reagents can be found in Section 5.7.3.

Imidazole buffer pH 7.8, no Brij-35
0.5 mol/L hydrochloric acid
2 % $CuSO_4$ solution
MilliQ
(2 mol/L nitric acid for strong acid regeneration. This is already made up and stored on board).

Alpkem regeneration procedure

1. Do not introduce air into the cadmium column during the regeneration process.
2. Pull apart the join in the tubing connecting the two ends of the column.
3. Fill the syringe marked "MQ", connect the syringe and flush the OTCR forcefully 2-3 times with MilliQ. Make sure the syringe is free of air bubbles.
4. Fill the syringe marked "HCl" with the 0.5 mol/L HCl solution, changing syringes carefully to avoid air entering the tubing. This can be achieved by holding the inlet end of the OTCR slightly lower than the outlet while the new syringe is being fitted. Flush the OTCR with approximately 10 mL. Quickly proceed to the next step.
5. Flush the OTCR with 20 mL of MilliQ.
6. Fill the syringe marked "$CuSO_4$" with 2 % copper sulfate Flush the OTCR slowly with about 50 mL of $CuSO_4$. You may observe a dark precipitate being flushed from the OTCR - this is precipitated copper.
7. Flush the OTCR with MilliQ 2-3 times, using reasonable force so that all precipitated copper is removed from the column.

8. Fill the syringe marked "Buffer" with Imidazole buffer pH 7.8, and flush with some force 2-3 times. The OTCR is now ready for use. Disconnect the syringe and carefully join the ends.
9. Ensure that the nitrogen line is connected to the nitrogen supply before going any further!!!.
10. The OTCR can be plumbed into the nitrate plus nitrite channel about 10-15 minutes after the reagents have been switched on line. Stop the flow on the pump, and plumb the OTCR into the reaction cartridge, being careful not to trap any air bubbles in the tubing upstream of the OTCR. Turn on the pump, and run until a regular bubble pattern is observed. Observe the stability of the baseline by recording the signal on the chart recorder.
11. If the baseline is acceptable, the OTCR should be stabilised by running a 35 µmol/L nitrate standard through the column 5 to 10 times, followed by a 35 µmol/L nitrite standard. This allows you to check that the column has been activated properly, and also serves to 'kick start' the column for the day's analyses. Use WinFlow to control the autosampler for this procedure.
12. Turn off the pump, and place the sample probe in a reservoir of 35 µmol/L nitrate standard. Restart the pump.
13. The nitrate standard running continuously through the column will cause the nitrate baseline to rise and plateau out. The signal should not drift significantly, and show minimal noise. There will be some perturbation due to hydraulic noise from the pump. If you are satisfied that the column is performing properly, stop the pumps and return the probe to the washpot after rinsing it with MilliQ.

If the OTCR performance is not satisfactory, the column should be regenerated and the procedure repeated. Most problems arise from air being introduced into the column after the regeneration step has been completed. If, after repeated attempts at regeneration, the column does not perform as required, the strong acid regeneration procedure can be used.

Strong acid regeneration procedure

Using a syringe vacuum, suck the following reagents through the column (NOTE: Use safety glasses, gloves, lab coat etc when using this method and ALWAYS draw the reagents into a syringe; do not push them through the column):

1. 2 mL of 2 mol/L HNO_3
2. 5 mL of concentrated HCl
3. 5 mL of MilliQ water
4. Repeat steps 2 and 3 four times (to give a total of 20 mL of each reagent)
5. 20 mL of MilliQ water
6. Fill column with buffer

The column can then be regenerated by the Alpkem method described above (with copper sulfate), and used in the system.

More information on OTCRs

Other things to keep in mind, and to try if having difficulties with OTCRs are:

- A new column, or one that has been stored for some time, should first be regenerated by the Alpkem method. It may need a few regenerations. As a last resort, try the strong acid regeneration.
- During a voyage, a column should be reactivated by the Alpkem method whenever the column efficiency falls below about 95 %.
- The reactivation should be undertaken with enough force so that on the last step (using the buffer) excess oxide particles are flushed out of the column.
- For short-term storage, columns should be stored in buffer without Brij-35. However, for long-term storage, use nitrogen, ensuring that the column is thoroughly dried by forcing the nitrogen through at high pressure.
- Use the minimal amount of Brij-35 in the buffer solution in the analysis, as Brij-35 is thought to coat the cadmium and therefore reduce the surface area available for nitrate reduction. Buffer solution can be made fresh daily if necessary.

In the case of blocked columns, one or both of the following can be tried:

- Unwind the column and use a copper wire to force out the blockage, taking care not to score the cadmium.
- Using the jeweller's saw and clamp built by CSIRO Marine Research Engineering Technical Services, trim the blocked end of the OTCR, and ensure that there are no sharp edges extending into the column at the cut edge (this will break up bubble patterns). The scraper provided with the clamp can be used for this. This technique can also be used for columns that cause severely broken bubble patterns or that cannot be reactivated to an acceptable level.

5.8.4 Acquiring data

Chart recorder

Turn the chart recorder on and insert the coloured pens into the appropriate bracket, using the colour codes

red for orthophosphate
blue for nitrate + nitrite
green for silicate.

Ensure that the detectors have been zeroed. Set the recorder channels to 'Measure', and place the pens on the chart. Position the pens so that they are about 10 % of full-scale deflection, and the individual traces can be distinguished from each other.

Set chart speed to 20 cm/h and press start.

Computer acquisition of data

Turn the computer on and open WinFlow (C:\Flow_3).

If the detectors are not switched on or there is a problem with communications between the software and the system, an error message will come up. If the error message appears and the Alpkem is on and running, check that all the detectors are on, then close the program and re-open it 30 seconds after turning all the power switches on.

5.8.5 Starting a run

Note that a quality control run should be completed early in a voyage. Table 5.9 gives an example of a typical QC run and Table 5.10 is an example of a typical sample run. The following procedure can be applied to either a sample run or a QC run.

Winflow preparation

For details on how to use WinFlow, see the WinFlow Manual.

1. Click on the sample table icon on the left side of the screen. If you already have a table prepared, open it. Otherwise build a table from the template file called 'csiro.tbl'. Tables can also be built in Excel and imported into WinFlow.
2. Check the details of the table, including sample order and cup numbers. Quality control (QC) and standard reference material (SRM) samples are regularly interspersed throughout the run to monitor the performance of each chemistry. A series of blanks or 'wash' tubes are also analysed to check that the baseline is not drifting excessively, and that there is no contamination of the carrier solution.
3. A SYNC peak is included to allow WinFlow to begin marking peaks. Carryover correction is allowed for with the inclusion of two cal 5 calibrants preceded by a wash. This is calculated later in the data processing procedures (see Section 5.9).
4. Samples should be run from least to most concentrated (generally corresponding to: shallow to deep). Each 24 sample station should be processed as a separate run, but shorter stations (eg, shallow stations) can be grouped together to cut down on processing time. Stations should not be run back to back, ie, deep samples should not be followed immediately by shallow samples. Use a wash to allow the baseline to return to background level, and minimise carryover before starting a new station. Ideally, run time should be no longer than 1.5 hours (Gordon et al., 1993); however, runs on the Franklin are commonly 2.5 to 3 hours long. Table 5.10 is about 3 hours long.
5. Save the .tbl file in the format FYYVVRR.tbl, and keep it in a folder with the voyage name. Print this table.
6. Ensure that in the Winflow method editior (csiro.mth), the carryover correction option is **NOT** checked.

Instrument, samples and calibrants

1. Get out QC samples from the freezer well in advance of the run (about 4 to 5 hours) and defrost at room temperature. Take out samples, calibrants and SRMs half an hour before beginning the run.
2. After setting up the instrument as described in Sections 5.8.1 to 5.8.4, ensure that the column is in line.
3. Zero the detectors on LNSW + background for all lines.
4. Ensure that the thawed samples, calibrants and SRMs are all mixed thouroughly before placing them in the rack. The freezing process causes brine to form and unmixed samples will cause errors in the results. Set up the calibrants, QC and samples according to your printed table, and put the rack into the sampler.
5. Hit the data collection button in the software. The run's file name should be saved in the form: f970312.rst.

6. After you are happy the baselines are stable, hit start (red arrow). Remember to swap the baselines at the appropriate times.

7. Baseline changes will need to be performed at the beginning of the run, and this takes around 30 minutes to complete. Note that if tube length is changed, timing will need to be re-done. The current timing for the changes are listed on the sampler and should be updated if they are changed.

   Cup 2 is denoted as the "baselines" cup in the runs given in Tables 5.9 and 5.10, and while the baselines are being run, the sampler is switched out of the system; therefore no liquid is being drawn from this cup. The cup should contain ASW in case the timing is out slightly and sample is accidently drawn from the cup.

   The baseline change order is as follows:

   - Start the run on ASW + colour reagents
   - Change to background reagents and LNSW, and switch the sampler out
   - Change from LNSW to MQ
   - Change to colour reagents
   - Change to ASW from MQ
   - Switch the sampler back in (calibrants begin)

Table 5.9 MDL.tbl is used to determine method detection limits and precisions at the start of a voyage.

