

**CSIRO Marine Laboratories**

**REPORT 237**

**Shipboard Analysis of Seawater for Salinity,  
Dissolved Oxygen and Nutrients.**

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# Abstract

This report describes the instrumentation and techniques employed by the chemistry laboratories of the CSIRO Marine Laboratories in Hobart and Marmion, and on CSIRO Research Vessels *Franklin*, *Sprightly*, *Soela* and *Southern Surveyor*. The methods include analysis of dissolved oxygen (by manual titration), salinity (using a YeoKal salinometer) and nutrients (nitrate + nitrite, nitrite, orthophosphate and silicate using a Technicon AAI autoanalyser) in marine waters.

The dissolved oxygen method described was used on CSIRO research vessels until 1994, the salinity method until 1997 and the nutrient analysis method until 1997 on the RV *Franklin*. The nutrient analysis method (with some modifications) is currently in use at the CSIRO Marine Research laboratories and on the RV *Southern Surveyor*.



# 1 Introduction

This report describes the instrumentation and techniques used in the chemistry laboratories of the CSIRO Marine Laboratories in Hobart and Marmion, and on CSIRO research vessels *Franklin*, *Sprightly*, *Soela* and *Southern Surveyor*. The analysis of seawater for salinity and dissolved oxygen has been previously described (Major et al., 1972) as have dissolved reactive nutrients (Airey & Sandars, 1987), however there have been important changes to the methods and instrumentation that are documented in these publications. These two publications therefore provide the background to this report.

The aim of this report is to provide a reference for oceanographic data generated by the CSIRO Divisions of Fisheries and Oceanography from 1984 onwards. The bulk of this data has been produced using the YeoKal 601 Mark III salinometer (salinity), manual dissolved oxygen titration apparatus (dissolved oxygen) and the Technicon AutoAnalyser II system (dissolved reactive nutrients). This publication only describes data that has been produced using these instruments.

During the period from 1984 onwards there have been major changes to instrumentation in all but the Marmion laboratory, and Table 1 outlines a schedule of these events. This information provides a cross-reference to the CSIRO Marine Laboratories oceanographic database, which enables database users to identify the instrumentation used to produce the data of interest.

**Table 1 History of instrumentation upgrades at CSIRO Marine Laboratories and on CSIRO Research Vessels**

Date	Event
Pre 1984	Installation of Yeokal 601 salinometer, manual dissolved oxygen titration apparatus and manual phosphate analysis apparatus on <i>FRV Soela</i> and <i>ORV Sprightly</i> .
August 1984	Removal of manual phosphate analysis apparatus and installation of Technicon AAII on <i>FRV Soela</i> .
October 1984	Removal of Technicon AAII from <i>FRV Soela</i> and installation of Yeokal 601 salinometer, manual dissolved oxygen titration apparatus and Technicon AAII at the CSIRO Marine Laboratories in Hobart.
May 1985	Installation of Yeokal 601 salinometer, manual dissolved oxygen titration apparatus and Technicon AAII on <i>ORV Franklin</i> .
November 1986	Change to the autoanalyser procedure for determining nitrate/nitrite from the Grasshoff buffer method (Airey & Sandars, 1987) to the imidazole buffer method (this report). Coastal station samples analysed in Hobart continue to be analysed using the Grasshoff buffer method as they were preserved with large amounts of mercuric chloride which caused a precipitate with imidazole during analysis.
June 1990	Commissioning of computerised nutrient data processing software DAPA (Terhell and Rayner, 1991) on <i>ORV Franklin</i> .

Date	Event
September 1990	Installation of Yeokal 601 salinometer and manual dissolved oxygen titration apparatus on <i>FRV Southern Surveyor</i> .
October 1993	Installation of Technicon AAII and computerised nutrient data processing software DAPA on <i>FRV Southern Surveyor</i> .
October 1994	Installation of automated dissolved oxygen titration apparatus on <i>ORV Franklin</i> .
August 1996	Installation of Guildline Autosal salinometers at CSIRO Marine Laboratories in Hobart and on <i>ORV Franklin</i> .
February 1997	Installation of Alpkem nutrient analyser on <i>ORV Franklin</i> .
April 1997	Installation of Guildline Autosal salinometer on <i>FRV Southern Surveyor</i> .
July 1997	Change from Grasshoff buffer to imidazole buffer nitrate/nitrite method for coastal station samples analysed in Hobart after a reduction in the level of mercuric chloride preservative added to samples.
February 1998	Installation of automated dissolved oxygen titration apparatus on <i>FRV Southern Surveyor</i> .

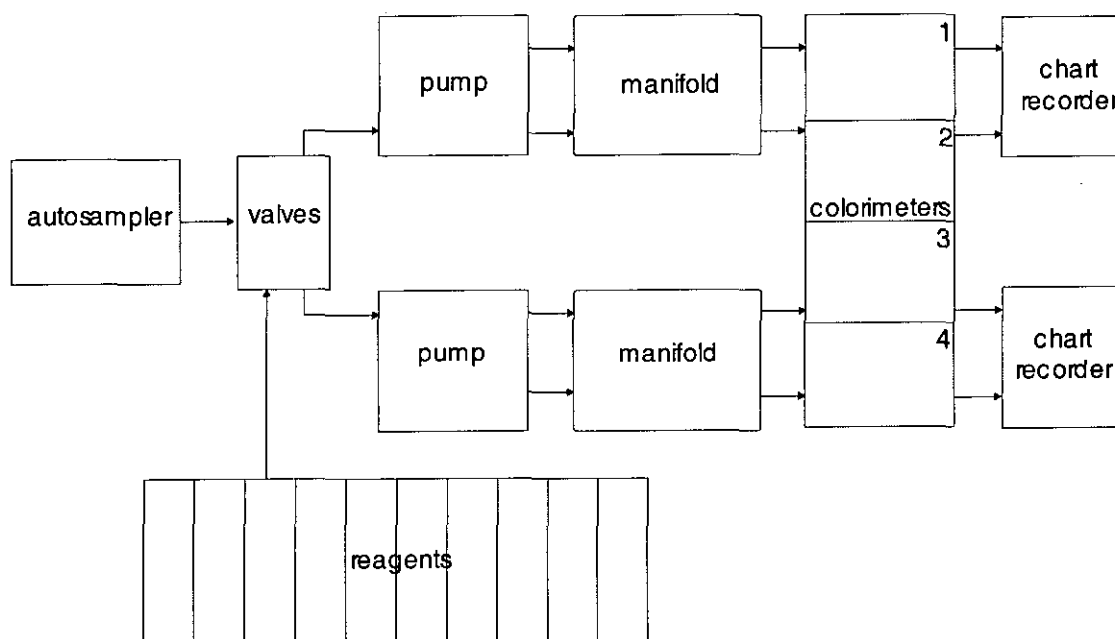
## 2 Automated nutrient analysis

### 2.1 The autoanalyser system

#### 2.1.1 The instrument

The autoanalysers used by CSIRO at sea on research vessels and in the laboratory are *constructed from commercially available Technicon AAII modules*. These modules are available in Australia from Bran and Luebbe (see Section 2.5), as are other consumable parts quoted in this manual which in many cases are also available from alternate suppliers. The system to be described consists of four channels for the determination of dissolved reactive silicate, nitrate + nitrite, phosphate and nitrite in seawater. Figure 1 shows a diagram of the four-channel system.

Figure 1 Block diagram of four-channel system

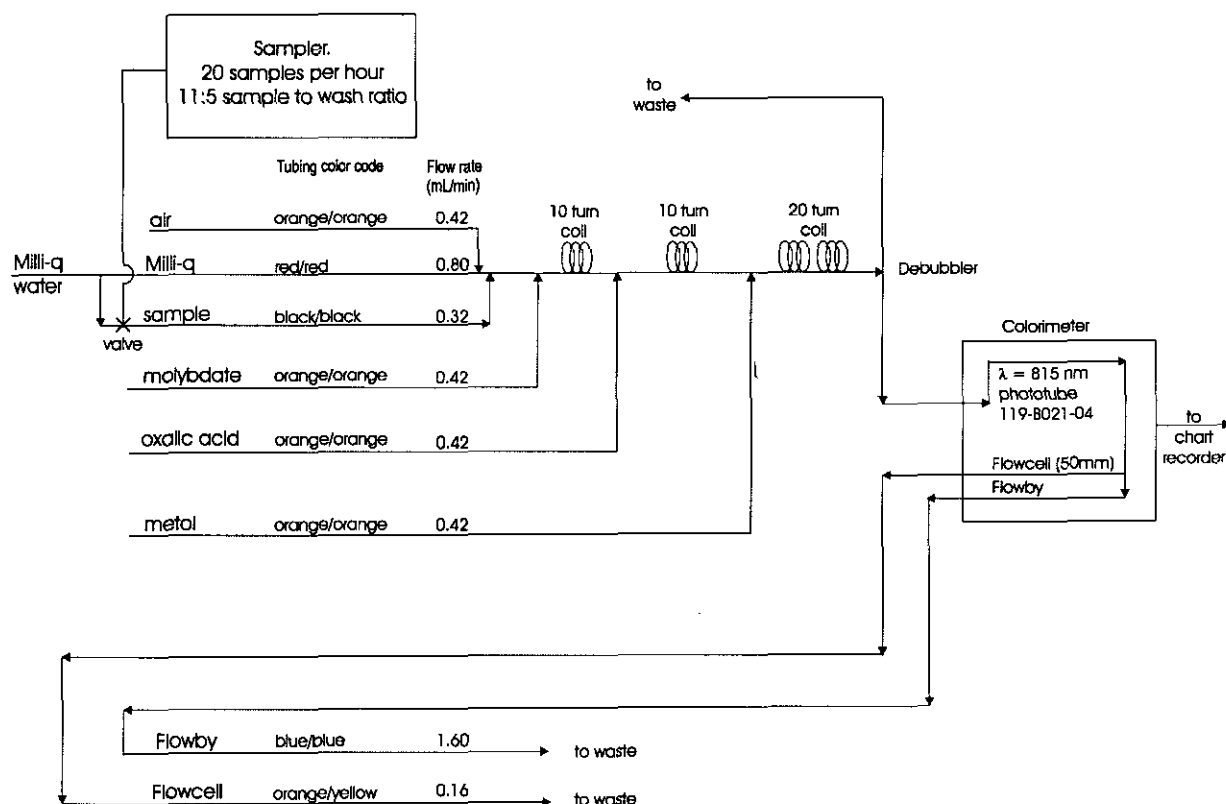


#### 2.1.2 The methods

##### Reactive silicate

Acid molybdate solution combines with reactive silicate at pH 2. At this pH, phosphate interference is overcome by the addition of oxalic acid which reacts with excess molybdate. The silico-molybdate complex is then reduced by metol (p-methylaminophenol) to form a blue heteropoly acid, the absorbance of which is measured at 815 nm. Figure 2 shows a diagram of the reactive silicate system.

