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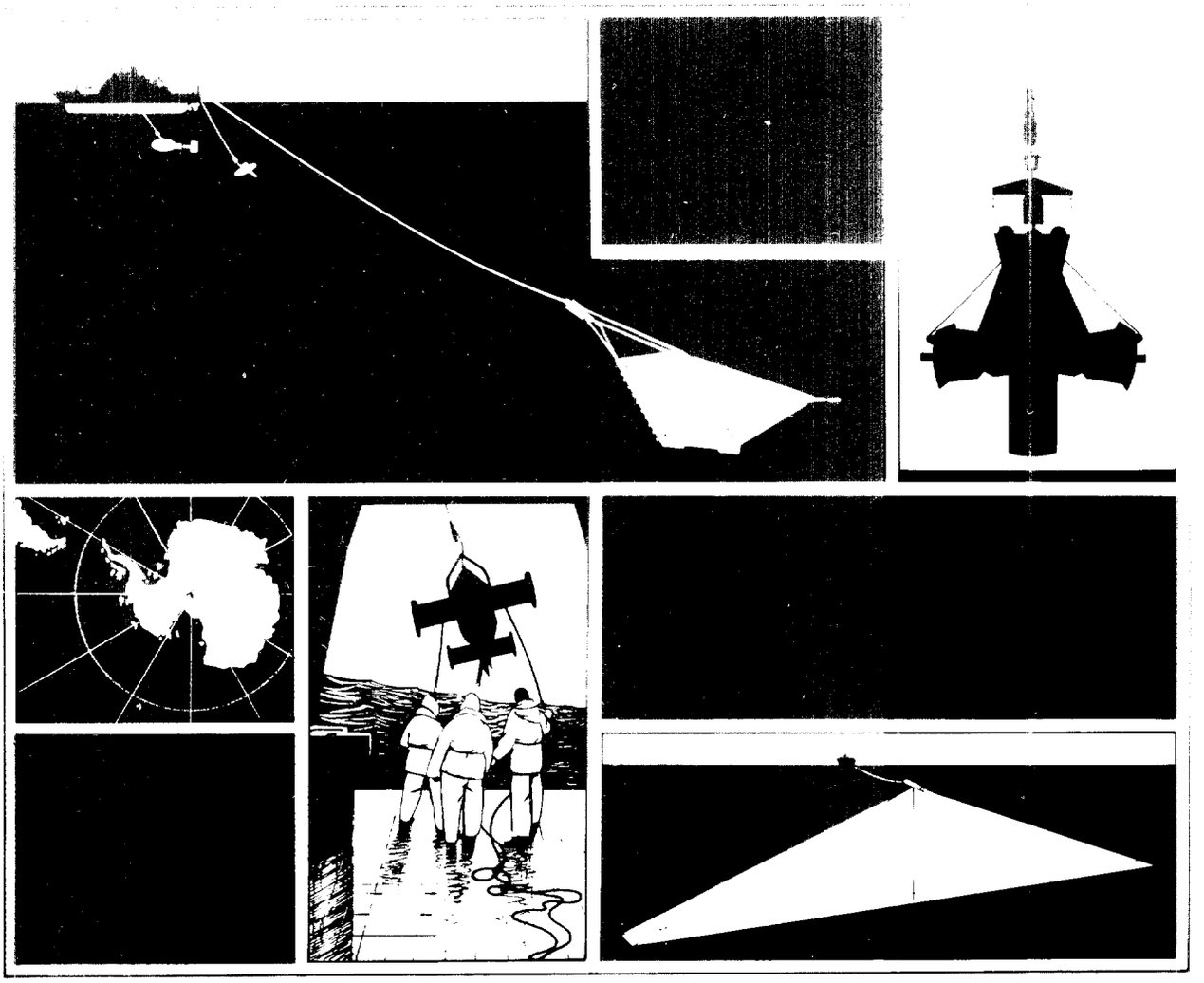
D Smythe-Wright, R Paylor & S E Holley

Report No 302 1992



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The measurement of silicate, nitrate and phosphate in seawater

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ABSTRACT

Staff of the James Rennell Centre for Ocean Circulation (part of the Institute of Oceanographic Sciences, Deacon Laboratory), have recently become involved in measuring nutrient species for chemical tracer studies. Such use of nutrients requires high quality data sets. With this in mind analytical techniques for the measurement of silicate, nitrate and phosphate have been developed using an RFA 300 autoanalyser system manufactured by the Alpkem Corporation. The analyses are based on the formation of coloured complexes; the intensity of the colour being directly proportional to the concentration of the nutrient in the seawater sample. The colour is measured at specific wavelengths using photometric techniques. Details of the design and full analytical methods are given.

KEYWORDS

ANALYTICAL TECHNIQUES
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NUTRIENTS
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1. INTRODUCTION

Cooper (1952) first suggested the use of silicate distribution to interpret the nature and movement of major oceanic water masses. The subject has since been widened to include the nutrients nitrate/nitrite and phosphate. Such species offer additional orthogonal information to the classical use of temperature, salinity and oxygen.

In general nutrient concentrations are affected both by biological activity and by ocean circulation and mixing. The former results in non conservative behaviour and so their use as chemical tracers must be with caution and requires sound background knowledge of the processes which control their distribution. In the deep ocean their distribution does not follow salinity but is controlled through the uptake by marine organisms in surface waters and ultimate replenishment at depth when the organisms die. When used in conjunction with conservative tracers, for example the time dependent CFCs or helium/tritium, and provided biological controls are taken into account, the nutrients provide powerful independent and complimentary information to resolve different aspects of ocean circulation.

Nitrate and phosphate are required by all marine organisms whereas silica is only needed by the siliceous radiolarians and diatoms. While there is generally a depletion of silicate where nitrate and phosphate are low, the process of resolution of siliceous structures does not parallel decomposition. Consequently it is not surprising to find that nitrate and phosphate co-vary while the general distribution of silicate in the sea differs somewhat from the other two with the Si : N and Si : P ratio variable. Since all nutrients are replenished to some extent when oxygen is utilised for degradation they show a distinct inverse relationship with oxygen at mid depths.

To circumvent the biological control of nutrients the oceanographer interested in water mass identity and movement routinely avoids the upper two hundred meters of the water column. This can induce errors because at depth, concentrations depend on the rain down of material from the surface. A more useful approach is to use the quasi conservative parameters PO and NO (Broecker 1974, 1991, Wilson and Wallace, 1990).

Notwithstanding the above there are distinct distributions of nutrients throughout the world's ocean which are directly related to water mass characteristics. It is not the scope of this document to go into these in detail, however, some broad indications are given below. These are included so that the analyst can have some feel for the levels of concentration to be expected.

Throughout all oceans the three nutrients are low in the upper few hundred meters with higher values at depth.

In the Atlantic Ocean (see Figure 1) the source of nutrients is from Antarctic Waters with concentrations in the south generally a factor of two higher than the north. Antarctic Intermediate Water and Antarctic Bottom Water are both characterised by high nutrient levels which decrease with latitude as the water masses move northwards. The concentration of silicate is particularly high in Antarctic Bottom Water and provides a good indicator of its spread throughout the Atlantic and Indian Oceans. Sandwiched between these two water masses is North Atlantic Deep Water with its low nutrient levels.