R=repeat, Dil=dilution, Wt=Weight factor

Cup	Name	Type	R	Dil	Wt	Vial	Comment
1	sync	SYNC	1	1	1		top
1	cal 5	C	1	1	1		
11	nitrite	CCV	1	1	1		
2	baselines	U	5	1	1		baseline changes
2	baselines	U	5	1	1		a cup containing ASW in #2
2	baselines	U	5	1	1		
2	baselines	U	2	1	1		
3	wash	U	1	1	1		for carryover correction
4	cal 5	U	2	1	1		for carryover correction
3	wash	BLNK	1	1	1		ASW
3	baseline	RB	1	1	1		ASW
5	cal 0	C	1	1	1		
6	cal 1	C	1	1	1		
7	cal 2	C	1	1	1		
8	cal 3	C	1	1	1		
9	cal 4	C	1	1	1		
10	cal 5	C	1	1	1		
11	nitrite	CCV	1	1	1		
12	wash	BLNK	1	1	1		
13	QC sample	U	1	1	1		
14	SRM low	U	1	1	1		SRM at 1uM P, 10uM N and S
15	SRM high	U	1	1	1		SRM at 3uM P, 30uM N and S
12	wash	BLNK	1	1	1		ASW
16	SRM blank	U	2	1	1		

Cup	Name	Type	R	Dil	Wt	Vial	Comment
12	baseline	RB	1	1	1		ASW
17	blank	U	3	1	1		ASW
18	blank	U	3	1	1		ASW
19	blank	U	3	1	1		ASW
20	blank	U	3	1	1		ASW
21	blank	U	3	1	1		ASW
22	cal 2	C	2	1	1		
23	cal 5	C	2	1	1		
24	wash	BLNK	1	1	1		
24	baseline	RB	1	1	1		ASW
25	sample 1	U	1	1	1		Samples taken from one niskin
26	sample 2	U	1	1	1		
27	sample 3	U	1	1	1		
28	sample 4	U	1	1	1		
29	sample 5	U	1	1	1		
30	sample 6	U	1	1	1		
31	sample 7	U	1	1	1		
32	sample 8	U	1	1	1		
33	sample 9	U	1	1	1		
34	sample 10	U	1	1	1		
35	sample 11	U	1	1	1		
36	sample 12	U	1	1	1		
37	sample 13	U	1	1	1		
38	sample 14	U	1	1	1		
39	sample 15	U	1	1	1		
40	wash	BLNK	1	1	1		
40	baseline	RB	1	1	1		
5	cal 0	C	1	1	1		
6	cal 1	C	1	1	1		
7	cal 2	C	1	1	1		
8	cal 3	C	1	1	1		
9	cal 4	C	1	1	1		
10	cal 5	C	1	1	1		
11	nitrite	CCV	1	1	1		
41	wash	BLNK	1	1	1		
13	QC sample	U	1	1	1		
14	SRM low	U	1	1	1		SRM at 1uM P, 10uM N and S
15	SRM high	U	1	1	1		SRM at 3uM P, 30uM N and S
41	wash	BLNK	1	1	1		ASW
16	SRM blank	U	2	1	1		
41	baseline	RB	1	1	1		

Table 5.10 Template for a typical sample run (csiro.tbl).

R=repeat, Dil=dilution, Wt=Weight factor

Cup	Name	Type	R	Dil	Wt	Vial	Comment
1	sync	SYNC	1	1	1		top
1	cal 5	C	1	1	1		
11	nitrite	CCV	1	1	1		
2	baselines	U	5	1	1		baseline changes
2	baselines	U	5	1	1		a cup containing ASW in #2
2	baselines	U	5	1	1		
2	baselines	U	2	1	1		
3	wash	U	1	1	1		for carryover correction
4	cal 5	U	2	1	1		for carryover correction
3	wash	BLNK	1	1	1		ASW
3	baseline	RB	1	1	1		ASW
5	cal 0	C	1	1	1		
6	cal 1	C	1	1	1		
7	cal 2	C	1	1	1		
8	cal 3	C	1	1	1		
9	cal 4	C	1	1	1		
10	cal 5	C	1	1	1		
11	nitrite	CCV	1	1	1		
12	wash	BLNK	1	1	1		
13	QC sample	U	1	1	1		
14	SRM low	U	1	1	1		SRM at 1uM P, 10uM N and S
15	SRM high	U	1	1	1		SRM at 3uM P, 30uM N and S
12	wash	BLNK	1	1	1		ASW
16	SRM blank	U	2	1	1		
12	baseline	RB	1	1	1		ASW
17	212	U	1	1	1		
18	211	U	1	1	1		
19	210	U	1	1	1		
20	209	U	1	1	1		
21	208	U	1	1	1		
22	207	U	1	1	1		
23	206	U	1	1	1		
24	205	U	1	1	1		
25	204	U	1	1	1		
26	203	U	1	1	1		
27	202	U	1	1	1		
28	201	U	1	1	1		
29	cal 1	C	2	1	1		
30	cal 4	C	2	1	1		
31	wash	BLNK	1	1	1		ASW
31	baseline	RB	1	1	1		ASW
32	312	U	1	1	1		
33	311	U	1	1	1		
34	310	U	1	1	1		
35	309	U	1	1	1		

Cup	Name	Type	R	Dil	Wt	Vial	Comment
36	308	U	1	1	1		
37	307	U	1	1	1		
38	306	U	1	1	1		
39	305	U	1	1	1		
40	304	U	1	1	1		
41	303	U	1	1	1		
42	302	U	1	1	1		
43	301	U	1	1	1		
44	cal 2	C	2	1	1		
45	cal 5	C	2	1	1		
46	wash	BLNK	1	1	1		
14	SRM low	U	1	1	1		SRM at 1uM P, 10uM N and S
15	SRM high	U	1	1	1		SRM at 3uM P, 30uM N and S
46	wash	BLNK	1	1	1		ASW
16	SRM blank	U	1	1	1		
61	SRM blank	U	1	1	1		
46	baseline	RB	1	1	1		ASW
47	412	U	1	1	1		
48	411	U	1	1	1		
49	410	U	1	1	1		
50	409	U	1	1	1		
51	408	U	1	1	1		
52	407	U	1	1	1		
53	406	U	1	1	1		
54	405	U	1	1	1		
55	404	U	1	1	1		
56	403	U	1	1	1		
57	402	U	1	1	1		
58	401	U	1	1	1		
59	wash	BLNK	1	1	1		
59	baseline	RB	1	1	1		
5	cal 0	C	1	1	1		
6	cal 1	C	1	1	1		
7	cal 2	C	1	1	1		
8	cal 3	C	1	1	1		
9	cal 4	C	1	1	1		
10	cal 5	C	1	1	1		
11	nitrite	CCV	1	1	1		
59	wash	BLNK	1	1	1		
13	QC sample	U	1	1	1		
14	SRM low	U	1	1	1		SRM at 1uM P, 10uM N and S
15	SRM high	U	1	1	1		SRM at 3uM P, 30uM N and S
60	wash	BLNK	1	1	1		ASW
61	SRM blank	U	2	1	1		
59	baseline	RB	1	1	1		

When deciding whether to re-use calibrants and QC samples, take into account the following:

- Use the same calibrants at the start and end of a run, and separate tubes for the gain checks through the run, remembering that these need to be done in duplicate and a cup can only be sampled up to three times
- Don't top up calibrant/SRM cups by adding leftovers together
- Replace washes for each run

5.8.6 Shutdown procedure

At the end of the day's work, the instrument should be flushed completely of all chemicals and stored with MilliQ in all lines.

1. The OTCR must be removed before going any further.
2. The OTCR should be flushed 2-3 times with MilliQ, applying the same techniques used in the regeneration. The OTCR should then be stored full of Imidazole buffer that is surfactant-free. For long-term storage, the OTCR should be thoroughly dried and stored full of nitrogen gas.
3. Turn off the pump and place all reagent tubes in a bottle of MilliQ. Turn the pumps on and allow MilliQ to pump through for at least 20 minutes.
4. Turn off the cartridge heaters and the chart recorder. The chart pens must be stored with the caps on or they will dry out.
5. When the instrument has been cleaned thoroughly, turn the pump off and release the tension on each of the platens. Turn off the instrument at the power supply and turn off all power points.
6. Return the reagents that need refrigeration to the refrigerator. These will be:

   Orthophosphate: reagent 1 and ascorbic acid
   Silicate: ammonium molybdate and ascorbic acid
   Nitrate+nitrite: NEDD reagent

7. Tip any unused sample to waste and dispose of the nutrient tubes. The tubes can be re-used if required, but must be scrupulously cleaned first.
8. Backup data files to the HYDRO PC, and turn off computer when all processing is completed.

5.9 Data processing

5.9.1 Theory of mathematical corrections to results

The following description is based on methods used in the WOCE Operations Manual, 1991, and explains why carryover correction, gain correction and baseline changes are required and how they are calculated.

A spreadsheet and macros have been developed to automatically calculate concentrations, taking into account carryover correction, refractive index and reagent blanks, and gain correction. The spreadsheet template is named Fyyvvrr.xlt and the document containing the macros is labelled Fyyvvrr.xls. For these macros to be available, Fyyvvrr.xls **must** be located

in C:\Program Files\Microsoft Office\Office\Xlstart. Details on how to use the spreadsheet are included in Section 5.9.4.