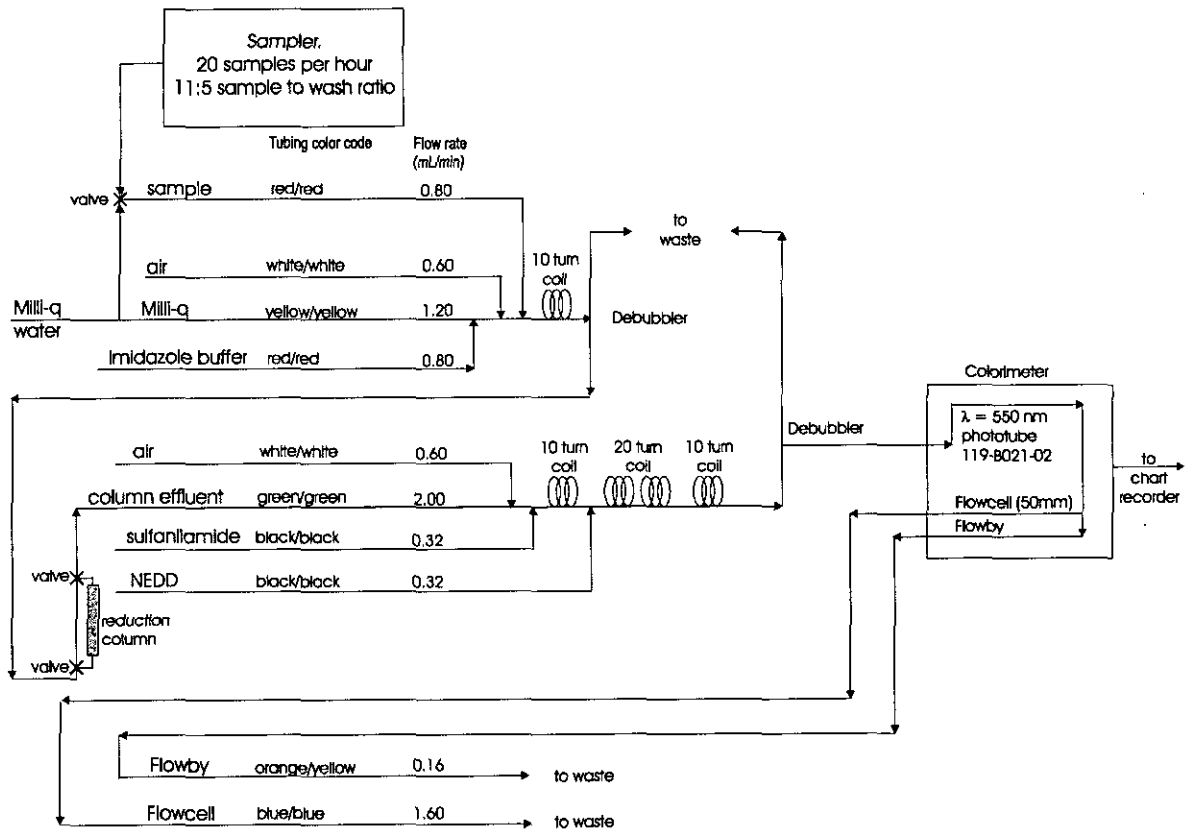
Figure 2 Manifold diagram of reactive silicate determination



## Nitrate nitrogen

Dissolved nitrate is reduced to nitrite via a cadmium reduction column. Nitrite reacts with sulphanilamide to form a diazonium ion, which couples with n-1 naphthylethylenediamine dihydrochloride to form a highly coloured azo dye. The absorbance of the dye is measured at 550 nm. The concentration measured by this determination represents nitrate plus nitrite; however nitrite concentrations in oceanic seawater samples are usually relatively low compared with nitrate. Nitrite levels are determined separately if necessary. In Figure 3, a diagram of the nitrate nitrogen system is shown.

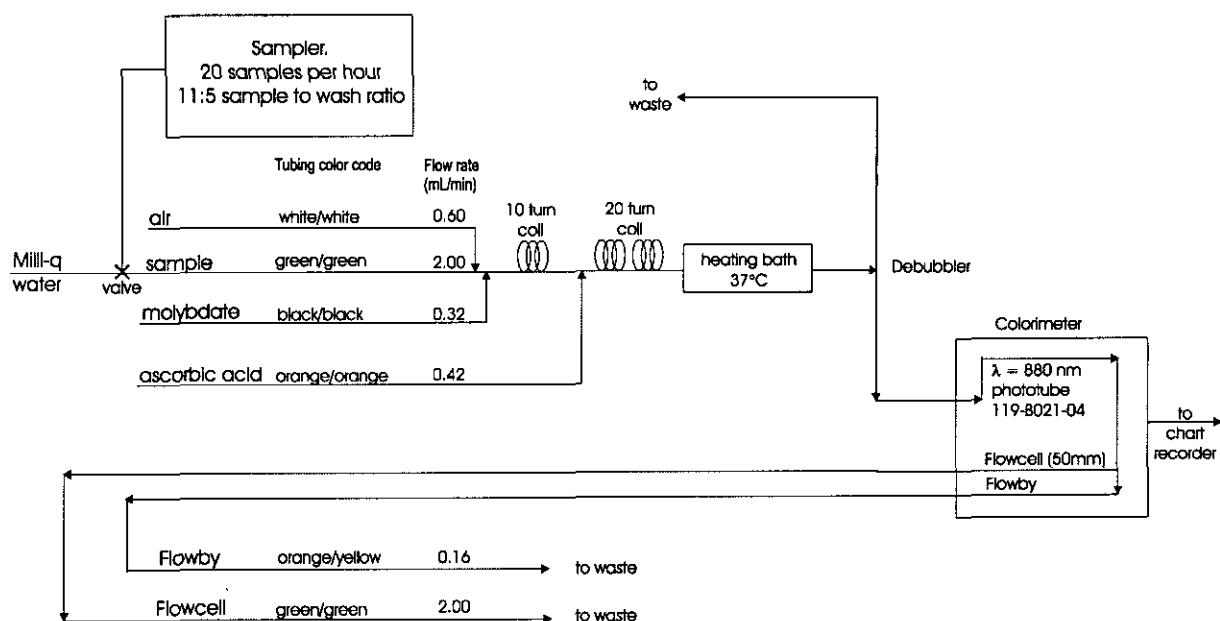
Figure 3 Manifold diagram of dissolved nitrate + nitrite determination



### Inorganic phosphate

Dissolved inorganic phosphate forms a phosphoantimonymolybdate complex with molybdate and antimony ions. The complex is reduced by ascorbic acid in a heated coil at 37 °C. The absorbance of the reduced complex is measured at 880 nm. Hydrazine sulphate is added to the ascorbic acid reagent as a preservative to inhibit the normal oxidation of the acid, being more easily oxidised than the ascorbic acid. The system is shown in Figure 4.

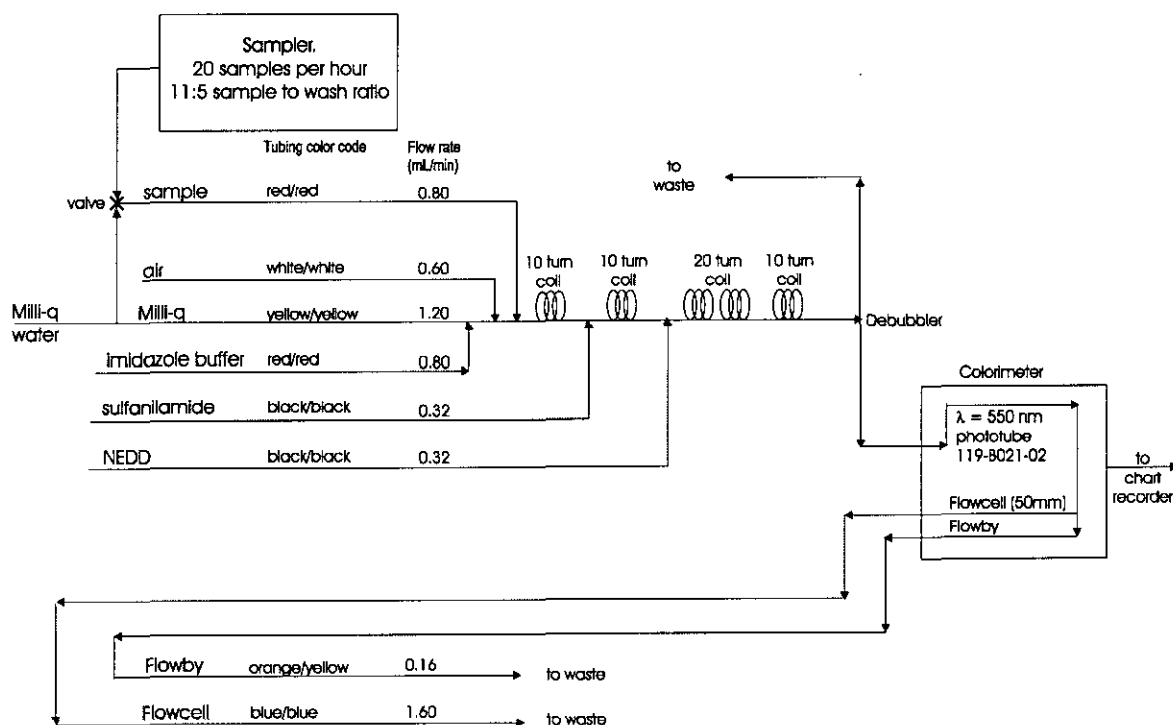
Figure 4 Manifold diagram of dissolved inorganic phosphate determination



## Nitrite nitrogen

The chemistry of this determination is the same as for dissolved nitrate (see above), without the cadmium reduction column. Figure 5 is a representation of the nitrite nitrogen system.

Figure 5 Manifold diagram of nitrite nitrogen determination



### 2.1.3 Special equipment

#### Water purification system

It is essential that a supply of high quality water is available for the autoanalyser, as water is the solvent for all reagent solutions as well as the reagent baseline from which all sample peaks are measured. The levels of nutrients and other contaminants in the purified water must be many times lower than the levels found in seawater which are sometimes at the detection limits of the autoanalyser. As an example, the detection limit for phosphate is less than 0.1  $\mu\text{M}$ .

A combination of water distillation and ion exchange has been found satisfactory. Tap water is firstly distilled into a polythene container using a Romer glass still (see Section 2.5), and distilled water is then pumped through a milli-q system (see Section 2.5) on demand. The milli-q system incorporates ion exchange cartridges to remove ionic impurities and carbon cartridges to remove organic impurities. The water is then filtered by the milli-q system to 0.45  $\mu\text{m}$ , and its conductivity is monitored to check cartridge function. All water collected should have a conductivity reading of around 18 megohms, and a slowly increasing conductivity reading indicates that new cartridges are necessary.

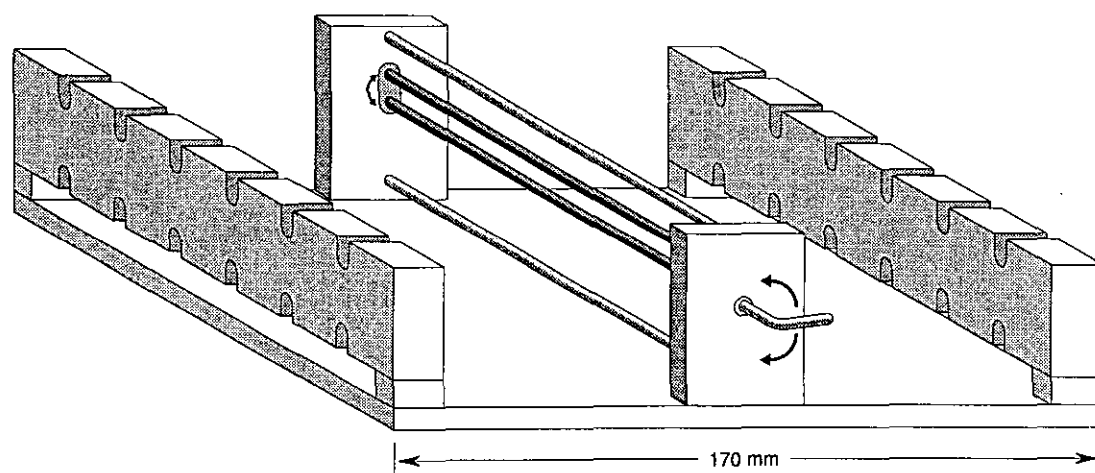
#### Reagent delivery system

Reagent containers used are Technicon 4 litre white polythene containers with polythene straws. White containers are used to hold all reagents and other solutions except the molybdate and metal reagents. These reagents are light sensitive, and are therefore stored in dark brown polythene containers.