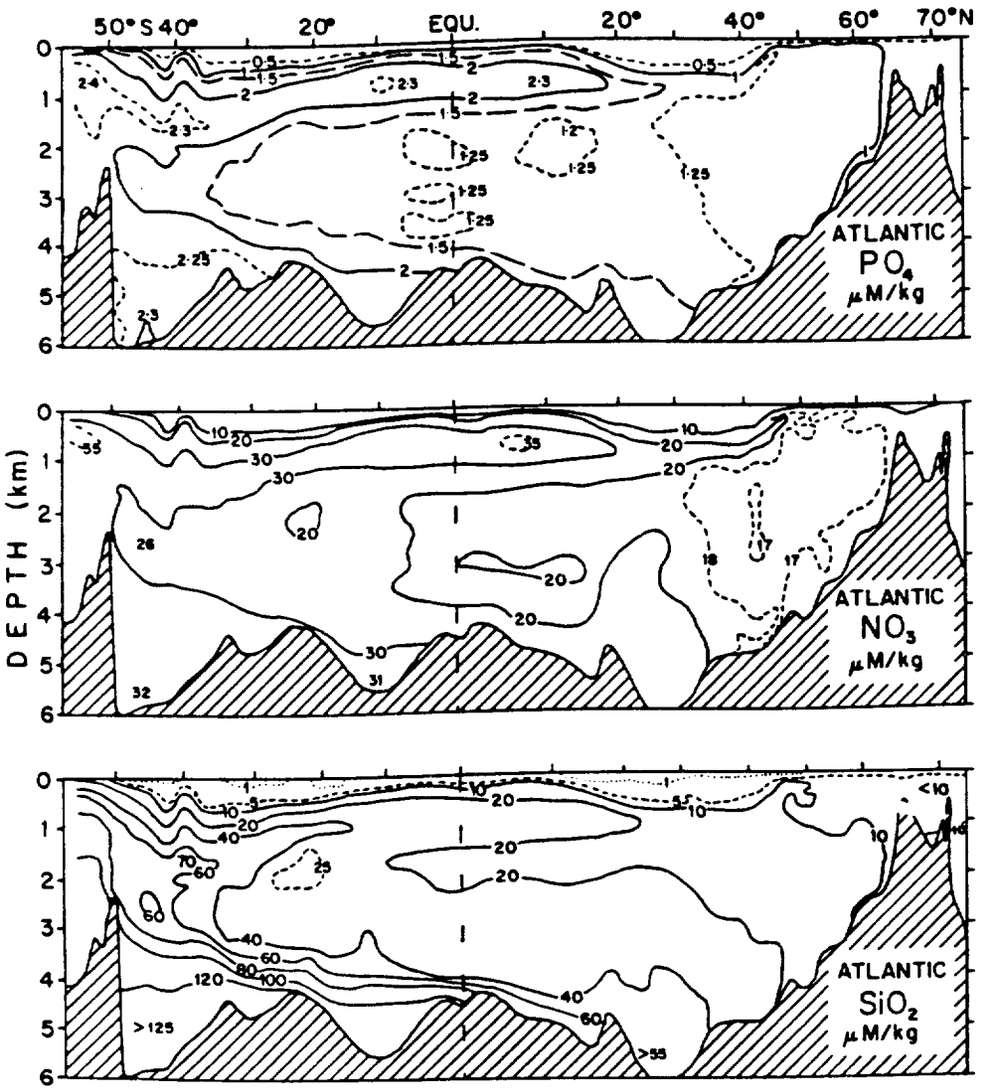

Figure 1 South-north vertical section of nutrients in the Western Atlantic Ocean (data from GEOSECS Atlas, Bainbridge, 1976).

In general the concentrations of nutrients in the Pacific are somewhat higher than in the Atlantic and in contrast to the Atlantic the highest values of nutrients are in the north (see Figure 2). In addition there are often enhanced surface values of nitrate and silicate in the equatorial Pacific due to upwelling at and north of the equator. This is associated with the divergence of the South Equatorial current at the equator and with the divergence of the North Equatorial Counter Current and the North Equatorial Current at about 10°N.

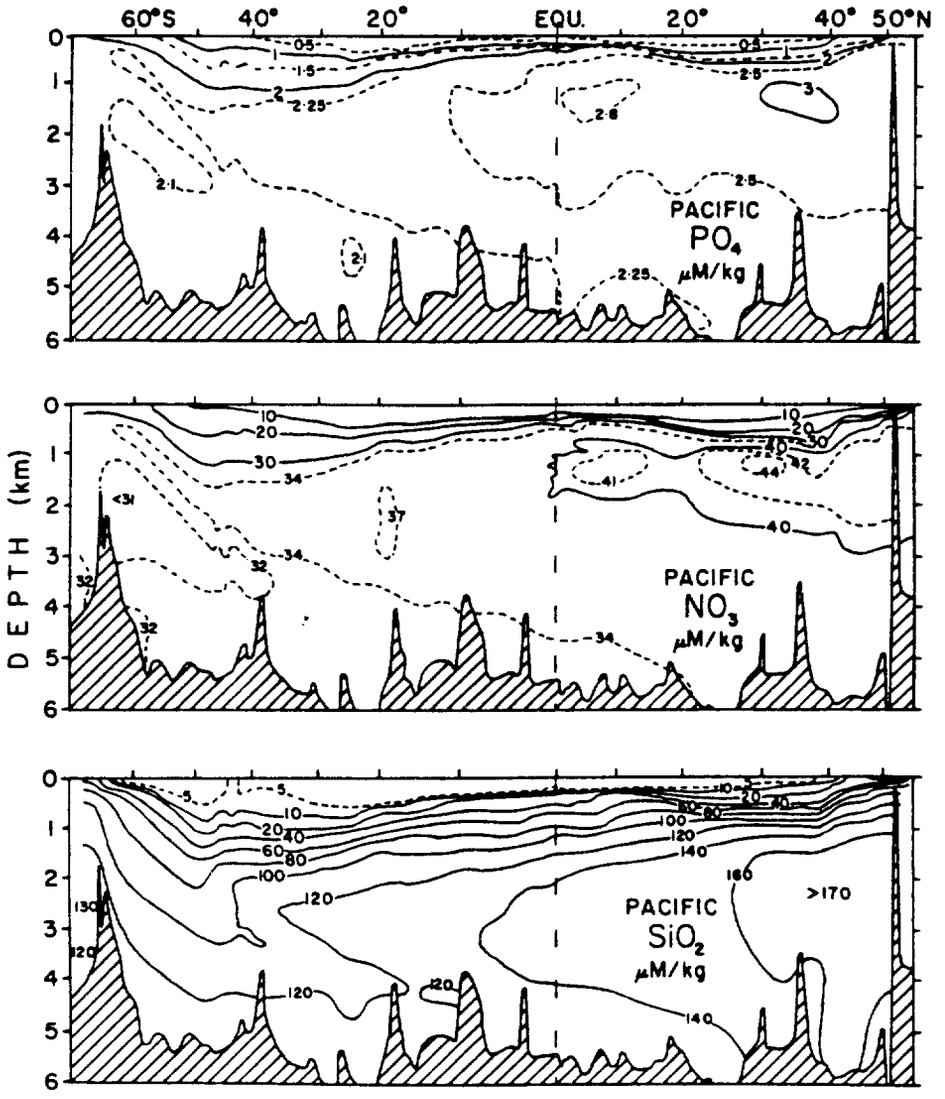

Figure 2 South-north vertical section of nutrients along the 180° meridian in the Pacific Ocean (data from GEOSECS Atlas, Craig et al., 1981).

Nutrient concentrations in the Indian Ocean fall somewhere between those in the Atlantic and the Pacific. There appears to be a source of phosphate (see Figure 3) from northern latitudes which extends downwards from 1000 m in the North to 2000 m at 25°S. All nutrient concentrations south of 5°S in the Indian Ocean show the influence of Antarctic Waters and in general there is an overall picture of concentration increasing with depth.

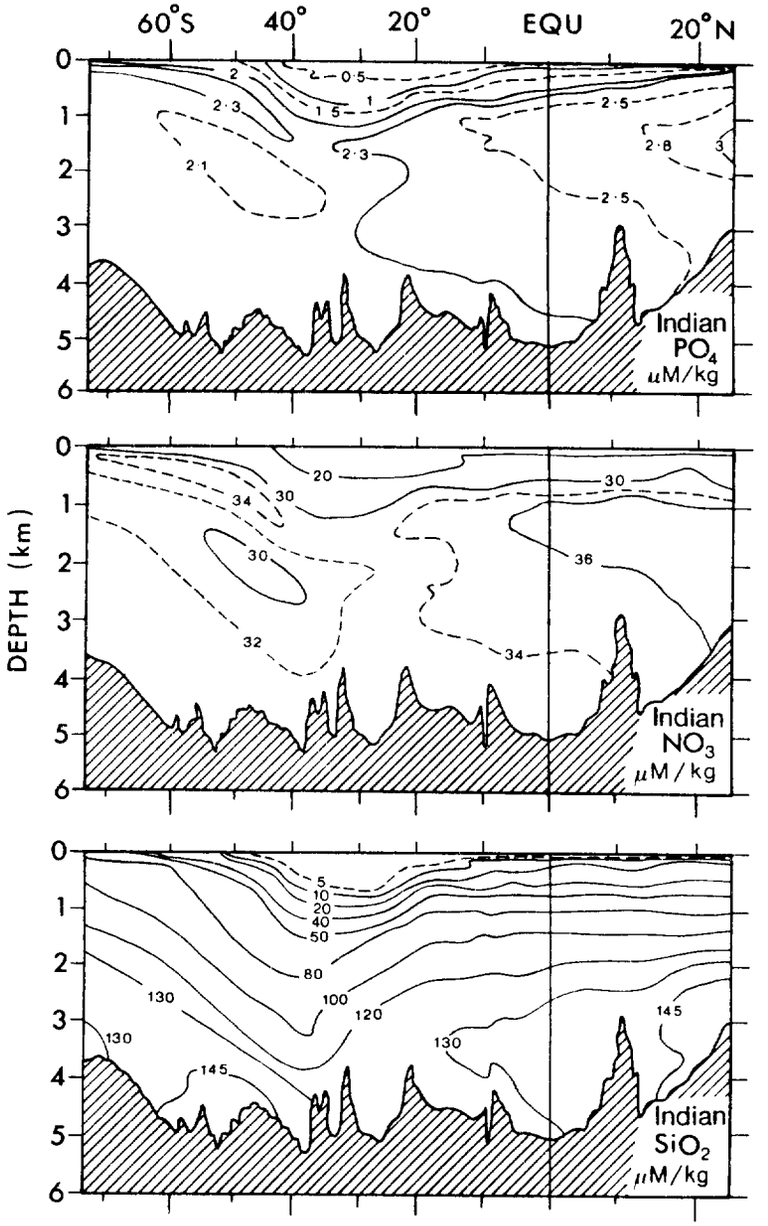

Figure 3 South-north vertical section of nutrients in the western Indian Ocean (data from GEOSECS Atlas, Spencer et al., 1982).