Carryover correction

Carryover correction checks are included at the start of a run and are used to account for the incomplete flushing between samples. The formulas for calculation of carryover require inclusion of a wash sample that is followed by two high-concentration calibrants made in a natural seawater matrix (in this case, cal 5) in the run.

The carryover coefficient, k, is calculated first

$$k = \frac{(x_2 - x_1)}{(x_1 - w)}$$

Where: k = carryover coefficient
x_1 = peak height of the first cal 5
x_2 = peak height of the second cal 5
w = wash preceding the first cal 5

Next, the carryover correction amount for each peak is calculated from:

$$\bar{o} = k(A_i - A_{i-1})$$

Where: $\bar{o}$ = carryover correction
k = carryover coefficient
A_i = absorbance of peak of interest
A_{i-1} = absorbance of peak preceding peak of interest

Finally, each peak is corrected by adding the carryover correction value to the original peak absorbance value.

$$A_c = A_i + \bar{o}$$

Where: A_c = carryover corrected peak height
$\bar{o}$ = carryover correction
A_i = original absorbance of peak

This method results in a different percentage correction to each peak, based on the carryover correction coefficient, k.

Refractive index and reagent blank corrections (baselines)

Two corrections are required after carryover correction when processing nutrient results. The first is a reagent blank correction, that is subtracted from all samples and calibrants to allow for absorbance caused only by the reagents. A reagent blank requires water containing zero nutrient. We routinely analyse samples in a seawater matrix, but it is impossible to obtain seawater with zero nutrient. Therefore, a MilliQ blank is used. ***It is extremely important that the MilliQ be free of nutrient.*** If it is suspected at any stage that there is a contamination problem with the MilliQ production system, analysis should be stopped until the problem is fixed. The reagent blank is subtracted from the carryover corrected absorbances:

$$\text{Blank Correction} = A_C - A_{MQ}$$

Where: A_C = carryover corrected peak height/absorbance
A_{MQ} = absorbance of MilliQ

The second correction to the nutrient results is to allow for the refractive index difference between MilliQ and seawater when using MilliQ as a blank. A component of the blank absorbance is due to refractive index, and the difference between the response of seawater and the response of MilliQ is subtracted from the sample and calibrant peak heights.

It is important that the refractive index difference between seawater and MilliQ be calculated **with respect to sign.** The procedure described in the WOCE manual (WOCE, 1991) is for alternating samples of MilliQ and seawater to be run at least ten times with the colour-forming reagents removed (ie, run on 'background', with NEDD removed from the nitrate+nitrite channel and ascorbic acid removed from the silicate and orthophosphate channels). The procedure should be run before a sample run. The average difference between MilliQ and seawater is calculated. The formula for calculation of refractive index (RI) is:

$$RI = \frac{\Sigma(A_{SW} - A_{MQ})}{n}$$

Where: RI = refractive index difference
A_{SW} = seawater absorbance
A_{MQ} = absorbance of MilliQ
n = number of repeat measurements

The method used by the CSIRO Marine Research Hydrochemistry group is an adaptation of the WOCE method, and includes reagent blank and refractive index provision at the start of every run, in the form of baseline changes.

There are several baseline changes, the order being:

ASW+colour
LNSW+background
MQ+background
MQ+colour
ASW+colour

where LNSW is natural seawater that is low in nutrient, and ASW is 35‰ NaCl.

All sample and calibrant peaks and baselines are measured from the ASW + colour baseline. The differences in baseline heights from the ASW+colour baseline allow calculation of the blank value and the refractive index contribution. The formulas used to calculate reagent blank and RI contributions by this method are:

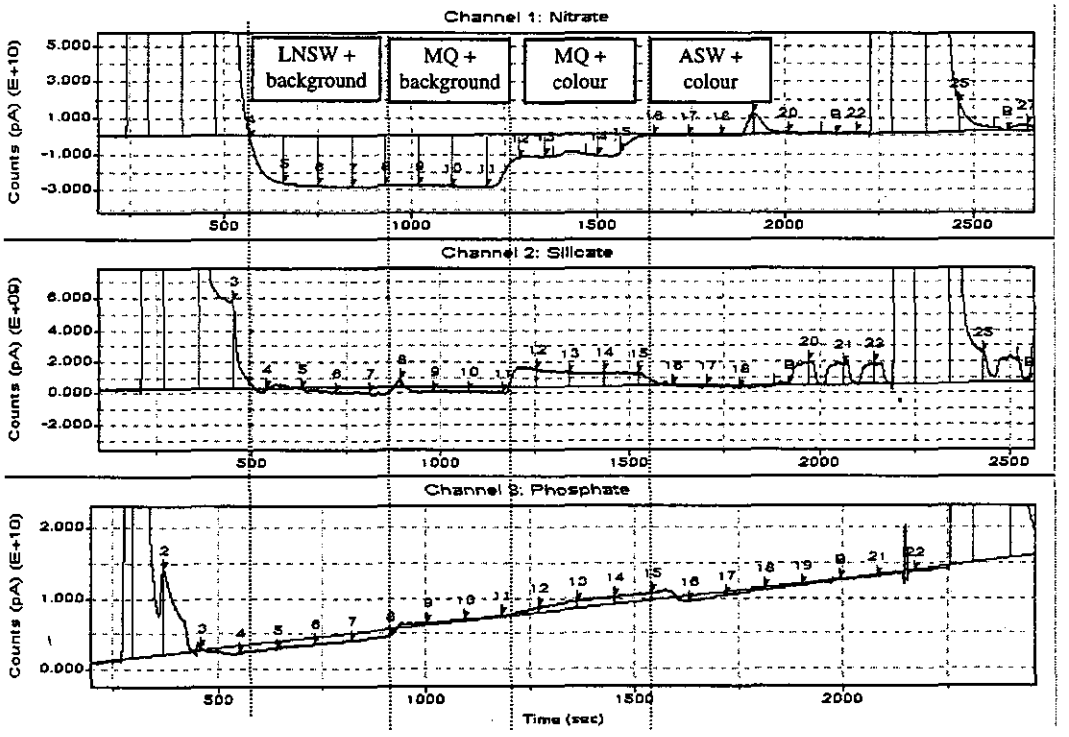
Blank = MQ+colour baseline – ASW+colour baseline
(as measured from the ASW+colour baseline)

Refractive index = LNSW background baseline – MQ+background baseline
(as measured from the ASW+colour baseline)

Typical baseline changes on the Alpkem Flow Solution III system using WinFlow are shown in Figure 5.6.

Figure 5.6 Examples of baseline changes in WinFlow.

Note that a 'B' (baseline mark) has been toggled on each baseline just prior to peak #22, which is the wash peak used in carryover correction. This is done to ensure accurate measurement of the wash peak. See Section 5.9.2 for details.



Gain correction

Gain correction is applied to all results; however, if calibrant peak heights are changing by more than 15 % from start to end (over a run of 3 hours), there is likely to be a problem with the system or chemistry. The problem should be fixed at the instrument, and the run repeated. **Gain correction should not be used to allow for large changes across a run.**

Gain correction is applied in one of two ways, depending on the shape of the calibration curve. The "response factor" for a linear fit is calculated as described in the WOCE manual, while a separate method has been developed for use with a polynomial fit.

For a linear fit, a response factor, **f**, is calculated from the calibrant absorbances. The response factor is actually the inverse of the slope of a linear fit, or the inverse of the sensitivity:

$$f = \frac{C_a}{(A_c - A_z)}$$

Where: f = response factor
C_a = added concentration of nutrient in the calibrant
A_c = absorbance of calibrant after carryover correction and blank/RI correction
A_z = absorbance of zero calibrant (unspiked natural seawater) after carryover correction and blank/RI correction

All response factors are then regressed over their position in the run. Sample absorbances that have been corrected for carryover, reagent blank and refractive index errors are then multiplied by the interpolated response factors, giving the final concentration of the samples.

For a polynomial fit, the gain correction is more complicated. A response factor cannot be used, as this requires a constant slope (or sensitivity) and therefore, linearity. In a polynomial fit, the slope is constantly changing and a different approach is required.

Each carryover-, blank- and refractive index-corrected calibrant absorbance is compared to the first full set of calibrants in the run. For example, in a typical run, cals 1 to 5 first appear in the run at positions 28 to 32, and each of these is given a value of one. All other calibrants are compared to this set. The formula is:

$$\text{Gain factor} = \frac{A_{cal\,x}}{A_{cal\,x(1)}}$$

Where: $A_{cal\,x}$ = carryover, blank and RI corrected absorbance of calibrant x at any position
$A_{cal\,x(1)}$ = carryover, blank and RI corrected absorbance of calibrant x in the first set of calibrants in the run

It is necessary to give the first calibrant appearing in the run a value of 1. Cal 5 appears in position 2 in the run, so this calibrant is now given a value of 1, and all other gain factors are divided by position 2's original gain value:

$$\text{Corrected gain factor} = \frac{GF_{cal}}{GF_{cal\,(2)}}$$

Where: GF_{cal} = gain factor of any calibrant at any position;
$GF_{cal\,(2)}$ = gain factor of the first calibrant appearing during the run.

The corrected gain factors for each calibrant are linearly regressed against their position number in the run. From this, an intercept and a slope are obtained.