In order to monitor reagent flows and to prevent air bubbles in reagent feeder lines, drip bags are installed on the reagent container straws. The liquid level in the drip bag is adjusted to be slightly above the internal filter, so that falling drops of reagent are observed during operation. Pinch valves (which are supplied with the drip bags) are installed below the drip bags, and are pinched up whenever instrument is not in use to prevent the reagent from siphoning through the system.

Reagents are fed from drip bags to the peristaltic pump via a simple valve (see Figure 6), which allows either reagent flow during operation or milli-q flow for flush out before instrument shut down.

Figure 6 Diagram of milli-q/reagent changeover valve



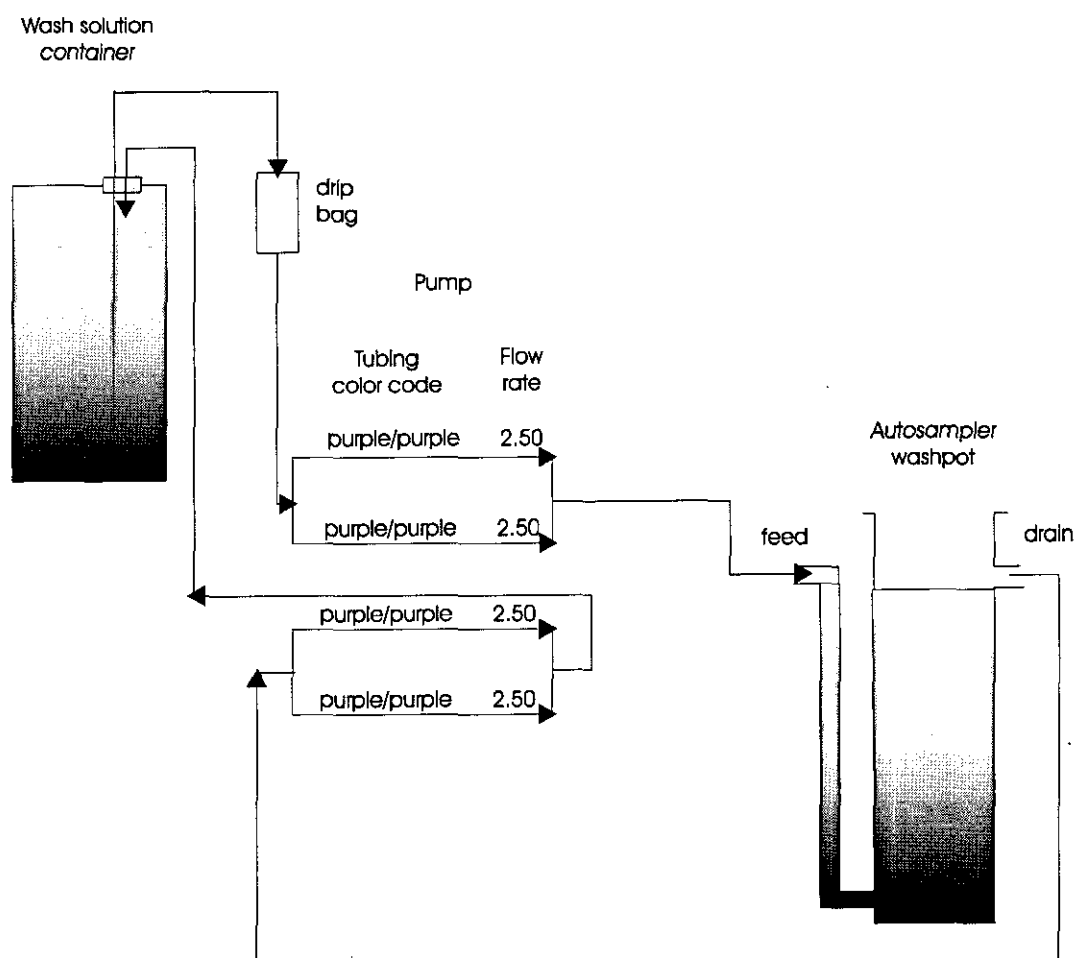
### Autosampling system

Some minor modifications of the Technicon Autosampler IV are necessary for the operation of this instrument at sea. Samples are collected in 15 mL plastic tubes (see Section 2.2.1), and the autosampler tray holes must be enlarged and a base installed to support these sample tubes. Stainless steel tubing (1/16 inch OD x 0.04 inch ID) is used as the autosampler probe. This diameter tube can deliver sufficient flow to supply all four channels via sample supply lines which are branched from the probe. The supply lines are led to each manifold via Omnifit 3-way valves, which allow either milli-q or solution from the autosampler probe to be pumped into each manifold.

Wash solution (see Section 2.2.4) is recirculated through the autosampler washpot and back into the wash solution reagent container (see Figure 7). Care must be taken to ensure that the rate of solution supplied to the washpot exceeds the demand by the autosampler probe, or the level of solution in the washpot will fall until the autosampler begins to draw air into the system. It is good practice to ensure that the rate of solution drawn from washpot overflow line is slightly higher than the supply rate to the washpot, so that all overflowed solution is returned to wash solution container.



Figure 7 Flow diagram of washpot recirculation system



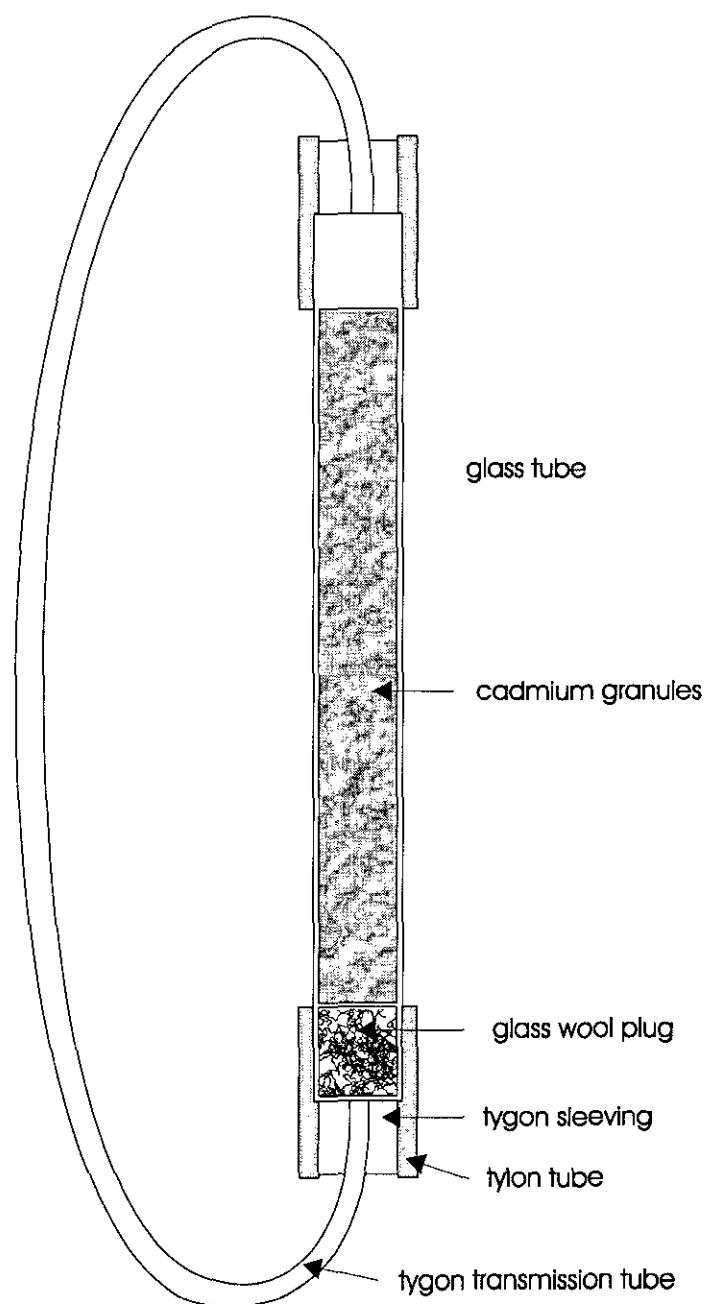
### Waste removal system

The waste system is designed to minimise back pressures in waste lines. Fluctuating back pressure leads to irregular flow, which is undesirable in an instrument dependant on reproducible conditions for satisfactory operation. Electrical conduit (19 mm PVC tube) has been found useful for constructing a waste removal system, and is available from most electrical retailers along with solvent glue and a range of bends and joints. The conduit can be drilled to fit standard Tygon tube, which can be fixed in place using cyclohexanone solvent (see Section 2.1.4).

The system is constructed to minimise the length of Tygon waste tubes from the peristaltic pump, as well as ensuring that all sections of conduit run downhill to the drain point. Waste solutions are acidic and release sulphur dioxide gas, so the drain point should be ventilated and well flushed to avoid gas and acid solutions building up. Figure 8 shows a schematic of the waste system.



2. Cut a 320 mm length of Tygon transmission tubing and two 10 mm lengths of Tygon sleeving (see Section 2.1.4). Coat the outer ends of the transmission tubing with cyclohexanone, and push the sleeving onto each end of the transmission tube so that the edges meet. Connect one end of the sleeved transmission tube to the end of the glass tube that contains the glass wool plug by coating the sleeving with cyclohexanone and sliding into the Tygon sleeving.
3. Fit a plastic funnel into the Tygon tube located on the free end of the glass tube. Clamp the entire assembly vertically with the funnel uppermost. Pour imidazole buffer solution (see Section 2.2.4) into the funnel, and remove all bubbles by gently tapping the glass tube.
4. Wash a small quantity of cleaned cadmium granules with imidazole buffer. Pour the granules into the plastic funnel, gently tapping the assembly to facilitate the passage of the cadmium into the glass tube. Continue tapping the assembly to settle the cadmium granules on the glass wool plug. Continue this process, until the cadmium granules are within 20 mm of the top of the glass tube. Maintain a continual supply of imidazole buffer in the funnel throughout, to keep air bubbles out of the column.
5. Raise the loose end of the transmission tubing to the same level as the top of the glass tubing. Remove the plastic funnel, wet the end of the transmission tube with cyclohexanone and insert it into the Tygon tube protruding from the top of the column. Do this carefully, so that no air bubbles enter the column or associated tubing.
6. Reduction columns can be prepared in batches of six, and are best stored vertically in a plastic bottle filled with imidazole buffer under refrigeration. This will ensure that no air can enter the columns, and they can be stored for many months under these conditions.
7. When columns are installed they should be mounted in a vertical position, and connected to Rheodyne 3-way valves or similar so that the column can be switched in and out of the manifold flow. This is an important feature which ensures that no foreign solutions (such as cleaning solutions) can enter the column. Care should also be taken not to introduce air bubbles into the column when cutting the transmission tubing for insertion into the autoanalyser system.