In well aerated basins where there is inflow at the surface the nutrient concentration is usually low. For example in the Mediterranean the nitrate and phosphate concentrations are about one half of those in the open North Atlantic. Consequently the Mediterranean outflow at approximately 900 m tends to reduce phosphate and nitrate concentrations at intermediate depth levels in the Eastern North Atlantic.

Anomously high nutrient concentrations are also commonly found at surface and intermediate depths on eastern boundaries due to the upwelling of nutrient rich deep water.

No matter what controls nutrient concentrations in the ocean, be it biological or physical processes, data quality is the underlying aspect of paramount importance. Without good data for end member and intermediate concentrations it is impossible to interpret the data meaningfully. To this end the WOCE Hydrographic Office has imposed an accuracy and precision of 1% full scale deflection. Such rigid controls are possible provided good analytical technique and procedures are followed. The main purpose of this document is to give full details of the analytical procedures used by the James Rennell Centre for Ocean Circulation. Sections 2, 5 and 6 deal with the analytical principle and methods, whilst sections 3, 4, 7, 9 and 11 are concerned with the system, its operation, common operational problems and maintenance. Section 8 deals with sample collection and storage and Section 10 gives a description of the SOFPAC commercial software used for quantification and data manipulation.

2. ANALYTICAL PRINCIPLE

Continuous flow analysis is the basic analytical tool for the determination of nutrients in seawater. The analyses depend on the formation of coloured complexes; the intensity of the colour being directly proportional to the concentration of the nutrient in the seawater sample. The colour is measured at specific wavelengths using photometric techniques.

A commercially available Alpkem Corporation RFA3 autoanalyser is used. The system uses a peristaltic pump attached to a dipping probe to aspirate a calibrated volume of sample through flow rated pump tubes. The whole system is in a steady state so that the only variable is the concentration of the analyte in the sample. The sample stream is delivered to an analytical cartridge where it is segmented with air and mixed with reagents. Mixing is achieved using glass coils and the development of colour may be enhanced by the use of heating baths. The intensity of the developed colour is read during its passage through a photometer and converted to an electronic signal which is displayed on a chart recorder. The analysis is quantitatively dependent on comparison with standard solutions. The ratio of sample to reagent concentration and volume is carefully set to ensure that a linear relationship of signal to concentration is obeyed throughout the concentration range of measurement.

The system is heavily dependant on the correct introduction and segmentation of bubbles into the sample stream. The bubbles serve a number of functions. They divide the sample stream into individual reaction cells for each sample, prevent carryover of the colour developed by one sample passing into the next, and clean the transmission tubing and mixing coils by their scraping action. For this reason it is essential that the bubble pattern is seen to be completely consistent throughout the instrument before any attempt is made to undertake analyses. Good reproducibility cannot be achieved without a consistent bubble pattern.

In contrast to other commercial equipment the Alpkem continuous flow analyser operates with bubbles in the flowcell. Their effect on the light path through the flow cell is compensated by an electronic gating technique. This only works satisfactorily when there is a consistent bubble pattern. During routine nitrate and silicate analysis it is unlikely that the original bubble maintains its geometry, consequently it is necessary to debubble and rebubble the nitrate and silicate channels prior to the flowcell. In contrast the phosphate flow line is short with solutions passing through quickly and so debubbling is unnecessary on this channel.

Silicate, nitrate and phosphate measurements are made simultaneously using a three channel system. The tube sizing and specification for each channel is critical and

ensures optimum operation. The system measures the concentration of three analytes at a rate of one sample every 80 seconds. While it is possible to speed up the analyses it has been found in the Alpkem system that precision may be compromised, especially with very low level determinations.

Detailed descriptions of the individual methods are given in Section 5. Diagrams with notes have been included to ensure correct tube sizing and flow path. All fittings must be carefully made, to ensure uninhibited flow through the system; this is critical to the operation.

3. OVERVIEW OF THE ANALYTICAL SYSTEM

The JRC continuous flow system is composed of the 5 separate Alpkem RFA-300 modules, namely the sample unit, the pump unit, the analytical cartridge unit, the power module and the photometer unit; 2 chart recorders; an Alpkem photometer-computer interface; an IBM PS/2 PC and an Okidata printer. These are orientated as shown in Figure 4. The five Alpkem modules are mounted on a wooden base and are transported fully assembled enclosed by a box lid which secures to the base and gives adequate clearance for the coils and tubing on the cartridge module. The two chart recorders, together with the PC interface box, are mounted vertically in a wooden framework next to the main system. The framework has demountable front and rear panels which serve to fully enclose the recorders for transportation. The PC and printer are positioned next to the chart recorders and are transported in separate packing cases.

Such an orientation allows the pump tubing to remain intact and minimum disconnection of electrical cabling during transportation. The only cables disconnected are those which link the photometer units to the chart recorders those, between the photometer and the PC interface and those between the interface and the PC. In consequence the system can be very quickly assembled at sea with the autoanalyser and the chart recorders remaining in their wooden frameworks and secured with bolts to the laboratory work surface.

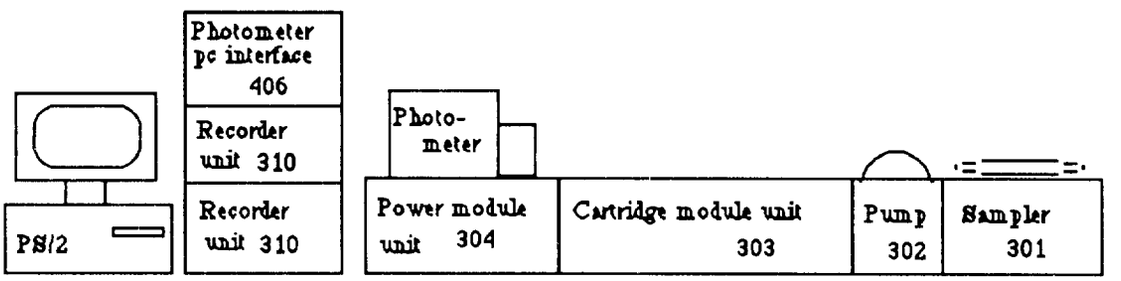

Figure 4 The JRC Alpkem RFA-300 autoanalyser system.

Since the analysis is temperature dependent the system needs to be sited in a draught free environment. Where possible this should always be in the constant temperature laboratory of the research vessel. Final results are quoted in μmoles kg^{-1} and this requires the temperature and salinity at the time of analysis to be known accurately. By siting the system in the constant temperature environment errors due to incorrect logging of temperature are kept to a minimum.

4. COMPONENTS OF THE CONTINUOUS FLOW ASSEMBLY

4.1 Sample unit

The sample unit automatically introduces samples into the analytical reagent stream. It comprises a dipping probe mechanism which takes individual samples and standards from disposable cups placed on a tray. Cups are available in varying sizes (0.5, 2.0 or 4.0 ml) depending on the amount of sample required for analysis. When using the analytical techniques described in this document 2 ml cups will suffice, and up to 90 samples can be placed on the tray at any one time. For routine water analysis the probe is 0.014" id stainless steel.

There are seven controls on the front of the sample unit. These are as follows:

4.1.1 Sample and wash times

These are push button binary-coded decimal switches which enable the operator to control the times (in seconds) of sample probe action. Since the amount of sample aspirated depends on the length of time the probe is in the sample, it is important to optimise sample time and therefore sample volume for good sensitivity on all three channels. It is also important to balance the sample and wash times to ensure best colour development and good separation of the analytical peaks. For the chemistries described in this document the sample and wash times should both be set to 40 seconds.

4.1.2 Stop count

A further digital setting indicates the position of the last sample cup. After sampling this cup the sample probe will remain in the wash solution. If samples are added or removed during an analytical run the stop count can be changed and the new value will be immediately recognised.

4.1.3 Tray rotate

This will advance the tray to the next cup position. Hold the switch down to advance multiple positions.

4.1.4 Tray advance

This will advance the tray to the next concentric ring. Repeat activation of the switch will bring the tray back to the inner home position.

4.1.5 Reset

This switch enables the operator to abort a run. When this switch is pressed the sample probe returns to the wash solution and the rosette to the home position. Reset should be pressed at the end of sampling and immediately before the start of a new run.

4.1.6 Start

This initiates the sampling as defined by the sample, wash and stop count parameters.

4.1.7 Power switch

This is an on off rocker switch.

There are also a number of controls and connections on the rear of the sampler unit. These are as follows.