The intercept and slope of the line of best fit are then used to calculate a gain factor for each position in the run. The gain factors will all approximate 1.

Using the gain factor for each position, carryover-, blank- and refractive index-corrected peak heights are adjusted over the run to give a gain-corrected peak height. The formula for this is:

$$\text{Gain corected peak height} = \frac{A_{sw}}{GF}$$

Where: A_{sw} = carryover, blank and RI corrected absorbance of the sample or calibrant
GF = calculated gain factor for that position

The carryover-, blank-, refractive index- and gain-corrected calibrant values are then plotted against concentration and a second-order polynomial curve fitted. From the curve equation, sample concentrations are derived.

5.9.2 WinFlow data handling

For full details on how to use WinFlow, see the WinFlow Manual. WinFlow data processing can be completed up to step 11 on all *.rst files before continuing, but step 12 on (importing and Excel processing) must be completed on one file at a time.

1. Open the saved *.rst file in WinFlow.
2. Open the nitrate channel and enlarge the peak plot on the screen.

3. Check that all peak markers are in the right place on the peaks. Sometimes they can be slightly out, and you may need to look closely with the magnifying tool.
4. Enlarge the baseline changes at the start of the run.
5. Move the peak markers to suit the baseline changes if necessary. Try to ensure that each peak marker is representative of that baseline height. If there are interruptions in the baselines due to air bubbles, etc, use the toggle baseline point tool to change a marker to a baseline point. Write down the peak markers that represent each baseline.
6. Ensure that the carryover correction samples (two cal 5's and a wash precedeing the cal 5's) are marked correctly. If necessary, a baseline point can be toggled in before the wash to ensure the wash is measured accurately (see Figure 5.6). Do this by picking up the cross-hair tool from the tool-bar, right-clicking with the mouse, then choosing 'Toggle baseline point'.
7. Go through the rest of the run and put the baseline markers on the baseline, moving them off peaks and troughs, and into a position typifying the baseline. Look at the baselines as you go and check they are not cutting off the bottom of peaks. If the baseline moves a lot and it is necessary to have more baseline points, it is possible to designate wash markers as baseline points by toggling a baseline point.
8. Recalculate, either by reducing the peak plot screen, or by hitting the recalculate button on the toolbar.
9. Note down peaks that cannot be used in the results, peak name changes and any other things that will need editing.
10. Save the file in the format: FYYVVRRa.rst. The 'a' is needed to distinguish the edited run from the original data file. WinFlow will not let you write over the original file.
11. Repeat steps 2 to 9 with orthophosphate and silicate before proceeding to the next step.
12. Ensure that the *.rst file is saved. Remaining *.rst runs can be processed up to this stage if you wish.

COMPLETE THE REMAINING STEPS ONE RUN AT A TIME

13. Size the nitrate results table to the full screen.
14. Select 'File', 'Export..'.
15. Save as 'nit.txt' in Flow_3. For example, c:/Flow_3/nit.txt. (You can save the file in any folder, but you will need to remember the path name).
16. Repeat steps 13 to 15 for orthophosphate and silicate, saving as 'sil.txt' and 'phos.txt'. Any name will do as the text files will not be retained and can be written over. Go directly to transferring data to Excel.

5.9.3 Transferring data to Excel

Once the nitrate+nitrite, orthophosphate and silicate data from the run have been saved as *.txt files:

1. Open Excel 97.
2. Click on 'File, New' and select the HYDRO templates tab. Select FRYYVVRR.xlt. This is the calculation template. The file will automatically save itself at a later step.

3. Click the first toolbar button indicated in the 'Start' sheet of the template to begin to import the data from the WinFlow text files. A dialog box will appear requesting run details. Fill in the appropriate information and click 'OK'. The box will not close until you click 'Cancel', unless the 'No results' option has been selected. If the box closes and you need to import more data, simply click on the first toolbar button again.
4. Repeat step 3 for the remaining two *.txt files.
5. The macro will produce a file with a worksheet for each nutrient for which you have results. Each sheet will be labelled as either 'linear' or 'polynomial'.
6. Click on the second toolbar button indicated on the 'Start' sheet. The macro will ask you questions regarding the calibrant ranges used for each nutrient.
7. The file will automatically save itself under the correct name in C:\Hydro\<Voyage>\Nutrients.

If the carryover correction samples (wash, cal 5, cal 5) are not labelled correctly, a message box will tell you these values cannot be found. In this case, re-label the carryover samples so that they read 'wash, cal 5, cal 5', and click on the second button again.

If you have problems during the processing (eg, you decide that the data should be fit to a linear fit after you have selected a polynomial fit), you can begin again by either re-importing the data (first button) and/or reprocessing (second button) as necessary. This can only be done before creating the summary sheet (third button, see Section 5.9.4).

5.9.4 Processing data in Excel

Start with the nitrate sheet. All changes that need to be made are in the green cells. However, you may need to delete gain correction values on occasions, and sometimes change peak heights by hand.

1. In the appropriate cells at the top of column F (rows 1, 2 and 3), adjust the formulas according to which peaks represent which section of the baseline for MQ+background, MQ+colour and LNSW+background.
2. Delete any peak-height information if needed, including the formulas in that row. Change any peak names and make any editing changes.
3. Go to the 'Gain correction' plot on the right of the spreadsheet. This part is used to calculate gain across the run and adjust the peak heights accordingly (see Section 5.9.1 for the theory behind these calculations).
4. Have a quick look at the plot for outliers. Erroneous peaks should be removed from the dataset. Do this by first checking that the error is real (by looking in Winflow to check the peak marker is on top of the peak), and then by deleting calibrant gain values if the peak height is real.
5. Go to the calibration plot and check the plot looks correct, and that the concentrations are correct. Concentrations for this plot (on the right of the chart) can be edited if necessary.
6. Look at the calculated concentrations for the SRMs, QC samples and calibrants and check that they are what is expected. If there is a problem, the first place to look is at the trace of the run in WinFlow, including peak markers and baseline positions, and then at the spreadsheet. If there is a problem with the WinFlow processing and you have re-processed the trace, there are two options for processing in Excel. You can

either manually type in new peak heights into the processed Excel spreadsheet or, if there are too many changes, you will have to re-start the importing and processing for all three nutrients for that run.

7. Give the sample values a quick scan to see if they make sense. Just because the quality control data indicates all is well, doesn't mean the sample values are okay!
8. Repeat steps 1 to 8 for the orthophosphate and silicate sheets.
9. Save the file.
10. Finally, click on the third toolbar button indicated on the 'Start' sheet. A summary sheet will be produced and the 'Start' sheet will be deleted. Three other sheets labelled 'silicate', 'nitrate' and 'phosphate' will remain. Print out the summary sheet.

5.9.5 Checking data quality

Currently there are two Excel spreadsheets into which quality-control data must be entered. The first, labelled 'StatsTempl.xlt', is used to calculate accuracy, precision, method detection limits and sampling precisions for the voyage. It is used to test the SRM results at a 99 % confidence interval, and therefore tell you if the data has determinate error. The second spreadsheet is labelled 'NutsQC.xls', and should be used to compare your QC data to historical records. It contains all the relevant QC data from previous voyages. As you process your results, the QC data can be entered into these two spreadsheets and therefore give you an idea of the quality of your results.

StatsTempl.xlt

The quality control data obtained from 'StatsTempl.xlt' should be included in the hydrochemistry voyage report, for later inclusion with the processed data. The hydrochemistry voyage report template (VoyageRep.dot) contains example text and tables that can be edited as required. Explanations of the calculations in 'StatsTempl.xlt' are given in Section 5.10.

1. Select 'File', 'New' in Excel and choose 'StatsTempl.xlt' from the Hydro templates.
2. The precision and accuracy sheets both have two tables (one green, one yellow) for high-range and low-range results. SRM values are used in both these sheets.
3. It is best to complete the setup for one nutrient, then copy the sheets for the other two and enter their results (as explained in the instructions in the template).
4. Insert or delete columns as required in the precision and accuracy sheets to give you the right number of columns for the number of runs completed. It is best to insert the columns before run 5 in the template, as all formulas on the sheets will be adjusted correctly. Re-number the run numbers as required.
5. Insert or delete rows in the tables to allow for the number of results per run.
6. Enter your data into the appropriate cells, entering blank corrected values in the accuracy sheet, and non-blank corrected values in the precision sheet. In the accuracy sheet, a t-value will need to be entered from the Student's t-table (see Appendix A), at a 99 % confidence interval. Look up the number of degrees of freedom on the table, and read the value that corresponds to an α value of 0.01.
7. If the data has determinate error at 99 % confidence, the orange cells below the accuracy tables will display 'Outside', which indicates the results are sufficiently

different from the expected value to indicate determinate error. Otherwise, the cells will display 'OK'.

8. Enter repeat blank results and repeat sample results into the appropriate sheets, and enter the t-value for the method detection limit calculations.