**Figure 9** Diagram of cadmium reduction column

### 2.1.4 Parts list with part numbers

#### Plastic tubing

- Tygon transmission tubing, 0.065", 116-0536-13
- Tygon sleeving, 1/4"OD 1/8"ID, 562-0005-01
- Tygon tubing, small, 0.025", 116-0536-06

#### Pump tubing

- Orange/yellow, 0.16 mL/min, 116-0549-05

- Black/black, 0.32 mL/min, 116-0549-07
- Orange/orange, 0.42 mL/min, 116-0549-08
- White/white, 0.60 mL/min, 116-0549-09
- Red/red, 0.80 mL/min, 116-0549-10
- Yellow/yellow, 1.20 mL/min, 116-0549-12
- Blue/blue, 1.60 mL/min, 116-0549-13
- Green/green, 2.0 mL/min, 116-0549-14
- Purple/black, 2.90 mL/min, 116-0549-16
- Purple/orange, 3.40 mL/min, 116-0549-17

### Connecting nipples

- Stainless steel, large, N16, 116-0061-04
- Stainless steel, small, N14, 116-0061-03
- Plastic, large-small, N5, 116-0002-01
- Plastic, medium-small, N6, 116-0004-01
- Plastic, small-small, N7, 116-0005-01

### Manifold glassware

- Debubbler, 116-0202-05
- T-piece, 116-0200-04
- Reagent injector, 116-B034-01
- Stream divider, PT10
- Glass tube, 8 cm, 116-0228-03
- Glass tube, 2.5 cm, 116-0228-01
- Glass tube, 15 cm, 116-0228-060
- Air/reagent injector, 116-0489-01
- 20 turn coil, 157-0248-01E
- 20 turn coil with reagent injector, 157-B089-01
- 5 turn coil, 170-0103-01
- Heating bath, 25-100 °C
- Heating bath thermometer, 35-60 °C, 157-0283-08QG

### Colourimeter parts

- Filter, 880 nm (phosphate)
- Filter, 820 nm (silicate)
- Filter, 550 nm (nitrate and nitrite)
- Phototube, 119-B021-04 (phosphate and silicate)
- Phototube, 119-B021-02 (nitrate and nitrite)
- Globe, 112-B007-01

- Flowcell, 5 cm, 199-B007-02

### Miscellaneous

- Cyclohexanone, 590-0026-01
- Scalpel and blades
- Pump endblocks, 26 tube, 133-0366-01
- Coil clamp, plastic, 509-0121-01
- Autosampler timing cam, nut and bolt, 127-B044-01
- Autosampler timing cam, plates, GG-127-1141-D
- Manifold tube holder, 170-0269-01
- Reagent straws, 157-B963-01
- Drip bags, 30.500

## 2.1.5 Hints for assembly

### Glass tubing

It is good practice to use as much glass tubing in the bubbled section of each manifold as possible. Plastic transmission tubing can be used to replace sections of glass tubing, however periodical replacement will be necessary. Plastic tubing tends to break up passing bubbles, causing disruption to smooth and regular segmented flow. Glass tubes are joined together using Tygon sleeving and cyclohexanone solvent. The ends of each glass tube should be square and smooth so they can be butted together neatly leaving no dead volumes. 10 mm lengths of sleeving are usually sufficient, and care should be taken when applying cyclohexanone so that no solvent enters the internal surfaces of the glass tubing.

### Plastic tubing

Many parts of the system can be connected with plastic tubing. Examples include milli-q supply to manifolds, milli-q/reagent changeover valves and milli-q/autosampler changeover valves. Transmission tube can be joined using solvent and Tygon sleeving, however solvent should not be used on plastic or metal connectors. Peristaltic pump tubes should be installed with connectors on each end to enable easy replacement, and all waste lines should be kept to minimum length before connection to the conduit waste system (see Section 2.1.3). Small Tygon tube (0.025") can be used for reagent delivery lines and other connections where low flow rate applications. Branching of autosampler probe line to milli-q/autosampler changeover valves on each manifold is done using the stream divider (part number PT10).

## 2.1.6 General hints

Any instrument used at sea must be well secured. Onboard CSIRO's research vessel *ORV Franklin*, the entire autoanalyser system is secured in shallow wooden boxes which are fastened onto a plywood base. Reagent containers are mounted on the wall above the level of the instrument, so that reagent flows are assisted by gravity and bubbles in reagent lines will rise into drip bags rather than enter the manifolds. In general, all lengths of tubing are kept to a minimum to reduce dead volumes and hence reduce flushing times. Electrical equipment such as colourimeter voltage stabilisers and the main

transformer are mounted under the autoanalyser bench for safety and to keep the system compact.

## 2.2 Pre-run preparations

### 2.2.1 Sample collection and storage

#### Hydrology cast

Hydrology samples (including nutrient samples) are normally collected from Niskin bottles (General Oceanics) deployed on a rosette sampler or hydrowire. A heavy weight should be used on the hydrowire to ensure the wire remains vertical during the cast and the samples are collected at the correct depth. Care should be taken to ensure the weight (or rosette) does not come into contact with the sea floor, as sediments can be disturbed resulting in sample contamination.

#### Taking the sample

Nutrient samples are collected in duplicate (from Niskin bottles) directly into labelled 15 mL polypropylene tubes with push-on caps (Disposable Products, number 21812), then stored upright in polystyrene racks (Kartell, number 1179). Tubes do not need to be rinsed, and are filled so that the tube does not make direct contact with the Niskin tap, which is usually wet with water from the sea surface and is handled frequently. It is very important that each tube is filled to only 80 % of total volume, so that no solution is lost during sample expansion on freezing. The tube is then capped and frozen in an upright position at -18 °C, unless the sample can be analysed within 2 hours, in which case refrigeration is recommended.

Samples can be stored frozen for up to 1 week, and after this time some loss of reactive silicate may occur. Oceanic seawater samples are not usually filtered, as their particulate loading is generally very low and the filtering process introduces a further risk of contamination. In coastal waters or during a plankton bloom, sample filtration may be advisable and Millipore 50 mL hand held syringes with 0.45 µm glass-fiber filters have proved suitable.

#### Sources of contamination

Meticulous care should be taken to ensure that at no stage during sample handling the internal surface of the sample tube or the sample come into contact with skin, sweat, saliva, or any other foreign matter. Rustkillers and other products found onboard research vessels at sea can pose serious contamination problems if allowed near sampling equipment or sampling areas. Smoking should not take place when sampling or analysing nutrient samples, and smokers should wash their hands before sampling operations begin. Beverages such as Coca-Cola™ contain high concentrations of phosphate and should also be kept well away from sampling areas.

### 2.2.2 Preparation of reagents

#### List of reagents

- Sulphuric acid, concentrated, AR.
- Hydrochloric acid, concentrated, 32 %, AR.
- Potassium antimony tartrate, AR.

- Sodium dodecyl sulphate (SDS), specially pure, BDH 44244.
- Ammonium molybdate.4H<sub>2</sub>O, AR.
- Oxalic acid, AR.
- p-methylaminophenol sulphate (METOL), LR (store under refrigeration).
- Sodium sulphite, anhydrous, AR.
- Imidazole, Sigma I-0125.
- n-1 naphthylethylenediamine dihydrochloride (NEDD), Sigma N-9125.
- Brij-35, 30 % solution, Technicon T21-0110-06 (store under refrigeration).
- Sulphanilamide, Sigma S-9251.
- Ascorbic acid, AR.
- Hydrazine sulphate, AR.
- Sodium chloride, AR.

### Dry reagents

Accurate weighing of reagents at sea onboard research vessels is not possible due to the continual motion of the vessel, therefore all dry reagents are pre-weighed into whirl-paks (see Section 2.5) before use at sea. Whirl-paks have been found non-contaminating and are sealed airtight after labelling. Pre-weighed reagents are stored in separate plastic bags in the dark, and Metol packets are stored under refrigeration.

### 2.2.3 The need for surfactants

Segmented continuous flow analysis depends on smooth, stable, and reproducible flow conditions for successful operation. Surfactants that are compatible with each chemistry are added to reagents to ensure smooth and regular bubble patterns, and to help each bubble maintain integrity whilst passing through the system. Without surfactant the flow becomes irregular and bubbles tend to break up on passage through plastic tubing. This causes uneven reagent concentrations in each individual “packet” of solution, and reduces the sweeping action of bubbles. The result is increased carry-over characteristics and reduced precision.

### 2.2.4 Reagent solutions

#### Prepare beforehand

- 5 N sulphuric acid: 140 mL conc. H<sub>2</sub>SO<sub>4</sub> + 860 mL milli-q.
- 50 % sulphuric acid: 500 mL conc. H<sub>2</sub>SO<sub>4</sub> + 500 mL milli-q.
- 0.3 % potassium antimony tartrate: Dissolve 3g of KSbTartrate in milli-q and dilute to 1000 mL (make fresh every day).
- Sodium dodecyl sulphate: Dissolve 10 g of SDS in milli-q and dilute to 200 mL (warm to dissolve).
- 10 % hydrochloric acid: Dilute 10 mL of conc. HCl to 100 mL with milli-q.

#### Wash solution

- Dissolve 78 g of sodium chloride in milli-q and dilute to 2000 mL.



**Silicate reagents**

- Ammonium molybdate: Dissolve 20 g ammonium molybdate in approximately 1600 mL of milli-q, add 80 mL of 5N H<sub>2</sub>SO<sub>4</sub>, 100 mL SDS and dilute to 2000 mL.
- Oxalic acid: Dissolve 14 g oxalic acid in approximately 1600 mL of milli-q, add 200 mL 50 % H<sub>2</sub>SO<sub>4</sub> and dilute to 2000 mL.
- Metol sulphite: Dissolve 24 g sodium sulphite and 20 g metol in approximately 1800 mL of milli-q. Dilute to 2000 mL.

**Nitrate and nitrite reagents**

- Imidazole buffer: Dissolve 17 g imidazole in approximately 3800 mL of milli-q, add 45 mL 10 % HCl and dilute to 4000 mL.
- NEDD: Dissolve 0.62 g NEDD in approximately 1800 mL of milli-q, add 2 mL Brij-35 and dilute to 2000 mL (refrigerate when not in use).
- Sulphanilamide: Dissolve 6.24 g sulphanilamide in approximately 1800 mL of milli-q, add 62 mL conc. HCl and dilute to 2000 mL.

**Phosphate reagents**

- Ammonium molybdate: Dissolve 12 g ammonium molybdate in approximately 500 mL of milli-q, add 1000 mL of 5 N H<sub>2</sub>SO<sub>4</sub>, 100 mL of 0.3 % KsbTartrate and 100 mL SDS. Dilute to 2000 mL with milli-q.
- Ascorbic acid: Dissolve 8 g ascorbic acid in approximately 1800 mL of milli-q, add 4 g hydrazine sulphate and dilute to 2000 mL (refrigerate when not in use).

Note: Reagents can be stored for up to 5 days under normal conditions.