4.1.8 Peck cycle

When switched on the sample probe will peck in the sample up to three times before aspirating. Such an action helps to prevent carryover from samples of high concentration. For routine seawater analysis this is unnecessary and can lead to complications. This is because the pecking action creates extra bubbles and for low level analysis the capacity of the debubbler fitting may be overcome due to the faster sample flow rate generated by an increased sample volume.

For routine seawater analysis this peck cycle switch should always be off.

4.1.9 Wash reservoir inlet

This is a quick luer fitting with a barbed end designed for 1/16" i d tubing.

4.1.10 Wash reservoir drain

This is a barbed fitting for 3/8" id 1/2" od tubing.

4.1.11 Sample interface connector

In the JRC system this is a redundant 9 pin 'D' connector used for flow injection analysis.

The sampler unit can be used in a number of modes which provide for up to quadruple sampling from a single cup. Details of these are given in the Alpkem manual. None of these modes are used by the JRC, primarily because with a 2 ml cup and a 40 second sample time there is little sample remaining in the cup after the first aspiration.

Further details regarding the use of the sample unit are given in Section 9.

4.2 Pump unit

Peristaltic pumps drive all solutions through the analytical system. They provide the metering of reagents, sample and bubble segments necessary for the chemical reactions that occur downstream. The pumps are roller assemblies over which the pump tubes are secured by collars to the shoulder grips. Four pump platens close over the roller assemblies and only when all four are closed will the system be operational. The platens are closed by lifting the spring rod handles into the slot in the platen and turning through 90°. The action of the handle forces the platen downwards onto the tubes. Metering is accomplished by squeezing the pump tubing between the rollers and the platen. By using a constant pump roller head speed and precise pump tube internal dimensions a fixed liquid flow is delivered. The roller head speed is maintained at a constant rate using an 1800 RPM synchronous AC drive motor. This type of motor derives its speed from the frequency of the AC power line. It will hold a fixed speed regardless of voltage surges or drops as long as the frequency remains constant. If voltage drops more than 25% for durations of greater than 4-5 seconds the motor may begin to lose speed due to a decrease in torque. It is therefore important to be aware of voltage changes particularly at sea where fluctuations can occur more often than on land.

During normal operation, the pump assembly should not be removed from the unit. Details of pump removal and servicing are not included in this document; the reader is referred to the Alpkem RFA300 service manual which covers this topic adequately.

The pump tubes are colour coded to indicate their respective flow rates. Details of this coding are given in Table 1.

An important component of the pump unit is the gas bubble intake system. Air, or nitrogen in the case of the nitrate/nitrite channel, is delivered to the system by means of solenoid operated valves. These are positioned in the flow system between the flow rated

TABLE 1

PUMP TUBE COLOUR CODING AND FLOW RATING

Colour	Abbreviation	Flow rate
orange/blue	ob	37 µl/min
orange/green	og	74 µl/min
orange/yellow	oy	118 µl/min
black/black	bb	226 µl/min
orange/orange	oo	287 µl/min
white/white	ww	385 µl/min
grey/grey	grg	568 µl/min
yellow/yellow	yy	642 µl/min
green/green	gg	947 µl/min

pump tubes on the platen which act as the gas intake and the upstream side of the first mixing coil of the analytical cartridge. The rating of the air intake pump tubes controls the size of the bubble.

When the valve is closed the pump forces a pressure build up in the gas flow line. This is released when an interupter circuit is triggered by a chopper wheel on the pump drive shaft and the gas surges through the valve and passes into the main analytical stream through an injector fitting. The dispersion of the gas pressure causes a bubble to be forced into the analytical stream.

It is very important that 0.015 id polyethylene tubing is used to connect the pump tubes to the valves and the valves to the analytical cartridge. Larger bore tubing produces a cushioning effect during pressure build up and this weakens the surging effect and so a regular bubble pattern is not achieved.

There are six gas valves to the rear of the pump unit; these are orientated as shown in Figure 5. Because of the necessity to bubble and debubble the nitrate and silicate lines (see Section 2) two valves are required for these channels but only one for the phosphate. It is important that the inlets from the pump tubes and the outlets to the cartridges are connected correctly. This can be somewhat confusing since the valve ports are configured inlet-outlet moving right to left along the three valves to the extreme rear of the pump unit whilst the three valves to the front are configured outlet-inlet moving right to left (see Figure 5 for clarification).

A bubble phasing potentiometer controls the firing of the valves. Valve opening duration can be adjusted from 2-20 milliseconds. In most instances the valve requires at least 4 milliseconds to completely open and so the valve opening duration should not be less

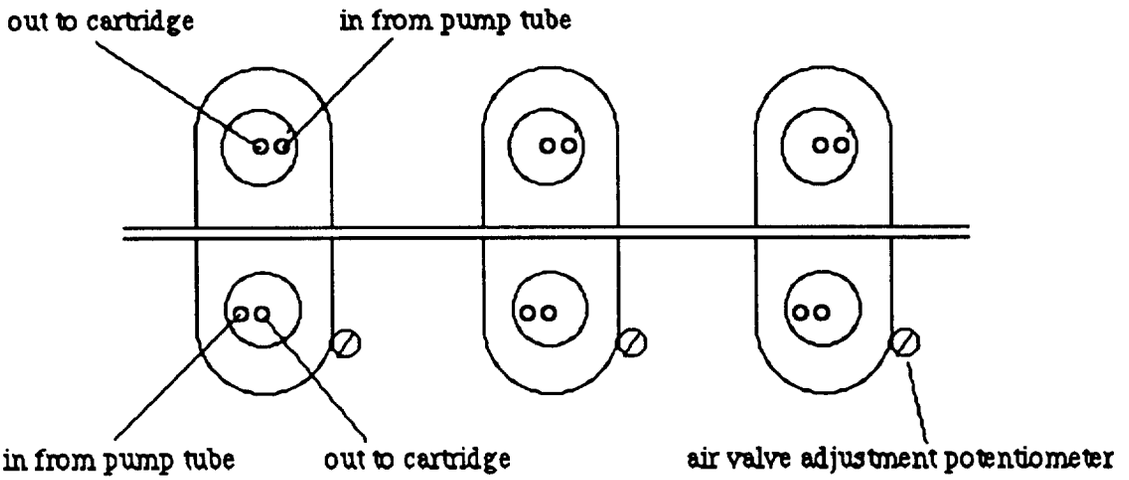

Figure 5 Orientation of the six gas valves in the Alpkem RFA-300 autoanalyser.

than 4 milliseconds. Normally the valves are set to fire every 2 to 3 seconds. However, if multiple bubble injections occur this will have to be adjusted as necessary. It is best to adjust the bubble injection so that it coincides with the period of backsurge. Backsurge is an unavoidable consequence of the pumping mechanism, occurring as each pump roller breaks contact with the pump tube. Whilst it can significantly affect the stability of the analysis, its effect can be dampened and even eliminated by timing the bubble injection correctly. To do this adjust the timing of the solenoid valve so that the solenoid clicks at the same time as the slight kickback occurs in the pull off line on each of the three channels.

Adjustments should be made very carefully by turning the screw controlling the phasing potentiometer by a half turn at a time. This will have a noticeable effect on the bubble pattern. If the phasing appears to be completely out, the phasing potentiometer should be turned fully clockwise until a faint click is heard. Eight turns anticlockwise will then phase the valve to about the right place. Phasing should only be attempted when the analyser is running on wash solution containing the appropriate surfactant. It will not be possible to phase or obtain a reasonable bubble pattern whilst running on water only.

4.3 Analytical cartridge unit

The analytical cartridge unit provides a mounting for the analytical cartridge trays, a drain to waste and electrical connections to the heating baths. It is designed in such a way as to allow access to the sample stream at all points.

The analytical cartridge trays contain all the necessary components to effect a chemical reaction and consequent colour development. They are specific for individual

chemistries. Details of the analytical cartridge trays used in the JRC system are given in the figures accompanying Section 5. The drain trough is mounted on the inside left of the cartridge module. Each tray drains into the trough and the waste is carried away from the instrument, via a strain relief gromet, using 3/8" id by 1/2" od tubing. Three 9 pin 'D' connectors on the rear of the unit connect the heating baths to the power unit (in the JRC system only two are used). Connections from the heating baths feed under the analytical trays to the 'D' connectors. See Section 9.1.2 for details of electrical connections.

4.4 Power unit

The power unit sits under the photometer unit and distributes all electrical supplies to the pump, heating baths, light source and photometer modules. It also houses the light source, fibre optics and heating bath temperature controllers. It has five rocker switches, three light emitting diodes and three temperature adjustment screws to the front and a series of seven 9 pin 'D' output connections, a fibre optic cable connection and the main power input connection to the rear. The unit is rated at 110 volts and is connected to the 240 volt UK mains and ship supply using a 240 to 110 transformer.

4.4.1 Rocker switches

The five rocker switches comprise the main on/off power switch, the photometer light on/off switch and three heating bath on/off switches.