NutsQC.xls

1. Three sheets contain the numerical results, the remaining sheets are plots of the results. The results sheets are labelled 'SRM', 'Duplicate' and 'QC'.
2. In all three results sheets, insert the required number of rows for the number of runs completed, at the bottom of the list of results.
3. Insert the voyage details, as per the previous voyages on the sheets.
4. Use one row for each run, and enter the data across the row. Nitrate+nitrite is first, and orthophosphate and silicate results are over to the right of the sheets. For the SRM results, enter the blank-corrected values. For duplicate results, enter the non-blank corrected SRM results, or any other duplicate data that you think is useful. The QC results entry is straightforward.
5. After entering the data, copy down the formulas in the coloured sections from the voyage above, to cover all runs from your voyage.
6. Look at the plots of the data, and compare your results with historical ones. Use this technique to evaluate your results as you go.

5.9.6 Transferring data to HYDRO

Once all the data have been processed and you are happy with its quality, the .xls files will need to be saved in Excel 4 format (*.xlw). There is a macro to do this included in the macro sheets. The steps to be followed are:

1. Make sure you have all files closed.
2. Select 'Tools', 'Macro', 'Macros' from the menu and run 'Fyyvvrr.xls!SavingXLW'. This macro will cycle through all the files and save them as *.xlw files. Note that the *.xls files are required to be in C:\Hydro\fyyvv, where fyyvv is the voyage name.
3. Transfer of data to HYDRO – currently there is a macro in HYDRO that will do this. Open HYDRO, make sure that Nutrient.xlm is open, select 'Macro', 'Run' from the menu and run 'Process_XLW_File'. This macro will go through every file to enter the data into the *.dat files.

5.10 Detection limits, accuracy and precision reporting

To be able to report the detection limits, accuracy and precision of the instrument for the data from any given voyage, procedures are included to determine these values. A spreadsheet in Excel contains templates for the calculations (StatsTempl.xlt). The description of each calculation is taken from Skoog et al. (1998).

5.10.1 Detection limits

Detection limits are determined by running repetitions (at least 15) of an ASW blank. These results are then used to calculate the detection limit at a 99 % confidence limit ($\alpha = 0.01$). Anything less than this value is considered not detectable. The following equations are used to calculate detection limit:

$$\text{Standard deviation of the measurements: } s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

Where: n = number of repeat measurements
x_i = the ith repeat measurement
$\bar{x}$ = mean of the repeat measurements.

$$\text{Degrees of freedom: } Df = N_1 + N_b - N_g$$

Where: N_1 = number of repeated measurements for any sample (in our case, usually 1)
N_b = number of repeated blank measurements
N_g = number of groups of measurements (ie, N_1 is one group and N_b is a second group, therefore, $N_g = 2$).

$$\text{Detection limit: } D_L = s \times t\left(\sqrt{\frac{(N_1 + N_b)}{N_1 N_b}}\right)$$

Where: N_1 = number of repeat measurements of a sample (in most cases, 1)
N_b = number of repeat measurements of the blank
s = standard deviation of repeat measurements
t = Student's t-distribution (from the table in Appendix A), based on the degrees of freedom (calculated above), and $\alpha = 0.01$ (99 % confidence interval)

5.10.2 Precision

Precision describes the agreement between two or more measurements that have been undertaken in exactly the same manner (ie, in the same run), and is presented as the standard deviation of the repeat measurements (see Section 5.10.1 for formula). To calculate precision of data for a series of nutrient runs across a number of days/weeks requires special treatment of the data. Also, as we do not undertake replicate measurements of all samples, an indication of the precision of a voyage's data set is obtained from a pooled standard deviation of replicate SRM determinations.

The SRM concentrations (before they are corrected for the SRM blank) are used to calculate precision for the repeat SRM determinations, and hence give an estimate of the sample precision for that run. Two concentrations of SRM are included in the run, one representing higher concentrations and one lower concentrations. They should be calculated seperately, as precision varies with concentration. When stating the precision estimates for all runs, a table should be included in the voyage report that contains the mean, standard deviation and coefficient of variation of the data (this can be taken directly from the precision calculation table in Excel). The coefficient of variation is calculated as:

$$CV(\%) = \left(\frac{s}{\bar{x}}\right) \times 100$$

Where: s = standard deviation of the uncorrected SRM data (see above for formula)
$\bar{x}$ = mean of uncorrected SRM data

An estimate of the precision for the entire voyage's data can be calculated from the standard deviations for all runs and pooling them. First, the absolute deviation from the mean of the run for each result is calculated:

$$\text{Absolute deviation from the mean: } AD = \left| x_i - \bar{x} \right|$$

Where: x_i = individual SRM value
$\bar{x}$ = mean of uncorrected SRM data for the run

Next, for each run, the sum of squares of the absolute deviations from the means is calculated. These calculations are included in a separate table in the Excel template:

$$\text{Sum of squares of absolute deviation from the mean: } SS = (AD_1)^2 + (AD_2)^2 + (AD_3)^2 + \ldots + (AD_n)^2$$

Where: AD_n = absolute deviation from the mean of SRM results 1 to n
n = number of results in the run

Finally, the pooled standard deviation is calculated for the voyage, using the sum of squares results for each run:

$$\text{Pooled standard deviation: } s_{pooled} = \sqrt{\frac{(SS_1 + SS_2 + SS_3 + \ldots + SS_n)}{(n - n_{ss})}}$$

Where: SS_n = sum of squares of runs 1 to n
n = total number of SRM results used (from all runs)
n_{ss} = number of sets of results (ie, one run = one set of results)

The pooled standard deviation can then be presented as the coefficient of variation (CV %), from the mean concentration of all SRM results (that have not been corrected for the SRM blank). The voyage report should contain a statement such as: "The mean concentration of precision measurements was 10.18 µM with a pooled standard deviation of +/- 0.075 µM (0.73 % coefficient of variation)." Data for both SRM concentrations should be included.

5.10.3 Accuracy

The accuracy of the method can be determined by including an external standard in the run (the OSI standard, or SRM), which is run at two concentrations. Estimates of accuracy should be reported for all runs and for the voyage data as both the absolute error and the percent error. Estimation of the absolute error:

$$\text{Absolute Error} = (\bar{x} - e)$$

Where: $\bar{x}$ = mean SRM concentration for the run or voyage
e = expected SRM concentration

The percent error can also be presented:

$$\text{Error (\%)} = 100 \times \left(\frac{(\bar{x} - e)}{e} \right)$$

Where: $\bar{x}$ = mean SRM concentration for the run or voyage
e = expected SRM concentration

To determine whether the absolute error associated with the run or the voyage results is significant and therefore due to determinate error, we compare the absolute value to a critical value that is calculated at a 99 % confidence interval from Student's t-distribution and the standard deviation of the results. The degrees of freedom calculation is required to select the correct t-value:

$$\text{Degrees of freedom: } Df = n_1 + n_2 + \ldots n_i - 1$$

Where: n_1 = number of results for run 1
n_i = number of results for run i

$$\text{Critical Value} = \pm\left(\frac{s \times T}{\sqrt{n}}\right)$$

Where: s = standard deviation of the SRM data (see above for formula)
T = Student's t-distribution (from the table in Appendix A), based on the degrees of freedom (equation shown above), and $\alpha = 0.01$ (99% confidence interval)
n = number of determinations

When referring to the accuracy in the voyage report, a table of absolute and percent errors and critical values for each run should be included. This can be taken directly from the coloured sections of the accuracy worksheet in Excel. Overall accuracies for both concentration SRMs used during the voyage can be included as a statement, eg, "At 10.00 µM the experimental mean was 10.05 µM and the absolute error was 0.05 µM (+0.5 %). Using Student's t-distribution at 99 % confidence, there was no demonstrated difference between the experimental mean and the expected mean." Alternatively, if the absolute error exceeds the critical value for either a run or the voyage, the cause of the error should be investigated. This may require some run data to be flagged as containing unacceptable errors and then excluded from the voyage statistics.

5.10.4 Sampling precision

To determine sampling precision, a run should be completed at the beginning of the voyage that includes at least 15 samples taken from one Niskin bottle by the nutrient sampler for the voyage. The mean, standard deviation and coefficient of variation of these results are calculated and should be included in the voyage report.

Table 5.9 shows the recommended run table for method detection limit and sampling precision. This run should be included at an early stage in the voyage so that any problems with sampling or the instrument can be identified and rectified. Results of precision, sampling precision, accuracy and detection limits should be presented in the voyage report.

5.11 Quality assurance/quality control

Quality assurance involves record-keeping and incorporation of procedures into the analysis protocol that assure the quality of the data (ie, leaving a 'paper trail'). Quality control is the inclusion of specific samples in a normal run to control the quality of the data obtained (ie, inclusion of QC samples and SRMs to check the accuracy of the results).

To maintain quality assurance, a log sheet needs to be completed for each run. An example of the log sheet is included in Appendix B. On the ship (either *RV Franklin* or *RV Southern*

Surveyor) details of the reagent preparation and SRM and working calibrant preparation are recorded. In Hobart, the details of stock standard preparation and chemical weighing details are recorded.

Quality control is included in each run in the form of duplicate measurements, QC samples, SRM samples and column-efficiency checks for nitrate+nitrite analysis. Table 5.10 shows the standard run setup with the quality control samples included. Data collected during a voyage are statistically tested and compared with historical data, as described in Section 5.9.5.

Following is the protocol associated with each quality-control element.