**2.2.5 Preparation of standard solutions****Preparation of stock standards**

Stock nutrient solutions for use at sea are prepared at the Hobart Laboratories. All salts are dried at 105 °C for 2 hours and allowed to cool before weighing. The phosphate stock solution is stored in Pyrex glass and all others in polythene containers. Stock solutions are refrigerated in the dark at 4 °C when not in use, and are stable for about 12 months under these conditions. All details are recorded in a standards log book whenever fresh standards are prepared.

**Method:**

Use A-grade volumetric glassware and calibrated scales.

- Silicate: 2.6328 g sodium hexafluorosilicate (Na<sub>2</sub>SiF<sub>6</sub>) is diluted to 1000 mL in an A-grade volumetric flask with milli-q. This gives 14,000 μM SiO<sub>4</sub>.
- Nitrate: 1.4154 g potassium nitrate AR (KNO<sub>3</sub>) is diluted to 1000 mL in an A-grade volumetric flask with milli-q. This gives 14,000 μM NO<sub>3</sub>.
- Phosphate: 1.6330 g potassium dihydrogen phosphate AR (KH<sub>2</sub>PO<sub>4</sub>) is diluted to 1000 mL in an A-grade volumetric flask with milli-q. This gives 12,000 μM PO<sub>4</sub>.
- Nitrite: 1.3799 g sodium nitrite AR (NaNO<sub>2</sub>) is diluted to 1000 mL in an A-grade volumetric flask with milli-q. This gives 20,000 μM NO<sub>2</sub>.

### Preparation of intermediate standards

Intermediate standards are made from stock standards, and stored refrigerated in the dark in separate 15 mL sample tubes. These solutions should be discarded every week.

#### Method:

- Silicate and Nitrate: Use stock standards directly.
- Phosphate: Pipette 10 mL stock standard and dilute to 100 mL in an A-grade volumetric flask with milli-q. This gives 1200  $\mu\text{M}$   $\text{PO}_4$ .
- Nitrite: Pipette 1 mL stock standard and dilute to 100 mL in an A-grade volumetric flask with milli-q. This gives 200  $\mu\text{M}$   $\text{NO}_2$ .

### Preparation of working standards

Working standards are prepared using an Eppendorf Multipette 4780 (fitted with 2.5 mL combitips) and 100 mL A grade borosilicate volumetric flasks. Working standard solutions are then transferred into 15 mL sample tubes, which are filled to 80 % volume and frozen for up to 1 week. 100 mL of each standard solution will fill 7 sample tubes, which enables multiple instrument runs to be performed after each batch of standards are prepared.

Silicate, nitrate, and phosphate can be prepared as a mixed standard, however nitrite standards must be prepared separately, as nitrite will interfere with nitrate standards. Extra top standards (the highest concentration of each standard used) are prepared, so that the colourimeter gain settings can be adjusted, as well as to keep a check on instrument calibration during a run.

All standards are prepared in wash solution (see Section 2.2.4). Enough wash solution should be prepared beforehand to enable all standards to be diluted with the same batch of wash solution, otherwise the zero (or blank) standard value may vary from one standard to the next. All volumetric flasks should be rinsed with this wash solution prior to use. Solutions should not be left standing in glass volumetric flasks, but transferred to plastic sample tubes as soon as possible, otherwise silicate will gradually leach into solution from the glass flasks.

#### Method:

Take 0, 50, 100, 150, 200, 250  $\mu\text{L}$  of working stocks using the Eppendorf Multipette (settings 0-5 with 2.5 mL tip) into 100 mL volumetric flasks, and make to mark with wash solution. Use a separate 2.5 mL combitip for each nutrient, and add a little wash solution to each volumetric flask after the addition of each working stock to avoid working stocks mixing together directly (this may lead to precipitation of potassium salts). This will give:

- 0, 7, 14, 21, 28, 35  $\mu\text{M}$   $\text{SiO}_4$
- 0, 7, 14, 21, 28, 35  $\mu\text{M}$   $\text{NO}_3$
- 0, 0.6, 1.2, 1.8, 2.4, 3.0  $\mu\text{M}$   $\text{PO}_4$
- 0, 0.1, 0.2, 0.3, 0.4, 0.5  $\mu\text{M}$   $\text{NO}_2$

In summary, each batch of standards would include six 100 mL flasks of mixed  $\text{SiO}_4/\text{NO}_3/\text{PO}_4$  range of standards, five flasks of mixed  $\text{SiO}_4/\text{NO}_3/\text{PO}_4$  top standards, six flasks of  $\text{NO}_2$  range of standards and five flasks of  $\text{NO}_2$  top standards.

## 2.3 Operating procedures

### 2.3.1 Starting up

#### Initial adjustments

1. If the autoanalyser system has been shut down for some time, the tubing and connections should be tested. Fill all reagent containers with milli-q and turn on the electrical systems. Clamp down the pump plattens and switch on the pumps. The cadmium reduction column should be left switched out of the manifold flow, and the reagent/milli-q changeover valve placed into the reagent delivery position. Pumps, heating bath, chart recorders, and colourimeters should now be operating. All tubing connections can be inspected to ensure there are no leaks or restrictions to flow. The heating bath temperature may need adjusting, and the colourimeters may need to be optically peaked (see the Technicon manual for details [Technicon, 1972]) if these components have been changed since last use; however these adjustments are not usually necessary during routine operation.
2. After 30 minutes warm-up the gain on each colourimeter should be set to "1", and the sample aperture "A" screwed down fully (open). The colourimeters should now be set to "measure" mode. Adjust chart recorders (see Section 2.1.3) to give output 50 % of full scale when switched to "zero".
3. Next, switch the chart recorders to "measure" and adjust the colourimeter reference aperture "B" to give 50 % of full scale chart recorder output. Adjust each colourimeter so that the output as seen on the chart recorder does not change when the colourimeter is switched from the "zero" to the "normal" mode. The "baseline" adjustment is used for this purpose. The colourimeters are thus zeroed, and their output should not change significantly when their gains are adjusted to top standard absorbances at a later stage.

The above adjustments are performed with milli-q pumping through the system, while a steady output is obtained. A common problem when starting up is that any small air bubbles trapped in the colourimeter flowcell will cause noisy output. This can be rectified by pinching the tube which supplies solution to the flowcell for about 30 seconds, and then suddenly releasing the tube. The resultant surge in flow will overcome the debubbling capacity of the manifold, and will cause large bubbles to be drawn through the flowcell, removing any small bubbles trapped in the flowcell.

#### Cleaning procedure

The manifold flows in a new system (or a system that has been shut down) are often irregular, and surges in flow indicate there may be some restriction to flow or that the system needs cleaning. The following cleaning procedure will often help to achieve a smooth flow and regular bubble pattern:

1. Prepare two solutions, one of 5 % w/v sodium hydroxide and the other 5 % v/v hydrochloric acid in milli-q, and ensure the cadmium reduction column valves are switched so no flow passes through the reduction column.
2. Lift and swing the autosampler arm over and lower the sampler probe into the beaker containing 5 % NaOH.
3. Turn the autosampler/milli-q changeover valves to autosampler probe position.
4. Allow 5 % NaOH to be drawn up for 10 minutes, then 5 % HCl for 10 minutes.

5. Place the autosampler probe back into washpot and allow washpot solution to be drawn up for 10 minutes to flush all HCl from autosampler probe lines.
6. Turn autosampler/milli-q changeover valves back to milli-q, and run the system for a further 20 minutes to re-stabilise.

### Final adjustments

1. Switch off the pumps and drain all reagent containers. Pour reagents, wash solution, and fresh milli-q into their respective reagent containers, and switch the pumps back on. Disconnect the tube returning the wash solution from the washpot and run to waste for 30 minutes, so as not to mix milli-q already present in the system with wash solution. Reconnect the tube. Adjust the reagent levels in the drip bags to be just above the internal filter. Reagent flow can be observed as falling drops.
2. When all bubbles in reagent and milli-q delivery lines have passed out of the system, switch the cadmium reduction column valves (see Section 2.1.3) so flow passes through the column. Adjust colourimeter gains to the following:  $\text{SiO}_4=6$ ,  $\text{NO}_3=2$ ,  $\text{PO}_4=7$ ,  $\text{NO}_2=10$ . The system can now be left to stabilise for 1 hour.
3. Whilst the instrument is stabilising, remove one set of standards and around 40 samples from the freezer and allow them to thaw at room temperature. Thawing will take place at a greater rate if the tubes are placed into the autosampler rack, where air can circulate more freely than in the polystyrene racks. When the tubes have thawed out, it is essential that they are inverted at least 10 times to thoroughly mix the contents (which will separate into brine and fresh water layers on freezing).
4. After the instrument has stabilised, adjust the chart recorder pens to 10 % of full scale deflection using the "position" adjustment, and lower the pens onto the paper with the paper drive engaged. The recorders should now draw smooth and level baselines with the exception of nitrite which tends to give noisy baselines due to the high gain on the colourimeter. Nitrite baselines may be improved by the use of "damp" modes on the colourimeter. Excessive noise or drift in the baseline can indicate problems (see Section 2.3.4). The baselines obtained in this way are known as milli-q baselines, and represent the reagent blank of the system.
5. Switch the autosampler/milli-q changeover valves switch to the autosampler probe position, and allow the system to run until the wash solution baselines are established. The interface between the milli-q baseline and wash solution baseline will be accompanied by some noise, which is due to the change in refractive index of the solution passing through the colourimeter flowcell.
6. Place a top standard tube in the autosampler tray, and place the stop-plug in the hole immediately inside the tube in the autosampler tray. This will stop the autosampler continuing past the tube and drawing air into the system. Activate the start button on the autosampler and the autosampler will take a sample from the tube. As each nutrient's peak appears on the chart recorder, adjust the gain on that colourimeter so that the peak produced has a height of 90 % full scale deflection on the chart recorders.
7. Run another top standard to check the gain adjustments, and then bring the system down to milli-q baseline again using the autosampler/milli-q changeover valves.
8. Stop the chart recorder drives and lift the pens from the paper. The system should now be ready to run samples.

## 2.3.2 Operational protocols

### Cup definition form

Every batch of samples that are run on the autoanalyser must have an associated cup definition form to enable peak identification (see Figure 10). This form is designed to allow for manual calculation of results if a computerised system is not available, and is printed on plastic (waterproof) paper. Run numbers are sequential over an entire field trip or research cruise. The date of standards preparation is noted on the form as are the colourimeter gain settings.

### Loading the autosampler tray

Before an instrument run can begin, the autosampler tray(s) must be loaded with tubes according to the cup definition form for that run (see Figure 10).

1. Use a top standard for the first tube in the run. This serves as a marker for the automated data acquisition system and to equilibrate the instrument plumbing with nutrient solution at the beginning of the run.
2. A wash (a tube filled with wash solution) follows the top standard, which ensures the baselines are re-established before the standards are analysed.
3. Six standards (the standard range) are then run, beginning with the zero standard (standards blank) and ending with the highest concentration standard.
4. Another wash follows to ensure baselines are re-established before samples are run. If this wash solution is not included, then the first sample (which is generally from the ocean surface) may be hidden by the tailing of the top standard peak.
5. Next, place the samples in the autosampler tray. The protocol is to run the shallowest to the deepest sample in each station, and a station will typically consist of 10-12 samples.
6. Approximately every 12 samples, insert a top standard followed by a wash to keep a *check on calibration drift during the run*. These top standards are usually placed after the last sample in a station. Follow this regime until 30-40 samples are loaded, and the last tube on the tray is a top standard.



last the entire run, and establish a steady milli-q baseline. Changing reagents or colourimeter gain settings during a run should be avoided, as the baselines will need to be re-established and the system re-calibrated.