4.4.2 Light emitting diodes

These indicate heating bath status. When the light is constantly on the bath is heating. Once flashing commences the bath is maintaining temperature. Constant off means the bath is cooling.

4.4.3 Temperature adjustment screws

Heating bath temperatures can be adjusted by rotating the screw clockwise to increase the temperature and counter clockwise to decrease temperature.

4.4.4 'D connectors

The seven 9 pin 'D' connections at the rear are for the three photometers, three heating baths, and pump. Only two heating baths are used in the JRC system and so centre

front controls and centre rear connector are redundant. All electrical connections are further explained in Section 9.

4.4.5 Fibre optic cable connection

This is a combined light source housing and fibre optic cable connection. It is connected to the unit via a knurled barrel assembly.

Warning the knurled barrel gets very hot during normal operation.

To replace the light bulb unscrew the knurled barrel and remove the fibre optic connection from unit. Remove old bulb from barrel and replace. Re-insert the connection into the photometer unit and tighten the knurled knob.

The fibre optic cable is very fragile and must be handled with care. Ensure it is not severely bent or broken, especially in transit.

4.5 Photometer unit

There are three dual beam filter photometers in the system, one for each channel. Each houses a wavelength filter, an analytical flow cell, and a reference cell together with all the necessary electronics to read photometrically the intensity of colour development and electronically convert and amplify the signal to output to the chart recorder. The three photometers are identical except for the filter and flow cell and so it is possible to interchange them for fault finding.

Light from the single tungsten-halogen lamp is split into six beams, two for each channel, by the fibre optic bundle. White light emerges from each fibre optic leg and passes through the flowcell containing the analytical stream. It then passes through the appropriate channel filter for wavelength selection. UV enhanced silicon diodes convert the light into an electronic signal. The manufacturers suggest that since wavelength discrimination occurs after the flowcell the flowcell requires minimal shielding. Experience has shown that this is not always correct and so the flowcell should be shielded where possible to prevent spurious light signals entering.

The photometer is fitted with an internal bubble-gate circuit which electronically ignores the bubble passing through the flowcell by taking readings only between bubbles. The bubble gated circuit may be disabled for applications where the analytical stream is not segmented. Such a situation does not apply when using the JRC methodology.

On the top panel of each photometer there are five controls and a liquid crystal display (LCD); the latter showing output voltage.

4.5.1 Function switch

This is a three position switch controlling the voltage readout displayed on the LCD. It is used in sample or reference mode when adjusting the sample and reference gain settings during set up and in the absorbance mode during normal operation. Adjustment of gain settings are detailed in the Section 9. In the absorbance mode the LCD shows a comparison of the reference and gain voltages.

4.5.2 Sample and reference gain switches

The photometer signal voltage should normally read 5 volts (the maximum linear output voltage of the photometer) when the system is operating on blank solutions. The appropriate gain switches are used to adjust the reference and sample cell raw voltages. The outer ring coarse setting (0.1, 1 and 5 x $10^{8)}$ should always be set as low as possible and the fine setting (upper knob) used to peak the instrument. Once both settings are at 5 volts, the absorbance reading should be stable at 0.00V ±0.02 . However, experience has shown that the absorbance reading can vary by more than this particularly into the negative and this will give incorrect readings and upset the software package used for data collection. To alleviate this the reference voltage is set marginally higher and the sample reading marginally lower than 5 volts suggested. The absolute values are adjusted so that when switched to absorbance mode a reading of +0.1 is obtained.

4.5.3 Damp switch

This switch determines the amount of low pass electrical filtering of signal passing to the recorder unit. It is used to electronically suppress high frequency baseline noise (unsteady signal). There are 5 settings of 0.1, 0.5,1, 2 or 4 seconds time delay and ideally the setting should be the lowest but this is not always achievable.

4.5.4 Standard calibration switch

This switch allows adjustment of the output signal from the photometers. To obtain maximum sensitivity over the concentration range of interest it is necessary to adjust this setting at the start of the run so that the highest calibration standard lies at about 90% absorbance on the chart recorder.

4.5.5 Gate delay

This is a small potentiometer screw above the LED. It must be carefully set to ensure that the signal is read when there are no bubbles in the flow cell. It is particularly important not to catch any of the bubble signal. Ideally the signal should be read between 180 and 200 milliseconds after the bubble has left the flow cell. There are two procedures to set the gate delay, one based on bubble entrance (procedure a), the other on bubble exit (procedure b), either are acceptable. Both require a segmented stream of water or reagent flowing through the flow cell.

a) Rotate the adjustment screw clockwise until the LED stops blinking then turn it counter-clockwise until it blinks continuously.

b) Rotate the adjustment screw counter-clockwise until the LED on top of the photometer reads over 5 volts when the function switch is in the absorbance position. Rotate the adjustment screw 8 turns clockwise which sets the update pulse to read at approximately 200 milliseconds.

Following adjustment turn on recorder and monitor the baseline signal. If spikes occur it is likely that the signal is being read just as a bubble is entering the flowcell. Turn the adjustment screw counter-clockwise half a turn and observe the baseline. Adjust a further half turn if necessary. Once set correctly the baseline should be smooth and consistent and the green LED light blinking consistently.

During normal operation the gate delay setting should not require adjustment once set.

There are a number of side panel components on the photometer, a 9 pin 'D' connector, an on/off bubble gate switch and outputs for the recorder, raw sample, raw reference and bubble-gate logic signals.

4.5.6 "D" connector

This provides power from the power unit.

4.5.7 The bubble gate on/off switch

This disables the bubble gate circuit. All bubbles must be removed from the stream prior to the flowcell if the bubble gating is off. For normal use the bubble gate on/off switch should always be in the on position.

4.5.8 Output sockets

The absorbance signal from the photometer is transmitted via the recorder output socket and during normal operation the chart recorder is connected to this. The remaining output sockets are for troubleshooting. The logic output enables visualisation of the bubble gate logic signal, the raw sample output of the sample diode signal and the raw reference output of the reference diode signal.

4.6 Recorder unit

There are two identical two channel chart recorders. Each channel receives a DC analog signal (0-5 volts) from the photometer which is amplified and plotted onto ruled strip chart paper. Normally silicate and nitrate are plotted on one recorder and phosphate on the other, but this is discretionary. Each recorder has a number of controls and components. These are mainly located on the top of the recorder. To the rear there is an independent power lead which is attached directly to the mains and not to the RFA power unit, a voltage selection switch giving the option of 110, 210 or 240 volts and a DIN socket for optional time based control. The recorders are normally run at 240 volts. The top controls are as follows:

4.6.1 Power switch

This recorder is on in position 1 and off in position 0

4.6.2 Input range switch

Various input ranges may be selected depending on the output voltage of the photometer. The 10 V position with VAR adjustment (see sections 4.6.4 and 4.6.5) is used for normal operation.

When the switch is in the '<o>' position the amplifier from the input signal is short circuited and the recorder zero may be set.

4.6.3 Recorder zero switch

Once a steady baseline has been established, this switch can be used to set the position of the pen on the chart recorder. Normally it is set at 5 graduations above the lower edge of the chart paper so that any negative shift in the baseline can be observed. Remember to set the input range switch to the '<o>'position before adjusting this knob.

4.6.4 Var/Cal switch

This switch allows the setting of the electronic span of the chart recorder. In the cal position it fixes the span to the input voltage switch setting. In the var position it allows full scale deflection to be obtained for any input value between 10 and 100% of the selected input range. The switch is normally used in the var position.

4.6.5 Var adjust knob

With the Var/Cal switch in the 'Var" position, the Var adjustment knob is used to set the recorder electronic span to match the photometer output.

4.6.7 Chart speed

The chart speed may be set at any value which is convenient for the operator. Good peak resolution is normally obtained when the chart speed is 1 cm min^{-1}.

4.6.8 Chart drive switch

Determines the direction of the chart drive. In the '▲' position the chart moves in the reverse direction, in the '0' position it remains stationary and in the '▼' position it moves forward.

4.6.9 Chart positioning switch

This is a push and hold switch which allows fast forward or reverse of chart paper, enabling recording to commence at a specific point. The chart speed is 3 cm min^{-1} for 3 seconds, automatically increasing to 60 cm min^{-1}. Release of the switch returns the recorder to the chart speed specified.

4.6.10 Photometer inputs

There are four DC input connections for the recorder photometer cables. These are negative-positive from left to right. The cables have been configured so that two leads pass into each pair of inputs. One links the photometer with the recorder and the other the recorder to the PC interface box.

4.6.11 Pen lift

This physically lifts the pens off the chart paper. It is important that pens are lifted when not in use and capped. If not the ink can be drawn out of the pen onto the chart paper and the pens quickly dry up and become non operational.

4.7 Photometer- Computer interface

This has four input sockets on its front face (in the JRC system only three are used) into which the cables from the chart recorders are plugged. The interface convert the analogue signal from the photometers to digitised format readable by the IBM PS2 PC.