5.11.1 Sample storage

Samples are to be stored frozen before analysis. Storage time should be no more than 2 weeks. If the principal scientist requires very accurate data, it may be necessary to analyse the samples fresh, in which case all calibrants and SRMs should also be made fresh for every run.

5.11.2 Log sheets

Log sheets must be filled out for every run, with enough detail to enable tracing of reagent details, etc, if necessary.

5.11.3 Calibrants

Calibrants and nitrites should be made in filtered LNSW (there is a supply of LNSW in the cool rooms on board both vessels). The blank absorbance is then subtracted from all calibrants and nitrite samples as part of the processing. This should help to avoid matrix differences between samples and calibrants.

5.11.4 QC samples and SRMs (OSI standards)

OSI standards are to be made up at the start of the voyage, just before the first run. Two concentrations should be made up (1 and 3 µmol/L for PO_4, and 10 and 30 µmol/L for SiO_4 and NO_3+NO_2, or as appropriate for the calibrant range used). 100 mL of a mixed standard is made according to Section 5.6.2. This will fill 10 nutrient tubes, which can be frozen along with the calibrants and used with each run (see Table 5.10). Also, fill 10 nutrient tubes with the LNSW used for dilution of the SRMs and freeze for use as a blank. The blank is subtracted from the SRM concentration to give the true concentration.

A QC sample should be included at the start and at the end of a run. These samples are spiked LNSW that has been frozen in 125 mL Nalgene bottles. These samples give some continuity between voyages, as well as being a useful check when the SRMs or calibrants appear to have been made incorrectly, or are contaminated.

5.11.5 Duplicates

Duplicate samples *per se* are not run; however, duplicate QC samples and SRM samples are included in a standard run, so they can be used to indicate the precision for that run.

5.11.6 Column checks

Column checks should be run at the beginning and end of a run. A column performing at less than 95 % needs to be regenerated or replaced.

5.12 Troubleshooting

The following is taken from Eriksen, 1997. It provides a useful basis for defining problems and lists the more common solutions to each type of problem. A trouble-shooting data file kept on the hydrochemistry lab PC on the *RV Franklin* can be used to access information and to add solutions to problems.

5.12.1 Basic troubleshooting guidelines

Don't overlook the obvious. Look for the simplest and most obvious cause of a particular problem. Check for correct power to all modules and that the power switches are on. Check for correct connection of all control and signal lines. Check tubing connections and flow-through system.

Rule out operator-induced errors. Consult the system manual for proper operating procedures and protocols. Review test methodologies and flow diagrams to verify operating parameters.

Isolate and define the problem. Categorise the problem or symptom as one of the following: chemistry, hydraulic, or electrical/mechanical. Once a problem has been clearly defined, the troubleshooting will become more efficient and the answer may become readily apparent.

Eliminate one variable at a time. Using good experimentation techniques, change only one component or variable at a time and note the changes and results. In this manner, the problem may be cured or the solution more clearly defined.

Document the solution. Keep a log of troubleshooting activity, including problems, symptoms, causes, and solution. This information will be helpful for future troubleshooting sessions, and for other users.

Table 5.11 Categorisation of typical problems

Chemistry	Hydraulic	Electrical/Mechanical
Reagents	Pump tubing	Circuit components
Calibrants	Bubble size and shape	Optics
pH	Surfactant	Photometer/detector
Temperature	Pump	Cabling
		Lamps

5.12.2 Problems and solutions

Common problems

The following tables list common problems and solutions for the Alpkem system.

Table 5.12 Bubbles trapped in flow cell

Possible cause	Potential solution
Bubble breakup in cartridge	Flush cartridge
Bubbles too small, or irregular pattern	Replace air/N_2 pump tube
Stray bubbles	Pinch flowcell waste line for 5 seconds
Not enough surfactant	Increase surfactant
Reagent degassing	De-gas reagents before use
Contamination in flow cell	Flush flowcell with MilliQ, or dismantle and clean

Table 5.13 Bubbles getting through debubbler

Possible cause	Potential solution
Too many bubbles	Correct air/N_2 flow problems
Not enough surfactant	Increase surfactant
Reagent tube pumping air	Refill or reposition reagent container
Debubbler pump tube incorrectly installed	Check/correct tubing installation
Sample tube pumping air	Check sample probe position, sample cup levels, and ASW levels

Table 5.14 Baseline noise

Possible cause	Potential solution
Particulates in reagents	Filter or remake solutions
Contamination in flow cell	Flush flow cell
Pump tube deterioration	Replace pump tubing
Contamination in cartridge	Clean cartridge
Bubble trapped in flowcell	Pinch flowcell waste line for 5 seconds
Source lamp deterioration	Check/replace lamp
Condensation under flowcell	Check flowcell o-rings seated properly

Table 5.15 Loss of sensitivity

Possible cause	Potential solution
Reagent deterioration	Prepare fresh solutions
Standard deterioration	Prepare fresh calibrants
Pump tube deterioration	Replace pump tubing
Contamination in flow cell	Flush flow cell
Wrong wavelength	Use correct wavelength

Possible cause	Potential solution
Wrong pump tubing	Use correct pump tubing
Damp or rise set too high	Use correct setting
Wrong AUFS range setting	Use correct setting
Source lamp deterioration	Check/replace lamp
Heater programmed to wrong temperature	Check/reset temperature

Table 5.16 Spikes on baseline

Possible cause	Potential solution
Electrical noise	Check electrical lines for good connections, freedom from noise, and solid earth ground
Bubbles in flowcell	Check operation of debubbler
Particles passing through flowcell	Filter samples and reagents as required to prevent particles from entering the flow stream. Inspect flow stream for formation of precipitates

Table 5.17 Drift or baseline shift

Possible cause	Potential solution
Contamination in flow cell	Flush flow cell
Reagent deterioration	Prepare fresh solutions
Pump tube deterioration	Replace pump tubing
Source lamp deterioration	Check/replace lamp
Bubble trapped in flowcell	Pinch flowcell waste line for 5 seconds
Condensation under flowcell	Check flowcell o-rings seated properly, and screw are tight

Table 5.18 Non-linearity

Possible cause	Potential solution
Reagent deterioration	Prepare fresh solutions
Standard deterioration	Prepare fresh calibrants
Pump tube deterioration	Replace pump tubing

Table 5.19 Carryover or poor washout

Possible cause	Potential solution
Insufficient wash time	Verify or increase wash time
Loss of intersample bubble	Check intersample bubbles maintain integrity throughout system

Table 5.20 No reaction (no colour product)

Possible cause	Potential solution
Reagent lines on "background" setting	Change to colour reagents
No analyte in sample or standard	Remake standard
Reagent deterioration	Prepare fresh solutions
Standard deterioration	Prepare fresh calibrants
Reagent lines not connected correctly	Check lines and consult flow diagrams
Wrong wavelength	Use correct wavelength
No reagent flow	Increase pump platen tension, or replace tube

Hydraulic problems

Table 5.21 Bubble break-up

Possible cause	Potential solution
Insufficient surfactant	Remake solution, or add more surfactant
Contamination in tubing	Clean or replace tubing
Tubing blockages	Clean or replace tubing
Incorrect pump tubing used	Use correct tubing

Table 5.22 Flow blockage

Possible cause	Potential solution
Contamination in tubing	Clean or replace tubing
Tube deterioration	Replace tubing
Contamination in cartridge	Flush cartridge

Table 5.23 Surges

Possible cause	Potential solution
Cartridge tubing blockage	Clean tubing, remove blockage
Insufficient surfactant	Remake solution, or add more surfactant
Heater temperature too high	Check and reset temperature

Table 5.24 Leaks

Possible cause	Potential solution
Loose connections	Tighten or replace
Poor fitting connections	Replace fitting
Holes in tubing	Replace tubing
Tubing blockages	Clean or replace tubing

Electrical/mechanical problems

Verify that: all cabling is secure
AC power is applied to the module
the line fuse is not open

5.13 References

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WOCE (1991), *WOCE Hydrographic Programme Operations Manual,* WHP Office Report, WHPO 91-1, WOCE Report 68/91, Woods Hole, Mass., USA

5.13.1 Further reading

Alpkem Corporation(1992), *Alpkem Flow Solution Operation Manual,* Alpkem Corporation, 9445 SW Ridder Rd, Wilsonville, OR 97070, U.S.A

6 Nutrient analysis (Technicon)

Rebecca Cowley

Contents

6.1 Introduction

The *RV Southern Surveyor* chemistry laboratory currently has a Technicon AAII Autoanalyser for the analysis of nutrients (nitrate+nitrite, orthophosphate and silicate) in seawater. The software used for data collection and preliminary processing is DAPA. The *RV Franklin* chemistry laboratory uses an Alpkem system with WinFlow software for data collection and preliminary data processing. Both ships' data require further processing using methods developed to allow for refractive index differences between wash and samples, and reagent blanks. This processing is completed using spreadsheets developed in an Excel environment. Because the software used for data collection is different on each ship, the macros used to import the data to the spreadsheets are different. Processing methodology in the spreadsheets is the same.

The standard run setup on both ships is different also, and this is a function of the time it takes to analyse each sample (90 seconds sample+wash on the Alpkem, 3 minutes sample+wash on the Technicon).

This section explains the standard run protocol for the running of nutrient samples, and processing of data collected using the Technicon Instrument and DAPA software. It does not go into detail on operational aspects of the Technicon System, however, these details can be found in Plaschke (1999).