2. Establish wash solution baseline by switching over the autosampler/milli-q changeover valves.
3. Ensure that the stop plug is in the hole directly inside the last tube on the autosampler tray, and start the autosampler.
4. When the autosampler has sampled all tubes, switch it off and allow wash solution baselines to re-established.
5. Milli-q baselines should then be re-established, so that any baseline drift can be taken into account.
6. Lift the chart recorder pens disengage the chart drive. Remove the chart from the chart recorder and label it with run number, date, cruise, and which nutrient is represented by each coloured trace on the chart.

### Dilution of samples

The concentration range of the standards (see Section 2.2.5) will cover most oceanic samples with the exception of silicate. Silicate concentrations increase with depth and do not reach a maximum as do nitrate and phosphate. As the 0 to 35  $\mu\text{M}$   $\text{SiO}_4$  standard concentration range deviates from Beers Law, the standard concentrations should not be increased further, and sample dilution becomes necessary. Any silicate samples which are off scale can be diluted as follows:

1. Fill a precision 10 mL dispenser (Optifix or similar) with wash solution. The dispenser should be fitted to a plastic bottle.
2. Set the 10 mL dispenser to 9 mL and run a number volumes to waste in order to expell any silicate which may have leached from the glass dispenser bore into the solution on standing.
3. Take a 1 mL aliquot of sample (with an automatic pipette fitted with a plastic tip) and dispense into a 15 mL sample tube.
4. Dispense 9 mL of wash solution into the sample tube and mix.
5. Diluted samples are usually placed last in the sample tray. A dilution blank is obtained by dispensing 9 mL wash solution into a 15 mL sample tube, and is placed in the autosampler tray immediately before the diluted samples. This will allow any traces of silicate in the wash solution diluent to be taken into account.

If all four nutrient manifolds are being run simultaneously, there is a danger that the autosampler probe will draw up the entire volume available when analysing dilutions, because only 10 mL of solution is available in diluted samples. This can be avoided by stopping the autosampler before dilutions are run, switching the nitrite channel to milli-q, then running dilutions.

### Nitrite backgrounds

Nitrite concentrations in ocean waters tend to be very low, and the autoanalyser must be set to maximum sensitivity to detect these levels. At this sensitivity, any small background absorbance should be taken into account, because it will represent a significant portion of the signal measured by the colourimeter. Background signals are created by small differences in light transmitted through the flowcell because of the different refractive

index values of seawater and milli-q. To measure this background absorbance, the following procedure can be used:

1. Disconnect the NEDD reagent line from the nitrite channel where the reagent is connected upstream of the pump. Connect the pump tube to milli-q supply.
2. Run the pump for 30 minutes until all traces of NEDD have been expelled.
3. Start the chart recorders, and run a milli-q baseline, then a wash solution baseline.
4. Run a top standard to ensure the system is not responding to nitrite, and then run 6 or so samples which are representative of the oceanic areas that have been sampled. The duplicate samples are suitable for this.
5. Re-establish wash solution baseline and then milli-q baselines.
6. The background value obtained this way does not usually change significantly and need only be checked periodically. Coastal waters (or other areas of very high productivity) may yield higher and varying background values, and a background determination will need to be done on every sample using the above method. The measurement and calculation of background values is described in Section 2.4.1.

### Log book

An instrument log book should be kept and updated every day the instrument is in use. This will establish an instrument history, which can be used for periodic maintenance and trouble shooting. The log book should contain the following information:

- Scientific cruise number and date.
- Total number of hours operated that day.
- Instrument operators name.
- Date of standards and reagents preparation.
- Details of any maintenance performed.
- Details of any problems experienced and how they were rectified.

### Typical results

Typical output traces collected using DAPA are shown for each nutrient in Figure 11. Peak shapes vary from one channel to another due to different manifold arrangements, with the most obvious being nitrate+nitrite which has an almost symmetrical peak shape due to dispersion in the cadmium reduction column. Peak shapes that vary significantly from those shown usually indicate a problem (see Section 2.3.4).





### 2.3.3 Instrument shutdown

#### End of run

Autoanalyser systems have relatively long stabilisation times, therefore it is advisable to wait until a large number of samples have accumulated before running the system. However, it is important that frozen storage times should not exceed one week. When all outstanding samples have been analysed, the instrument is ready to be shut down.

1. Turn off all colourimeters, as their globes and phototubes have a finite life.
2. Switch the reagent/milli-q changeover valve to milli-q and run the system for a few hours to flush all reagents from the system. Discard all reagents and wash solutions and rinse the reagent containers with milli-q, leaving containers half filled with milli-q.
3. Turn the pumps off, and squeeze up all pinch valves to stop any solution siphoning through the instrument. Unclamp the pump plattens and remove from the pumps to prevent the pump tubes becoming deformed.
4. Although the instrument appears to be shut down at this point, the heating bath in the phosphate manifold is still operating, so turn off all power at the mains.
5. Update the instrument log book.

#### Maintenance

Servicing that is performed on a frequent basis is described below; however, the Technicon Manual should be consulted for major servicing of instrument components.

- Peristaltic pump tubes should be replaced every 100-200 hours depending on their performance. Worn pump tubes tend to produce unsteady flow, which gradually decreases until flow ceases or the tube begins to leak.
- Manifold tubing should be cleaned at least daily using sodium hydroxide and hydrochloric acid cleaning solutions (see Section 2.3.1).
- Reagent delivery lines tend to become discoloured and clogged with precipitate after a number of weeks. Molybdate and metal reagents are usually the cause, and the drip bag and delivery tubing should be replaced as required.
- Pump lubrication should be performed on a monthly basis, and before each scientific cruise using a lubrication kit supplied with the pumps. Lubricate the chain by applying a few drops of oil to the foam pads which run against the chain on each side of the pump. The pump rollers require lubrication with semi-fluid grease at each end of the rollers where they make contact with the spacing bar. If this is not done regularly the casing on the rollers will wear away and the spacing bar will grind into the rollers. This lubrication should leave minimal amounts of lubricant on the rollers, as excess lubricant will tend to smear over pump tubes during operation. A small amount of lubricant will be found on the pump tubes and platten after extended operation and this can be removed by wiping with a tissue soaked in alcohol.
- All plastic tubing should be inspected monthly, and any sections that exhibit undue discolouration, deterioration, or suspected blockage should be replaced.

### 2.3.4 Troubleshooting

The Technicon Manual (Technicon, 1972) includes a detailed section on troubleshooting; however the sections below describe some of the more common problems encountered. The problem observed is listed first, then possible causes and remedy.

#### Chemical problems

- **Baselines high or inverted silicate peaks:** Contaminated milli-q supply due to milli-q cartridge failure. Silicate is usually the first nutrient to pass through exhausted cartridges. Replace all cartridges in the milli-q system.
- **No peaks or only small peaks obtained:** Reagents made incorrectly, reagent feed line blocked, or reagents expired. Re-make the reagents and/or check reagent flow.
- **Spiking before and after peak:** Wash solution made incorrectly or sample salinity is low. Re-make the wash solution or check the sample salinity.
- **Noisy baseline:** Air or particulates in flowcell, or reagent or sample/wash solution supply to manifold partially blocked. Run a bubble through the flowcell, or check for blockages in the supply lines to manifolds. Note that an increase in background noise levels will be experienced at sea in bad weather, because of the continual movement of the vessel.
- **Large spikes on baseline:** Air entering the flowcell due to air in reagent or sample lines. Bleed out the reagent lines (reagent may have run out) and check sufficient volume of reagent and sample is available for autosampler requirements.
- **Poor quality standard curve:** Poor quality standards or an instrument problem. Run several samples from a mixed sample to check the instrument precision. If precision is good then re-make the standards. If precision is poor then check flow through the autosampler probe lines and reagent lines.
- **Changes to the standard curve, slope and/or a non-zero intercept is recorded:** Diluent (wash) solution not of the same batch for all standards. Make fresh wash solution and re-make the standards.
- **First phosphate standard low:** This problem has never been fully solved and appears from time to time. It is not due to poor quality standards. Washing the phosphate channel with sodium hydroxide solution seems to help. Fresh potassium antimony tartrate reagent has also been shown to help.
- **Drifting baseline:** Insufficient flushing time allowed for dead volumes to be purged.

#### Electrical problems

- **Drifting baseline:** Colourimeter phototube expired. Swap phototubes around, and if direction of drift reverses one tube is faulty. Install a new phototube of correct type (see Section 2.1.4).
- **Random sharp spikes on baseline:** Electrical problem in the colourimeter requiring repair by the agents.
- **Colourimeter light fails to operate:** Blown colourimeter globe. Replace the globe.
- **Loss of sensitivity after moving or servicing colourimeter:** Perform optical peaking procedure (see Technicon Manual [Technicon, 1972]).

## 2.4 Data interpretation

### 2.4.1 Manual method

#### Marking baselines

All baselines are drawn with a stainless steel meter rule using green, blue and red fine point pens. The colour of each trace on the chart is the colour chosen when drawing baselines for that nutrient. The milli-q baseline is drawn between the milli-q baselines established at the beginning and end of each run, which will allow for any instrument drift during the run. It is essential that the chart is placed on a flat surface and stretched tightly from end to end to ensure a straight line and not a curve is drawn. The line is only marked on the chart under the sample peaks and not under the standards.

Standards baselines are drawn at the same slope as the milli-q baseline and begin at the zero standard peak, which will usually be at a similar level to the wash solution baseline. This line is only drawn under the standards. Dilutions baselines are drawn from the dilution blank at the same slope as the milli-q baseline, and are marked on the chart only under peaks of diluted samples.

#### Measuring peak heights

Peak heights are measured with a 30 cm engine divided ruler for greatest accuracy. Standard peak heights are measured in millimeters from the standards baseline, and recorded in the "standards" section on the cup definition form. Samples are measured in millimeters from the milli-q baseline, and are recorded under the corresponding nutrient section on the cup definition form. Dilutions are measured from the dilutions baseline, and are recorded in the column of that nutrient in brackets (this is usually only done for silicate).

Peaks are normally measured from the highest point on each peak, unless a noise spike or other disturbance in the trace exists on a peak. In this case the peak height is estimated based on the shape of other peaks of the same nutrient. On some occasions, samples can contain less nutrient than the wash solution, in which case an inverted peak is obtained and the peak height is then measured from the lowest point on the peak.

#### Calibration curves

The peak heights obtained from the standards are used to draw a calibration curve of concentration versus peak height, and correlation coefficients of 0.99 and over are not unusual. The slope is calculated by averaging the slope at each point from intercept zero. Occasionally one point on the graph will be in obvious error, and can be ignored.

#### Calculation of concentration

Sample concentrations are obtained by simply dividing the sample peak height by the slope of the corresponding calibration curve, and are recorded on the cup definition form. Dilution concentrations are calculated by dividing the peak height obtained from the dilutions baseline by the calibration curve slope, and then multiplying the result by the dilution factor (usually 10).