5. ANALYTICAL METHODS

5.1 Silicate

The colourimetric determination of silicate in seawater depends upon the formation of yellow β-silicomolybdic acid when the sample is treated with an acidic molybdate reagent. Since the β-silicomolybdic acid is unstable and has only a low molar absorbance it is subsequently reduced to the stable and more adsorbant molybdenum blue complex measured at an absorbance of 820 nm. The reduction can be carried out using a number of reducing agents for example stannous chloride, metol (p-methyl-aminophenol sulphate) and sodium sulphite, and absorbic acid. Liss and Spencer (1969) found little difference between the results obtained with stannous chloride using the technique of Armstrong (1969) and those obtained from the method of Strickland and Parsons (1968) in which metol is used. At the James Rennell Centre we found little difference in the use of stannous chloride and absorbic acid. However we elected to use absorbic acid since the reagent is much more stable and easier to use. Stannous chloride solution rapidly deteriorates and needs changing at least every twenty four hours.

Phosphate and arsenate produce a similar blue colour to silicate, but interferences from these species in the seawater sample can be prevented by incorporating oxalic or tartaric acid in the reducing agent; both decompose the phosphomolybdic and arsenomolybdic acids adequately.

Figures 6 and 7 show the cartridge currently used by the James Rennell Centre to measure silicate concentrations from 0 to 50 μmol Kg^{-1} and 0 to 150 μmol Kg^{-1} together with details of the sampling, flow and photometer parameters. It is important that these parameters are set as indicated. Higher or lower concentration ranges can be accommodated using the same strength reagents but changes in pump tube sizing and hence flow rates are necessary. These serve to adjust the sample: reagent ratio required for the different ranges.

Silicate measurement is very temperature dependent. Up to 3% deviation in measured value is observed with 1°C change in temperature. For this reason a 2 ml coil within a heating bath at 37°C is incorporated in the silicate channel. This is a modification to Alpkem's recommended method. Comparisons between non-heated and heated analysis show a distinct improvement in precision and sensitivity during the latter.

Care must also be taken to allow for salt effects. Some workers have reported up to a 10% reduction in absorbance at salinity 34 compared to salinity 35. Comparisons of standard solutions prepared in sodium chloride solution over salinity range 33 to 37 have shown no difference in recovery with the Alpkem system. However JRC workers have noticed reduced recovery of up to 10% when comparison is made between standards

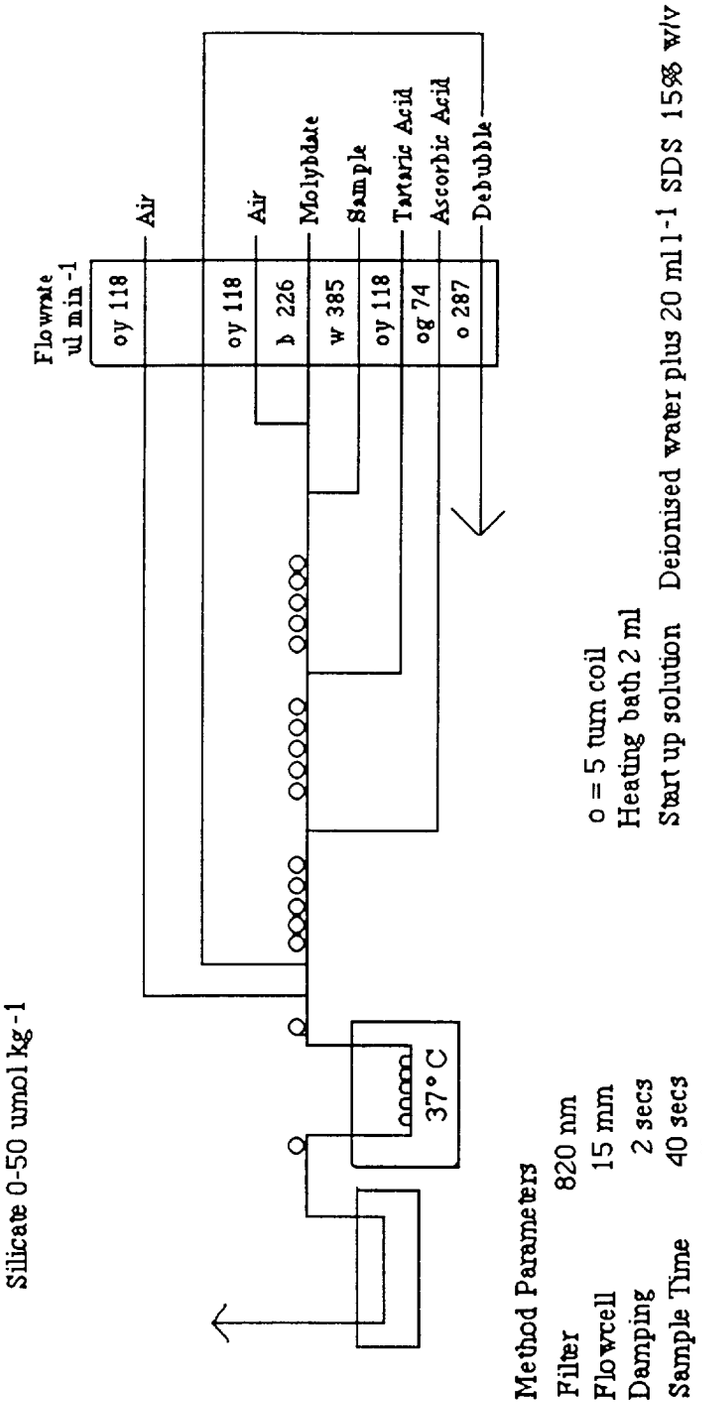

Figure 6 The analytical cartridge used by JRC for the measurement of silicate from 0 to 50 µmol kg^{-1}.

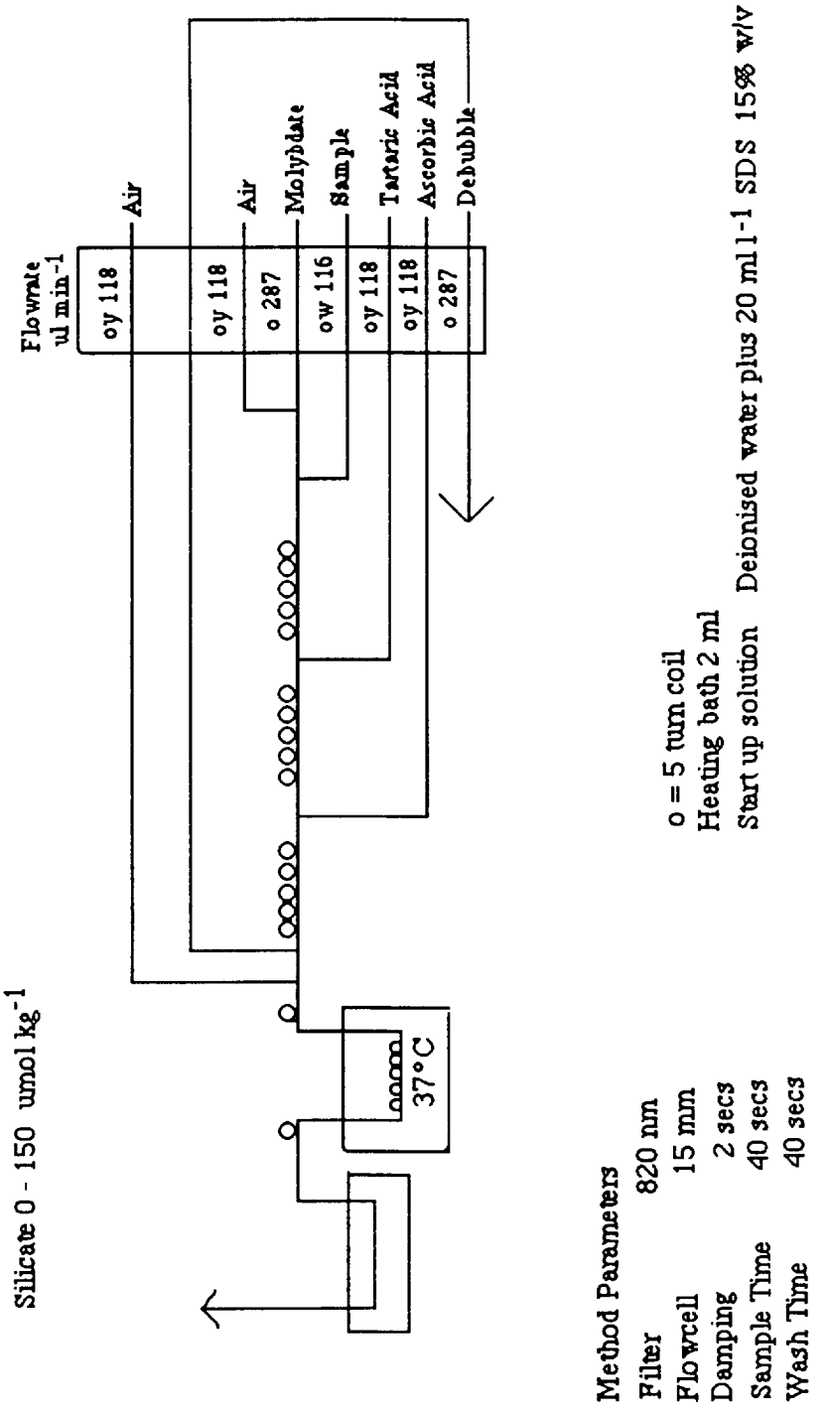

Figure 7 The analytical cartridge used by JRC for the measurement of silicate from 0 to 150 µmol kg^{-1}.

prepared in depleted seawater and sodium chloride solution which needs further investigation.