6.2 Run setup

6.2.1 Standard run table

Due to the time taken to complete a run using the Technicon instrument, fewer quality control samples are included in a standard run than are included in an Alpkem run. A Quality control run must be completed at the beginning of a voyage. Table 6.1 shows the quality control run setup, and Table 6.2 shows a typical sample run on the *Southern Surveyor's* Technicon.

For information on the quality assurance/quality control procedures in use, see Section 5.11. Section 5.8.5 is also useful for run setup information, even though it is written for the Alpkem system.

Table 6.1 Standard *Southern Surveyor* Technicon nutrient run for sampling precisions and detection limits.

Sample number	Sample	Comments
1	Trigger	Cal 5
2	Cal 5	
3	Nitrite	
4	seawater	
5	seawater	
6	seawater	
7	MilliQ	
8	MilliQ	
9	MilliQ	
10	MilliQ	
11	wash	
12	wash	For carryover
13	cal 5	For carryover
14	cal 5	For carryover
15	wash	
16	cal 0	
17	cal 1	
18	cal 2	
19	cal 3	
20	cal 4	
21	cal 5	
22	Nitrite	
23	QC sample	
24	SRM	
25	wash	
26	SRM blank	
27	Sampling precision samples	15 repeat samples
42	wash	
43	ASW samples repeated (15)	For method detection limit
58	QC sample	
59	SRM	
60	wash	
61	SRM blank	

Sample number	Sample	Comments
62	cal 2	Or cal 3
63	cal 2	Or cal 3
64	cal 5	
65	cal 5	
66	Nitrite	
67	wash	

Table 6.2 Standard *Southern Surveyor* Technicon nutrient run.

Sample number	Sample	Comments
1	Trigger	Cal 5
2	Cal 5	
3	Nitrite	
4	seawater	
5	seawater	
6	seawater	
7	MilliQ	
8	MilliQ	
9	MilliQ	
10	MilliQ	
11	wash	
12	wash	For carryover
13	cal 5	For carryover
14	cal 5	For carryover
15	wash	
16	cal 0	
17	cal 1	
18	cal 2	
19	cal 3	
20	cal 4	
21	cal 5	
22	Nitrite	
23	QC sample	
24	SRM	
25	wash	
26	SRM blank	
27	samples 1 to 12	First station's samples
39	wash	
40	samples 1 to 12	Second station's samples
52	cal 5	
53	cal 5	
54	wash	
55	samples 1 to 12	Third station's samples
67	wash	
68	QC sample	
69	SRM	
70	wash	
71	SRM blank	
72	cal 2	Or cal 3

Sample number	Sample	Comments
73	cal 2	Or cal 3
74	cal 5	
75	cal 5	
76	Nitrite	
77	wash	

6.2.2 Baselines

Background solution recipes

In order to measure a blank response and to conform with the procedures used on the Alpkem system (see Section 5.9), background reagents are included in the baseline procedures at the beginning of a run. Below is a description of the background solutions used with the Technicon chemistries (Plaschke, 1999).

Silicate background

24 g sodium sulfite (Na_2SO_3) is diluted to 2 L with MilliQ water. Interchange with the metol solution.

Phosphate background

MilliQ water only (do not add hydrazine sulfate, as it also acts as a reducing agent). Interchange with the ascorbic acid solution.

Nitrate+nitrite background

Add 2 mL of Brij-35 to 2 L of MilliQ. Interchange with NEDD solution.

Baseline change order

The method uses dummy 'samples' to allow baseline changes to be included in the run. The following procedure should be used as a guide at the beginning of a run. Timing may need changing and should be checked before starting sample analysis.

- Begin with a MQ+background baseline
- Start DAPA
- Change to ASW+colour and run two cal 5's and a nitrite standard
- Change orthophosphate and silicate from colour to background during wash cycle after nitrite standard, and ASW to LNSW
- Change nitrate from colour to background after first seawater sample
- Change to MilliQ during wash cycle before first MilliQ sample
- Change all three nutrients to colour during wash cycle between second and third MilliQ samples
- Change to ASW during wash cycle after last MilliQ and before first ASW sample

At the end of a run, the baselines of all three nutrients must be changed to MQ+background, so that DAPA has the same point of reference to measure from at the start and the end.

6.3 Data processing

6.3.1 DAPA setup

Below is a brief description of the settings for each section of DAPA required to process nutrient data. For more detail on the use of DAPA, refer to the *DAPA Cookbook* (Terhell and Rayner, 1991) and the *DAPA Manual*.

Integration action table (*.act)

DATA INTEG. SETUP MENU

Integration Fn	F.1/E
Peak start Fn	10
Integ. action Table	<Select appropriate table>
Area reject (Min)	50
Area reject (Max)	0
Integ. full trace	Y
View start<minutes>	0
View end <minutes>	245
Slope Function	10
Lesser Changed Options.	
Tangent ratio	100
Integration type	H
Save Integration	Y
Least Changed Options.	
BaseLine Reset %	.1
Reset B/Line Aft.Rej	N

Items that may need to be altered in the above table to suit the run are:

- *View end* – Increase or decrease as necessary to view the entire trace.
- *Slope Function* – Increase or decrease as necessary to allow DAPA to pick up on smaller peaks (eg, increase the value if the integration is making a lot of noise, decrease it if smaller peaks are being missed).

When constructing an integration action table, you must complete two records. They enable the baseline to be drawn from the MQ+background baseline run at the start, through to the same baseline at the end of the run. All peaks and other baseline heights are measured from this baseline.

New Method Channel [G] Method {DAPA}

INTEGRATION ACTION EDIT MENU Table ID:P31

Run time options	
Record number	1
Action code letter	F2/24.4
Retention Time	2
New value	0

```
+----------------¦ New Method Channel [ G ]  Method {DAPA} ¦----------------+
        INTEGRATION ACTION EDIT MENU   Table ID:P31
¦-------------------------------------------------------------------------¦
          Run time options
          Record number       2
          Action code letter  F24.4/248
          Retention Time      24.4
          New value           0
¦-------------------------------------------------------------------------¦
```

The settings that need to be changed in the above tables are Action code letter and Retention Time. For the first record, these settings are:

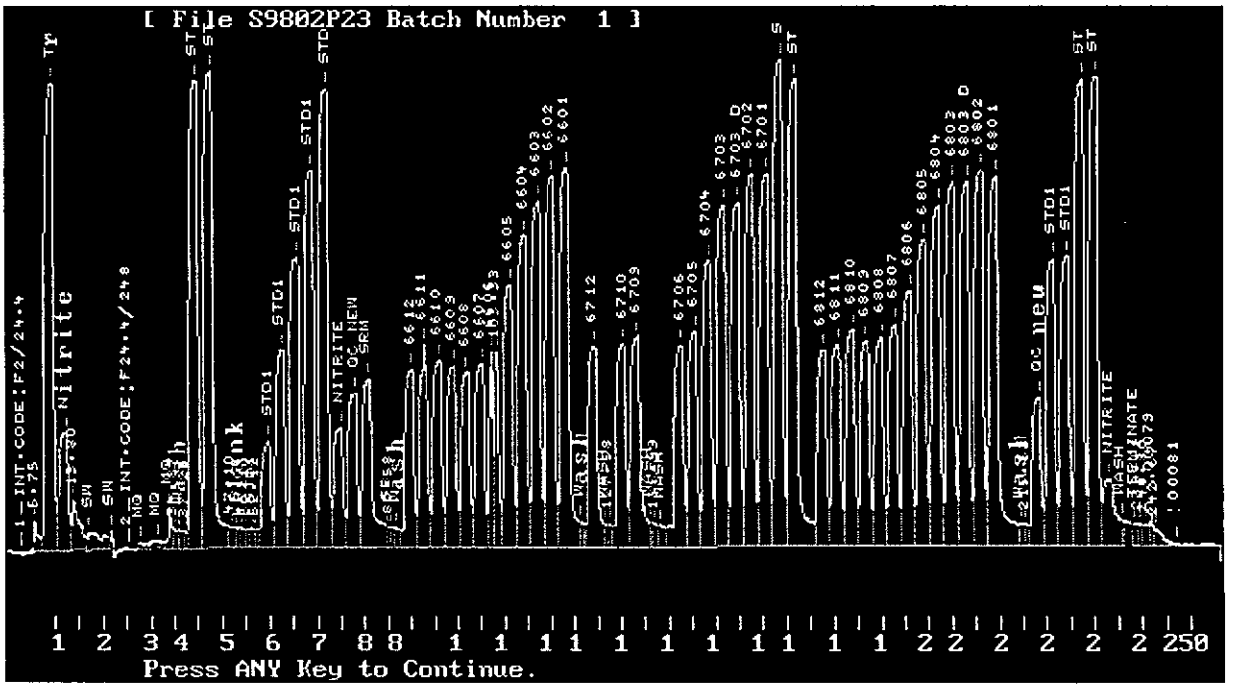
- *Action code letter* – F<start MQ+bgrd baseline time>/<middle MQ+bgrd baseline time>
- *Retention Time* – <start MQ+bgrd baseline time>

For the second record, these settings are:

- *Action code letter* – F<middle MQ+bgrd baseline time>/<end MQ+bgrd baseline time>
- *Retention Time* – <middle MQ+bgrd baseline time>

Figure 6.1 shows an example of a orthophosphate trace that demonstrates the baseline markings using the above integration action table settings.