#### Nitrite calculations

Section 2.3.2 describes the method used to run *nitrite backgrounds*. The *nitrite background* run has a milli-q baseline drawn on the chart (see Section 2.4.1). The background peaks obtained are usually inverted (ie, smaller than the wash solution baseline) and of similar size. Measure the background peaks from the lowest point on the

inverted peak to the milli-q baseline. As long as the peak heights do not differ by more than 1 mm, an average value can be used.

This value (usually about 10 mm), is subtracted from the sample peak height before dividing by the curve slope to obtain concentration (see Section 2.4.1). If the backgrounds vary significantly (as in estuarine waters), then each background must be run and measured so that every sample will have an individual background value rather than an averaged value.

## 2.4.2 Automated data interpretation

See *The DAPA Cookbook* (Terhell and Rayner, 1991) for details on how to analyse data using DAPA.

## 2.5 List of suppliers

- Drip bags: available from medical suppliers as Solution Administration Set number 30.500.
- Technicon equipment and consumables. Bran and Luebbe Pty Ltd, Private Bag No. 40, Post Office HOMEBUSH NSW 2140.
- Milli-Q water purification system. Millipore Waters Pty Ltd, Private Bag 18 LANE COVE NSW 2066.
- Romer glass water still: Romer Glass, PO Box 727, KOGARAH NSW 2217.
- YEW chart recorders: Imbros Pty Ltd, PO Box 427, MOONAH TAS 7009.
- Rheodyne valves: Alltech Associates Pty Ltd, 8/25 George St., HOMEBUSH NSW 2140.
- Omnifit Valves: Activon Scientific Products, PO Box 2153, NORTH BRIGHTON VIC 3186.
- Sample tubes: Disposable Products, 439 Nicholson St., CARLTON VIC 3054.
- Eppendorf automatic pipettes: Crown Scientific, PO Box 334, KINGSTON TAS 7050.
- Whirl-paks: Masur Sales, PO Box 105, POINT LONSDALE VIC 3225.

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## 3 Salinity analysis

### 3.1 Equipment requirements

- Inductively coupled salinometer, Yeo-Kal model 601 - mark III. Manufactured by Yeo-Kal Electronics Pty Ltd, Unit 7, No. 150, Old Pittwater Rd., BROOKVALE NSW 2100.
- Brown glass salinity bottles, 300 mL, with caps and rubber inserts, mould number 51525. Available from ACI International Ltd., PO Box 293, MOONAH TAS 7009.
- Teflon coated galvanized wire crates, stackable, to hold 24 bottles each. Available from R.E. Walters, 1 Jessie St., RICHMOND VIC 3121.
- International standard seawater vials. Available from I.A.P.S.O. Standard Seawater Service, Ocean Scientific International Ltd., Brook Rd., Wormley, SURREY GU85UB UK.
- Salinity sub-standard bottle, 10 L, glass, with magnetic stirrer.
- Liquid paraffin, heavy, BDH 29437.

### 3.2 Sampling

Samples are drawn from Niskin bottles deployed on a rosette or hydrowire. Dissolved oxygen samples, if required, should be taken first, followed by salinity samples. Salinity bottles usually contain some previous sample, which keeps the sample bottle equilibrated with seawater.

Inspect the rubber insert in the sample bottle cap to ensure it is correctly positioned and will provide an airtight seal. Any seawater in the sample bottle is discarded, and the bottle and cap rinsed three times with sample from the niskin bottle. Care should be taken to ensure the sample bottle is held away from the niskin tap at all times, so that no water from the external surface of the niskin bottle makes contact with the sample. After these three rinses, the sample bottle is filled to a headspace level of 25 mm, and the cap is tightly secured.

When a crate of 24 sample bottles has been filled, transfer the crate to the chemistry laboratory next to the salinometer. This allows the samples to equilibrate to the temperature at which salinity analysis will be done.

### 3.3 Analysis

#### 3.3.1 The sub-standard

After initial instrument calibration using international standard seawater, further calibrations must be made during the analysis using sub-standard seawater, so that any drift in instrument calibration can be determined. Sub-standard seawater is simply seawater that is specially stored to ensure no changes in salinity will occur. Surface seawater is collected in a clean 10 L glass bottle, and liquid paraffin added to form a layer 10 mm thick, which will prevent any evaporation occurring. A hard plastic tube is inserted before the addition of paraffin, and a magnetic stirrer is used to ensure the sub-standard is homogenous.

### 3.3.2 Initial preparations

1. All salinity samples, sub-standard, and international standard seawater vials must be stored together next to the salinometer for at least six hours to allow these solutions to equilibrate to the same temperature. Because of the expense of international standard seawater, and time taken to perform calibrations, it is usual practice to accumulate at least 48 samples (2 crates) before analysis is commenced. The salinometer is switched on and left to warm up during this period, with the aspirator and stirrer switched off.
2. The magnetic stirrer is operated for 10 minutes before analysis begins to thoroughly mix the sub-standard, then switched off because the stirrer will become warm and increase the temperature of the sub-standard if continually operated. It is important to operate the stirrer on a slow speed, to avoid the formation of a vortex which will draw globules of paraffin down to the bottom of the bottle. If this occurs, some paraffin will rise into the outlet tube and eventually be drawn into into salinometer cell, causing serious problems with cell drainage and bubble entrapment.
3. A salinometer log book is used to record date, salinometer number, date sub-standard filled, international standard batch, sub-standard temperature dial reading, sub-standard temperature compensation setting, standardise knob readings, sub-standard conductivity ratio and operators comments. This information is used later to check instrument performance during the life of a batch of sub-standard, as well as providing a record of international seawater standards used (there have been problems with some batches in the past).
4. A plastic tray is placed under the salinometer cell, and a tube connected to vacuum is installed so that any liquid drained from the cell, vacuum bowl, or cell filler tube will be contained and removed to waste.
5. All details other than standardisation information are recorded on the inductive salinometer log sheet, including station and sample bottle numbers (see Figure 12).

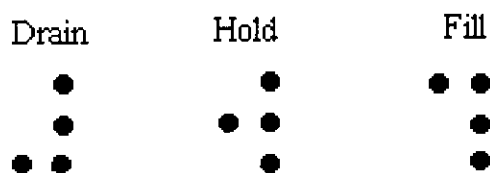
Figure 12 Inductive salinometer log sheet

C.S.I.R.O MARINE LABORATORIES											
INDUCTIVE SALINOMETER LOG SHEET											
INTERNATIONAL SEAWATER BATCH: _____ DATE: _____ COND: _____					CRUISE: _____ STATIONS: _____ DATE COLLECTED: _____ DATE MEASURED: _____ INSTRUMENT No: _____						
STANDARDISATION:					ANALYST: _____						
STANDARD	TEMP. DIAL READING	TEMP. COMP.	STD'ZE	COND. RATIO	NOMINAL SALINITY	$\Delta S$	CORRD SALINITY				
INTERNATIONAL											
INTERNATIONAL											
SUB.STD											
-- SUB.STD											
BOTTLE NO.	TEMP. DIAL	COND. RATIO	NOMINAL SALINITY	$\Delta S$	CORRD SAL.	BOTTLE NO.	TEMP. DIAL	COND. RATIO	NOMINAL SALINITY	$\Delta S$	CORRD SAL.
SUB.STD						SUB.STD					
SUB.STD						SUB.STD					
SUB.STD						SUB.STD					
SUB.STD						SUB.STD					



### 3.3.3 Instrument calibration with international standard seawater

1. Scratch the ends of a vial of international standard seawater with a file, and carefully snap off part of the glass tip on one end using a cloth or similar to protect from any sharp glass on the broken ends. Making sure the salinometer cell tap is in sample hold position (see Figure 13), insert the opened glass tip of the vial into the fill tube on the salinometer cell to make a watertight seal.
2. Holding the vial upright with the fill tube connected to the bottom of the vial, snap off the upper glass vial tip. Turn on the air aspirator pump to ensure calibration is performed under the same conditions as analysis.
3. Drain the salinometer cell contents to waste (see Figure 13), then raise the vial above the cell and turn the cell tap to the fill position. The cell will now fill with international standard seawater, and the rate of filling can be controlled by raising or lowering the vial relative to the salinometer cell. Any bubbles in the cell fill tube can be removed by turning cell tap to the hold position and gently tapping the fill tube to make bubbles rise back into vial. The flow of standard back into vial should be avoided by keeping vial at a higher elevation than the salinometer cell at all times. When the cell is filled and standard seawater flows into the overflow tube, the cell tap should be turned to the sample hold position.
4. Turned on the stirrer/detector switch. The stirrer should spin freely. Turn the temperature/salinity knob "temperature" and adjust the ammeter needle to read zero by rotating the temperature dial.
5. Note the reading of the temperature dial. The dial reading on some instruments needs to be divided by 2 to give the correct temperature. When the temperature has been determined, cross reference the temperature reading with the corresponding temperature compensation setting with the table on the front of the salinometer. Adjust the temperature compensation knobs to this setting. The instrument must be left on this temperature compensation reading until full calibration is performed again (usually when the next batch of samples is analysed).
6. Adjust the conductivity ratio knobs on the salinometer to read the ratio quoted on the international seawater standard vials.
7. Set the temperature/salinity knob to "salinity", and adjust the ammeter needle to read zero with the "standardise" knobs. Switch off the stirrer/detector.
8. Drain the cell to waste, and refill with fresh standard from the vial. Switch on the stirrer/detector again and readjust the "standardise" knobs to zero the ammeter. This process is continued until the reading is reproducible. A second vial of international standard should then analysed to ensure correct calibration, as some vials have been found to be in error.
9. Enter all details obtained so far into the salinometer log book and the salinity log sheet.

**Figure 13 Salinometer cell tap alignment positions**

### 3.3.4 Calibration of sub-standard

After calibration of the instrument using international standard seawater has been carried out, determination of the sub-standard salinity is performed.

1. Approximately 500 mL of stirred sub-standard (see Section 3.3.1) is run to waste, to flush any dead volume in the delivery tubing. Connect the sub-standard delivery tube to the cell fill tube, so that sub-standard runs directly into the salinometer cell.
2. Turn the cell tap to the fill position, and control the flow of sub-standard into the cell by pinching the sub-standard delivery tube. When the cell is full and sub-standard flows from the overflow tube, turn cell tap to hold position.
3. Determine sub-standard temperature as described in Section 3.3.1 and note this value. The temperature compensation should not be altered. Use the conductivity ratio knobs to zero the ammeter in "salinity" mode, and drain the cell to waste and refill until a reproducible ratio is obtained for the sub-standard.
4. Standardisation details are then completed in the salinometer log book and on the salinity log sheets.

### 3.3.5 Analysis of samples

1. Select the first sample, and gently invert twice to mix any condensate inside the bottle back into the sample. Turn the cell tap to the fill position, so that any solution in the cell will drain to waste through the cell fill tube.
2. Pour a small volume of sample over the external surface of the fill tube, to rinse any droplets of previously used standard or sample that are left on the cell fill tube. Seal the vacuum bowl holes with your fingers, and insert the fill tube into the sample by raising the sample bottle. The rate of fill can be adjusted using the valve on the aspirator tubing, so that the cell will fill slowly to minimise any chance of bubbles remaining in the cell.
3. When sample flows from the overflow tube, rotate the cell tap to the hold position and release the vacuum bowl. Measure the sample temperature and record it on the log sheet. This will only need to be done every 10 samples assuming samples are isothermal. If the temperature drifts into another temperature compensation range (as determined from table on the front of the salinometer), analysis must be stopped until the ambient temperature is stabilised and the instrument recalibrated.
4. With the salinometer in "salinity" mode, adjust the conductivity ratio to zero the ammeter, and switch off the stirrer/detector. Drain the cell contents to waste by rotating the cell tap to the waste position, and then back to the hold position. Apply the vacuum again by sealing the vacuum bowl, and rotate the cell tap to the fill

position. Refill and drain the cell until a reproducible conductivity ratio is recorded. There should be sufficient sample to obtain at least 4 rinses.