5.2 Nitrate/nitrite

There is no completely satisfactory method for the direct measurement of nitrate in seawater despite considerable analytical work by a number of workers. It is hampered by the fact that although the nitrate ion has an intense absorption band in the far ultraviolet at 202 nm there is strong absorption in this spectral region by bromide ions and dissolved organic compounds. In contrast the nitrite ion can be satisfactorily measured as an azo dye at an absorbance maximum of 543 nm. Therefore the analysis of nitrate in seawater is based on the initial reduction of nitrate to nitrite and the total nitrate/nitrite in the sample measured.

A number of methods of nitrate reduction have been suggested (Riley,and Skirrow 1975), the most satisfactory is the use of cadmium in the form of an open tube cadmium reactor (OTCR). The nitrite is subsequently used to diazotise the primary amine sulphanilamide which is then coupled with naphthyl ethylene diamine dihydrochloride at an acid pH to give the pink azo dye. Interferences from copper, zinc and other trace metals are eliminated by the use of Imidazole buffer; there does not appear to be any salt water matrix effect.

Figure 8 shows the cartridge used for the concentration range 0 to 40 $\mu mol\ l^{-1}$; it is unlikely that concentration levels above this will be found in the open ocean. Particular attention must be paid to the use and handling of the cadmium reactor. Oxygen must be eliminated from the inside of the tube, consequently the intersample bubble on the nitrate channel is fed from a cylinder of oxygen free nitrogen. In addition, the cadmium tubes loose their activity with repeated use and must be regenerated or replaced. The reader is referred to Section 7 for the use and maintenance of the OTCR.

5.3 Phosphate

Orthophosphate is the most routinely measured form of phosphorus in sea water. The analytical method is based on the formation of 1,2-molybdophosphoric acid which is subsequently reduced to a phosphomolybdenum blue complex. Although several reducing agents are capable of bringing about the reduction only a few produce a complex with sufficiently high absorbance for use at normal seawater concentrations. Stannous chloride and ascorbic acid have both been used in the past. The former has a number of disadvantages such as instability of colour, a rate of reduction dependent on temperature

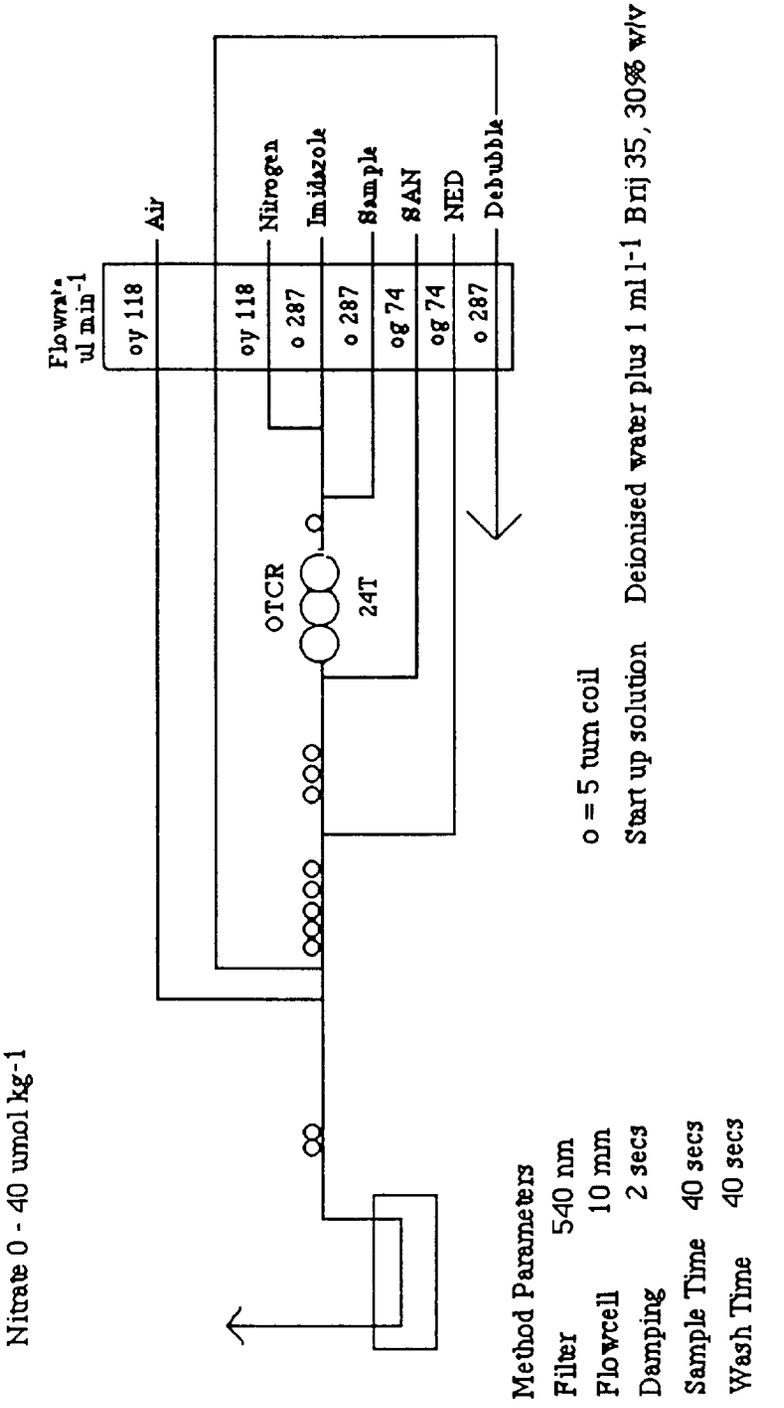

Figure 8 The analytical cartridge used by JRC for the measurement of nitrate from 0 to 40 μmol kg^{-1}.

and salinity and a considerable salt error. The latter suffers from a very slow reaction with the risk of hydrolysis of the labile, organic forms of phosphorus. To overcome this antimony is incorporated into the reagent and this produces a rapid reaction to give a very stable purple blue complex which contains antimony and phosphorous in a 1:1 atomic ratio. The reduction of the phosphoantimonyl molybdenum complex with asorbic acid gives a mixed valence complex with an absorbance maximum of 880 nm. It is important that the final acidity of the solution should be sufficiently high to prevent reduction of the molybdate itself but low enough to minimise the hydrolysis of the organophosphates.

Figure 9 shows the cartridge used for the concentration range 0 to 3 μmol l^{-1}; it is unlikely that concentration levels above this will be found in the open ocean. Interferences from other compounds which form hetropoly acids with molybdate are rare for example there is no interference from silicate provided its concentration does not exceed 170 μmol l^{-1}. However, arsenic (V) does yield a hetropoly blue complex with a similar molar absorbance to that of orthophosphate. In the open ocean where arsenic (V) levels are low this is unlikely to be a problem. If encountered it can be overcome by increasing the sulphuric acid concentration from 2.5 to 3.6 N or by reducing the arsenic to the non-reactive 3+ oxidation state with metabisulphite before addition of the combined reagent.

The molybdenum blue complex is prone to plating out on the walls of the continuous flow system. This can be overcome by frequent washing the phosphate channel system with 1% sodium hydroxide solution.