Figure 6.1 Example of baseline marking in a DAPA file.



Peak naming table (*.nam)

The naming rules for the peak naming table are much the same as described in the DAPA cookbook. The calibrants should be labelled with a compound status of 'S1', and DAPA will name the calibrants automatically as STD1. The Excel spreadsheet used later in the processing requires the calibrants to be labelled as cal 0 to cal 5, and therefore the calibrants will need to be re-named after the data has been imported to Excel.

Other options that will require setting are the output options. A Lotus file (*.prn) is needed, and a printout of the *final* results is needed so that a hard copy of peak heights is available for later processing steps.

6.3.2 Processing nutrient data with DAPA

After setting up the integration action and peak naming tables for a run, select the required trace from the Integration menu and integrate.

Ensure that all peaks are marked correctly by checking the following:

- Calibrant peaks are marked.
- Baselines are marked at the flattest spot. One baseline point that is an accurate representation of the baseline is sufficient.
- All SRM blanks, baselines and zero calibrants should be labelled in the trace, and ensuring they are marked at this stage will save time later. If they are not marked on the trace, the printout will list a '*Mid-Point*' marker, which does not always give accurate results. The graphical integration facility of DAPA should be used to mark peaks that have been missed by the automatic integration. After you have marked missed peaks, select 'Reprocess Data' from the integration menu. (Altering the 'Slope Function' of the Integration menu can often help with marking of smaller peaks).
- The integration action table is set up correctly and has therefore drawn the baseline in the correct place.

After you are happy with the data, and all peaks are marked, use the method editor to change the 'Print Report' option in the Output menu to 'Y', and print the report. Check that all peaks are labelled correctly with their name. There should now be a hard copy of all the peak heights and a *.prn and *.int file for the run.

At this stage, CHECK THAT THE *.INT FILE IS THE CORRECT VERSION BY CHECKING THE TIME IT WAS CREATED. Dapa sometimes does not save the correct version of the *.int file, so all your peak marking could be wasted!!

DAPA's calibration facility is not of interest to us, as the Excel spreadsheet does all the calibration and gain corrections etc. Therefore, do not worry about settings such as forcing the calibration through zero. The only information extracted from the DAPA files are the names of the peaks and the peak heights.

6.3.3 Transferring data to Excel

1. Ensure that the *.prn and *.int files are labelled in the standard format (eg, S9802n02.prn) and are located in C:\Hydro\<Voyage name>\Nutrients. Have the hard copy of the results in front of you.
2. Open Excel 97.
3. Click the first toolbar button indicated in the 'Start' sheet of the template to begin to import the data from the WinFlow text files. A dialog box will appear requesting run details. Fill in the appropriate information and click 'OK'. The box will not close until you click 'Cancel', unless the 'No results' option has been selected. If the box closes and you need to import more data, simply click on the first toolbar button again.

4. Click the first toolbar button indicated in the 'Start' sheet of the template to begin to import the data from the DAPA files.
5. Select the appropriate settings from the dialog box according to the nutrient data you are transferring.
6. If the DAPA files cannot be found, a message will pop up asking you to check they are present, and the macro will end.
7. A dialog box will appear during the transfer asking for the trigger retention time, and if there are peaks missing labels, you will be asked to enter the peak height. Therefore, the fewer '*Mid-Point*' markers, the fewer peak height data to be entered.
8. Repeat step 5 for the remaining two nutrient files.
9. The macro will produce a file with a worksheet for each nutrient for which you have results. Each sheet will be labelled as either 'linear' or 'polynomial'.
10. Rename STD1 peaks as cal 0 to cal 5 for all three nutrients.
11. Once all the data are transferred, click on the second toolbar button indicated on the 'Start' sheet.
12. The file will be saved under the appropriate name in the directory C:\Hydro\<Voyage>\Nutrients.

If the carryover correction samples (wash, cal 5, cal 5) are not labelled correctly, a message box will appear letting you know that these values cannot be found. In this case, re-label the carryover samples so that they read 'wash, cal 5, cal 5', and click on the second button again.

If you have problems during the processing (eg, you decide that the data should be fit to a linear fit after you have selected a polynomial fit), you can begin again by either re-importing the data (first button) and/or reprocessing (second button) as necessary. This can only be done before creating the summary sheet (third button, see Section 5.9.4).

6.3.4 Transferring hand-measured data

In the case that the data requires measurement by hand, data can be entered directly into the spreadsheet in the appropriate worksheet (nitrate linear or polynomial, silicate linear or polynomial and phosphate linear or polynomial). Enter the peak names and heights into each sheet required and click on the second button to continue processing.

6.3.5 Excel processing, data quality and transfer to Hydro

For information and instructions on these topics, refer to Section 5.9.

6.4 References

Terhell, D., Rayner, M. (1991), *DAPA Cookbook* Version 1.0, 26 February. Unpublished.

Skoog, D. A., West, D. M., Holler, F. J. (1998), *Fundamentals of Analytical Chemistry*. Fifth Ed, Saunders College Publishing

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7 Acknowledgments

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Appendix A

Student's t-table.

Degrees of freedom	α (Confidence interval, %)			
	0.20 (80 %)	0.10 (90 %)	0.05 (95 %)	0.01 (99 %)
1	3.078	6.134	12.706	63.657
2	1.886	2.920	4.303	9.925
3	1.638	2.353	3.182	5.841
4	1.533	2.132	2.776	4.604
5	1.476	2.015	2.571	4.032
6	1.440	1.943	2.447	3.707
7	1.415	1.895	2.365	3.500
8	1.397	1.860	2.306	3.355
9	1.383	1.833	2.262	3.250
10	1.372	1.812	2.228	3.169
11	1.363	1.796	2.201	3.106
12	1.356	1.782	2.179	3.055
13	1.350	1.771	2.160	3.012
14	1.345	1.761	2.145	2.977
15	1.341	1.753	2.131	2.947
16	1.337	1.746	2.120	2.921
17	1.333	1.740	2.110	2.898
18	1.330	1.734	2.101	2.878
19	1.328	1.729	2.093	2.861
20	1.325	1.725	2.086	2.845
21	1.323	1.721	2.080	2.831
22	1.321	1.717	2.074	2.819
23	1.319	1.714	2.069	2.807
24	1.318	1.711	2.064	2.797
25	1.316	1.708	2.060	2.787
26	1.315	1.706	2.056	2.779
27	1.314	1.703	2.052	2.771
28	1.313	1.701	2.048	2.763
29	1.311	1.699	2.045	2.756
30	1.310	1.697	2.042	2.750
infinity	1.282	1.645	1.96	2.576

Appendix B

Example of a quality control log sheet for a nutrient analysis run.

Autoanalyser Check List

Run Number:	12
Cruise Number:	f9905
Date:	24/07/99

Nutrient Stocks

Nutrient	Stock Date	Made by
Silicate	21/05/99	Bec
Nitrate	21/05/99	Bec
Phosphate	21/05/99	Bec
Phosphate intermediate	17/07/99	MSR
Nitrite	21/05/99	Bec

Working Standards and SRM

Nutrient	Date prepared	Made by	Range	SRM code
Silicate	17/05/99	MSR	0-70	
Nitrate	17/05/99	MSR	0-35	
Phosphate	17/05/99	MSR	0-3	
Nitrite	17/07/99	MSR	35	
SRM N3	23/07/99	MSR	10.0, 30.0	
SRM P	23/07/99	MSR	1.0, 3.0	
SRM Si	23/07/99	MSR	10, 30	
SRM N2				

Reagents (fill in if prepared fresh this run)

Silicate

Reagent	Date prepared	Dry chemical used	Date	Weighed by
Ammonium molybdate	22/07/99	Ammonium molybdate	Feb-99	VL
Ascorbic acid	21/07/99	Ascorbic acid	Feb-99	VL
Ascorbic acid bgd	22/07/99			
Oxalic acid	22/07/99	Oxalic acid	Feb-99	VL

Nitrate

Reagent	Date prepared	Dry chemical used	Date	Weighed by
Imidazole buffer	21/07/99	Imidazole	1/02/99	VL
NEDD	18/07/99	NEDD	Feb-99	VL
NEDD background	17/07/99			
Sulphanilamide	22/07/99	Sulphanilamide	Feb-99	VL
Brij 'opened'	10/07/97			

Phosphate

Reagent	Date prepared	Dry chemical used	Date	Weighed by
Ammonium molybdate stock	19/07/99	Ammonium molybdate	1/02/99	VL
Antimony potassium tartrate stock	29/06/99	Antimony potassium tartrate	?	RP
Ascorbic acid stock	19/07/99	Ascorbic acid	1/02/99	VL
Reagent 1 stock	22/07/99			
Reagent 1 working solution	24/07/99			
Reagent 2	22/07/99			

Reagent 2 background	23/07/99		
SDS	29/06/99		

ASW

Reagent	Date prepared	Dry chemical used	Date	Weighed by
ASW	24/07/99	sodium chloride	Feb-99	VL

QC samples

Batch date: no date	11/07/99