5. After a reproducible reading has been recorded, drain the cell to waste through the fill tube in preparation for the next sample. Leave some sample in the salinity bottle to make sure the bottle remains equilibrated with seawater.
6. The salinometer log sheet allows up to 10 samples to be analysed before another sub-standard determination must be carried out. An instrument drift of up to 0.00004 ratio units between sub-standard determinations is normal; however a larger drift may indicate a problem and recalibration with international standard seawater may be necessary.
7. Analysis is completed by a sub-standard determination, even if less than 10 samples have been analysed since the previous sub-standard.

### 3.3.6 Maintenance and storage

At the completion of analysis, rinse and fill the cell with a dilute solution of mild detergent in milli-q, to prevent corrosion of the stirrer bearing, and to keep the cell clean. A small amount of light lubricant (such as CRC) can be applied to the stirrer shaft bearing to keep the stirrer spinning freely. If the cell tap becomes difficult to operate, remove the tap and lubricate with a small amount of good quality grease such as Apiezon.

Yeo-Kal model 601 salinometers need to be calibrated by the manufacturer whenever a new temperature thermistor is fitted. This usually only happens if a thermistor is accidentally broken.

## 3.4 Calculation of salinity

Results are entered into a VAX mainframe computer which calculates salinities from conductivity ratios, and applies any correction due to drift between sub-standard determinations by linear interpolation.

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  $32.4356 \times 10^{-3}$ , at the same temperature and pressure. 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:

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

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

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).

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## 4 Dissolved oxygen analysis

### 4.1 Reagents and apparatus

#### 4.1.1 Apparatus

- Thumbwheel Burette, 0-10 mL, glass barrel with 2-way valve, Metrohm.
- Fluorescent light source.
- Magnetic stirrer and 1 3/4" ringed spinbar.
- Vacuum dispenser, Pyrex, 100 mL. Available from Romer Glass, PO Box 727, KOGARAH NSW 2217.
- Wheaton DO Bottles with glass stoppers and rubber leashes, Cole Parmer cat. number R-3660-00.
- Boxes to hold 24 DO bottles, plywood with lid.
- DO beaker, tall, 300 mL, Kimax 14030.
- Sampling tube, assembled from Tygon tube: 250 mm (OD 8 mm/ID 5 mm) (cat. 1014), 10 mm (OD 10 mm/ID 7 mm) (cat. 1020), 20 mm (OD 12.5 mm/ID 9 mm) (cat. 1024).
- Dispensers for DO reagents, glass, adjustable 0 to 2 mL, with 500 mL brown glass bottles, OPTIFIX (require 3).

#### 4.1.2 List of reagents

- Sulphuric acid, concentrated, AR.
- Vitex, indicator for iodine, AJAX Chemicals 620.
- Sodium hydroxide pellets, AR.
- Potassium iodide, AR.
- Manganese sulphate tetrahydrate, AR.
- Sodium thiosulphate pentahydrate, AR.
- Potassium iodate, AR.

#### 4.1.3 Reagent solutions

- 0.1 N potassium iodate standard: Dry 4 g potassium iodate at 105 °C for 2 hours. After cooling weigh 3.5666 g and make up to 100 mL with milli-q in an A grade borosilicate volumetric flask. Record all details in a log book and store the standard solution in a brown glass bottle at 4 °C.
- 40 % manganese sulphate: Dissolve 400 g of manganese sulphate.4H<sub>2</sub>O to 1000 mL with milli-q. Store in a plastic bottle at 4 °C.

- Alkaline potassium iodide: Dissolve 300 g of potassium iodide in 300 mL milli-q and separately dissolve 360 g of sodium hydroxide in 400 mL milli-q. After cooling the hydroxide solution, mix both solutions and make to 1000 mL with milli-q. Store in an airtight plastic bottle away from light at room temperature.
- 0.1 N sodium thiosulphate: Dissolve 49.6 g of sodium thiosulphate.5H<sub>2</sub>O in milli-q and make to 2000 mL. Store in an airtight plastic bottle at 4 °C.
- 0.01 N sodium thiosulphate: Dilute 200 mL of 0.1 N sodium thiosulphate solution to 2000 mL with milli-q. Shake well before each use.
- Starch solution: Dissolve 1 heaped teaspoon of Vitex in 250 mL milli-q in a 250 mL plastic wash bottle. This solution must be freshly prepared before each use.

## 4.2 Sampling

### 4.2.1 Reagent preparation

Adjust three automatic dispensers to deliver 0.5, 1.0, and 2.0 mL and fill them with 40 % manganese sulphate, alkaline potassium iodide, and concentrated sulphuric acid respectively. Purge the dispensers to waste to remove all air bubbles and ensure delivery of the correct reagent volume.

### 4.2.2 Sample collection

Samples are collected from Niskin bottles deployed on a hydrowire or rosette sampler. Dissolved oxygen samples are always collected first to minimise the possibility of contamination from atmospheric oxygen. Sampling commences with the deepest sample which generally will contain relatively low oxygen concentrations.

1. Connect the sampling tube to the Niskin tap, and open the tap by pushing the spigot towards the Niskin bottle. Any flow of seawater from the tap indicates that the Niskin end-caps have not sealed properly, and a note should be made on the CTD log sheets. This information can be used at a later stage when checking the data set for irregular results. After observing the sample tube for any leaking seawater, open the Niskin air-bleed to allow water to flow freely through the sample tube. After a few seconds when all the air bubbles have been expelled from the sample tube, pinch the tube shut near the niskin tap. This should leave the sample tube filled with seawater, but not flowing.
2. Carefully insert the end of the sample tube into the neck of a sample bottle, and raise the bottle until the tube makes contact with the bottom of the bottle. Allow the seawater to flow slowly at first into the bottle (by partially releasing the pinched sample tube) and then free flow once half of the bottle has been filled. When the bottle is full, run another half bottle volume to waste to expel all sample that has made contact with the atmosphere. Withdraw the tube slowly while the sample is still flowing. Stop the sample flow by pinching the sample tube, and remove it from the bottle while the sample is slowly flowing. Minimise any agitation of the seawater surface inside the bottle, and avoid any air bubbles entering the sample.
3. The sample bottle should now be full to the brim, and is ready for reagent addition.

### 4.2.3 Reagent addition

1. Immediately after sampling, add 0.5 mL of 40 % manganese sulphate followed by 1.0 mL of alkaline potassium iodide solution to the sample. Make sure the dispenser tip is below the sample surface when delivering the reagents, to avoid any air bubbles being pushed into the sample.
2. Carefully lower the sample bottle cap into place to avoid trapping air bubbles. Any overflowed solution will contain a small amount of reagent, so rinse the bottle under a flowing tap. Vigorously shake it 20 times (keeping pressure on the glass stopper) to suspend the manganese hydroxide precipitate throughout the sample. Place the sample into a box away from light.
3. When the precipitate has settled to at least half the bottle volume, add 2.0 mL of concentrated sulphuric acid in the same manner as the other reagents, followed by another rinsing of the bottle once the cap has been replaced. Shake the bottle again to dissolve the precipitate, and the colour will change to the brown of liberated iodine. The sample is now ready to be titrated, and is again stored in a box away from light.

## 4.3 Analysis

### 4.3.1 Standardisation of 0.01 N thiosulphate solution

1. Remove the 0.1 N potassium iodate standard from refrigeration and allow it to come to room temperature.
2. Fill the titrant bottle on the Metrohm burette with 0.01 N sodium thiosulphate solution and dispense 10 burette volumes to waste to purge any old solution from the burette. Switch on the fluorescent light source and place a piece of clean white paper over the magnetic stirrer to aid in endpoint detection.
3. Dispense exactly 1.00 mL of 0.1 N potassium iodate standard into a 300 mL DO beaker using an Eppendorf Multipette (fitted with a 12.5 mL tip), and add 100 mL of milli-q.
4. With the spinbar in the beaker, add 1.0 mL of alkaline potassium iodide and mix in by swirling. Add 2.0 mL of concentrated sulphuric acid and place the beaker on the magnetic stirrer, adjusting it to give a rapid stirring of the solution. Add 0.01 N sodium thiosulphate titrant rapidly at first until a pale straw colour is reached. Add a squirt of starch from the wash bottle. A bright blue colour will form. The remaining iodine in the solution is titrated until the last trace of blue just disappears. Note that the blue colour will often slowly reappear with further stirring but this does not indicate an incorrect titration.
5. With the burette refilled, repeat the titration until values agree to within 0.03 mL. Record all standardisation information on the oxygen log sheet (see Figure 14).





### 4.3.2 Titration of samples

1. Record all sample bottle numbers to be titrated on the oxygen log sheets (see Figure 14) along with their corresponding station numbers.
2. Apply a vacuum to the 100 mL dispenser and select the first sample. Shake the sample to ensure homogeneity, and pour an aliquot into the dispenser until excess is aspirated away to waste.
3. Drain the 100 mL aliquot into the titration beaker allowing at least 5 seconds of extra time for solution to drain from the dispenser walls.
4. Perform the titration as per Section 4.3.1 (proceeding directly to the addition of titrant). A duplicate titration should be carried out approximately every 12 samples to check the titration precision. The titration beaker should be well drained between titrations. Rinsing is not necessary unless the previous titration has been overshoot.
5. On completion of titrations rinse the 100 mL dispenser and leave it filled with milli-q. The stirrer and fluorescent light should be turned off.

### 4.3.3 Blank titration

Analytical grade reagents available today are normally of excellent purity and contain insignificant concentrations of interfering substances. A blank titration may be performed to check for interferences if reagent purity is in doubt.

1. To 100 mL of milli-q, add 2 mL of sulphuric acid, followed by 1 mL of alkaline potassium iodide. Add 0.5 mL of 40 % manganese sulphate and allow the solution to stand for 10 minutes.
2. Add starch indicator and titrate the solution with 0.01 N sodium thiosulphate. A titration of 1 drop or less indicates insignificant concentrations of oxidising iodate, hypoiodate in alkaline potassium iodide reagent, ferric salts in manganese sulphate reagent, or nitrogen oxides in the sulphuric acid.
3. If no free iodine shows when starch is added then dilute 1 mL of 0.1 N potassium iodate standard to 10 mL with milli-q and add one drop or more to test for reducing sulphur dioxide in the sulphuric acid. The blue starch-iodine colour should show immediately after the first drop of iodate solution.

## 4.4 Calculation of results

### 4.4.1 Results as milliliters dissolved oxygen per liter

The following is taken from Major et al. (1972).

$$\text{Dissolved oxygen} = A/S * 5.64 \text{ mL/L}$$

where

A = mL of 0.01 N sodium thiosulphate required to titrate 100 mL of sample.

S = mL of 0.01 N sodium thiosulphate required to titrate 1.00 mL of 0.1 N potassium iodate standard.

#### 4.4.2 Results as micromolar dissolved oxygen

Dissolved oxygen = mL/L (calculated above) \* 44.673 uM.

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## 5 References

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