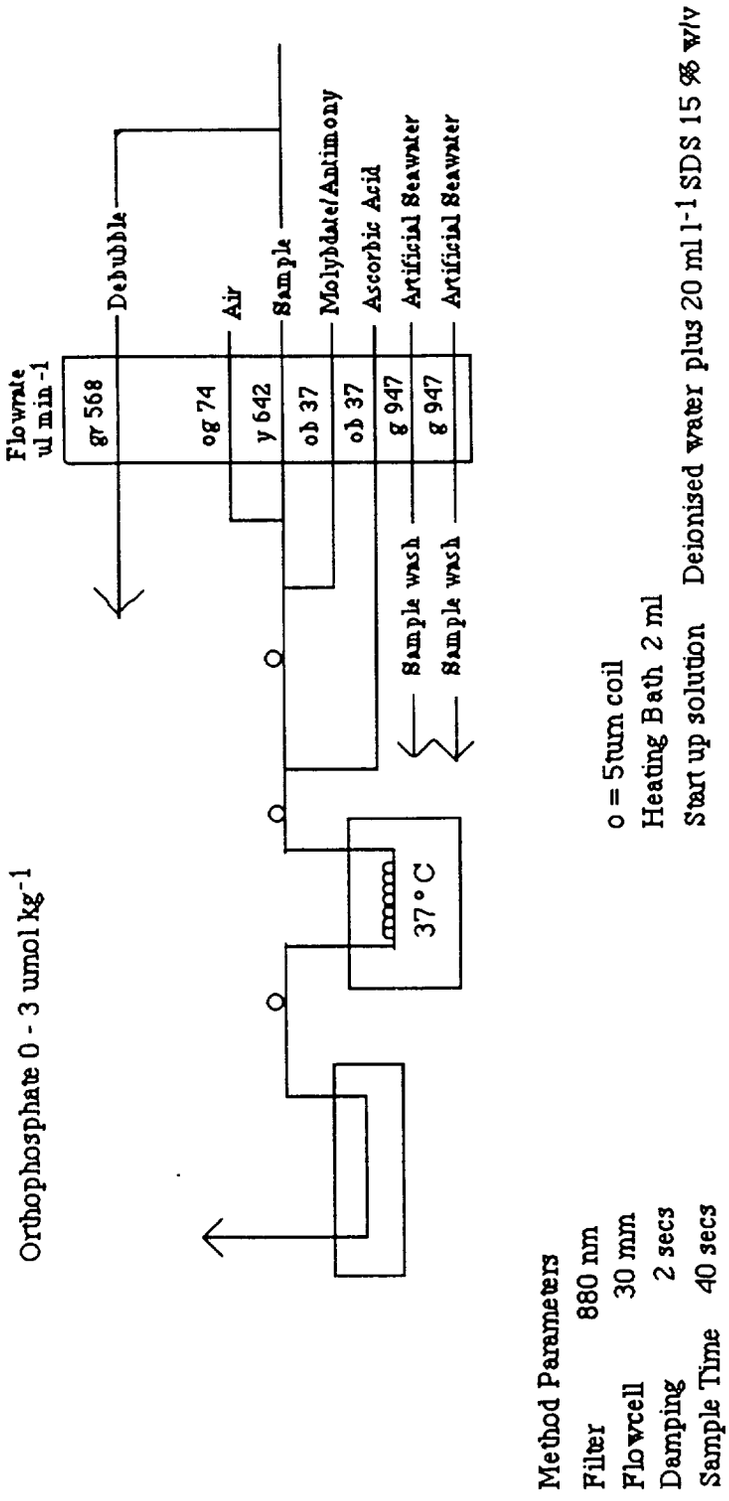

Figure 9 The analytical cartridge used by JRC for the measurement of phosphate 0 to 3 µmol kg^{-1}.

6. ANALYTICAL REAGENTS AND CALIBRATION STANDARDS

This section details the preparation of all analytical reagents and standards required for the measurement of seawater silicate, nitrate and phosphate. Standards are prepared in class A Volax volumetric flasks using analytical grade chemicals (Merck) and immediately transfered to plastic containers. The glassware has an accuracy of ±0.1%. All reagents and stock standards are made to volume using 18 megohm cm^{-1} conductivity at 25°C deionised water. Dry reagents are weighed to an accuracy and reproducibility of 0.01g using an Oxford series S top pan balance. Dry standards are weighed on a 5 place balance to an accuracy of 0.0001g. Transfer pipettes for the preparation of working standards are air displacement Gilson pipettes with a manufacturers specified accuracy of 0.3%

Unless indicated, all dry reagents are taken to sea preweighed and in sealed vials.

Once prepared the stock standards should not deteriorate over a period of two months and where possible it is beneficial to use the same stock standard throughout a cruise. However it should be quality checked every two to three days using commercially available nutrient standards or against a second prepared stock standard. When a stock standard is changed either in the laboratory or at sea it is imperative that the old and new standard are thoroughly compared prior to changeover. This ensures that the new standard has been prepared correctly and is a second check to any deterioration of the old.

Working standards should be prepared for each analytical run by dilution of the stock standard using large diluant volumes to reduce error. Five working standards are usually prepared with concentrations evenly spaced to cover the anticipated sample concentration range. Normally the standards are prepared in 100 ml volumetric flasks and are used directly from the flask. There is some debate as to the diluant used. Some workers prefer deionised water, others 35% sodium chloride solution and others nutrient depleted seawater. Deionised water can often result in a salt (refraction) error which is particularly pronounced for phosphate. In turn the sodium chloride does not have the same matrix as seawater and it can be argued that depleted seawater is not representative of newly sampled seawater. In the present JRC methology we are using sodium chloride solution.

Working standards are only stable for a few hours. They should be used for just one single analytical run.

6.1 Silicate reagents

6.1.1 Stock Sodium Dodecyl Sulphate (15% W/V)

Sodium Dodecyl Sulphate 15 g

Deionised water

Dissolve the Sodium Dodecyl Sulphate in 85 ml of deionised water in a 250 ml plastic beaker. The beaker may need warming to ensure complete solution. Make up to 100 ml and store at room temperature in a sealed polypropylene bottle and renew weekly.

6.1.2 Stock Ammonium Molybdate

Ammonium Molybdate	5.4 g
Sulphuric Acid	1.4 ml
Deionised water	

Carefully add the concentrated Sulphuric Acid to 400 ml of deionised water in a 500-ml volumetric flask. Add the Ammonium Molybdate and dissolve. Finally make the solution up to 500 ml with deionised water. Store in a polypropylene bottle at room temperature. Discard after one week or sooner if the reagent becomes discoloured.

6.1.3 Working Ammonium Molybdate

Stock Ammonium Molybdate	100 ml
Stock Sodium Dodecyl Sulphate	2 ml

Pour out 100 ml of the stock Ammonium Molybdate solution into a measuring cylinder and transfer to a polypropylene bottle. Add 2 ml of the Sodium Dodecyl Sulphate solution. Renew the reagent each day.

6.1.4 Tartaric Acid (10% W/V)

Tartaric Acid	25 g
Deionised water	

Add the Tartaric Acid to 200 ml of deionised water in a 250 ml volumetric flask. Mix the solution well and make up to 250 ml with deionised water. Store at 4°C in a polypropylene container. Renew weekly.

6.1.5 Ascorbic Acid (18 g l^{-1})

Ascorbic Acid	9 g
Deionised water	

Add the Ascorbic Acid to 400 ml of deionised water in a volumetric flask. Mix well and make up to 500 ml with deionised water. Store in a polypropylene container at room temperature and renew daily.

6.2 Silicate standards

6.2.1 Stock silicate standard 10 mmol l^{-1}

Sodium Hexafluorosilicate	0.9600 g *
Deionised water	500 ml

Add exactly 0.9600 g* of Sodium Hexaflourosilicate to 300 ml of deionised water in a 500 ml volumetric flask. Mix to obtain complete solution and make the solution to volume Store the standard in a sealed **plastic** container. This will give a stable 10 mmol l^{-1} solution.

***NB This quantity depends on the assay of the chemical. Adjust according to the manufacturers specification.**

6.2.2 Working silicate standards

Silicate concentrations can vary widely both geographically and with depth and so working standards must be prepared to cover the anticipated concentration range. It is usual to prepare the following:

0-25 µmol l^{-1} range

50 µl stock standard in 100 ml deionised water	5 µmol l^{-1}
100 µl stock standard in 100 ml deionised water	10 µmol l^{-1}
150 µl stock standard in 100 ml deionised water	15 µmol l^{-1}
200 µl stock standard in 100 ml deionised water	20 µmol l^{-1}
250 µl stock standard in 100 ml deionised water	25 µmol l^{-1}

0-50 µmol l^{-1} range

100 µl stock standard in 100 ml deionised water	10 µmol l^{-1}
200 µl stock standard in 100 ml deionised water	20 µmol l^{-1}

300 µl stock standard in 100 ml deionised water 30 µmol l^{-1}

400 µl stock standard in 100 ml deionised water 40 µmol l^{-1}

500 µl stock standard in 100 ml deionised water 50 µmol l^{-1}

0-150 µmol l^{-1} range

250 µl stock standard in 100 ml deionised water 25 µmol l^{-1}

500 µl stock standard in 100 ml deionised water 50 µmol l^{-1}

750 µl stock standard in 100 ml deionised water 75 µmol l^{-1}

1000 µl stock standard in 100 ml deionised water 100 µmol l^{-1}

1500 µl stock standard in 100 ml deionised water 150 µmol l^{-1}

6.3 Nitrate/nitrite reagents

6.3.1 Stock Imidazole Buffer (0.1 M)

Imidazole 6.81 g

Concentrated Hydrochloric Acid

Deionised water

Dissolve the Imidazole in 900 ml of deionised water in a volumetric flask. Adjust the pH of the solution to pH 7.5 by adding the Hydrochloric Acid dropwise; check the pH of the solution after each addition using narrow range pH paper. Make up to 1litre with deionised water and mix. Store at 4°C in a tightly sealed polypropylene container.

6.3.2 Stock hydrated Copper II Sulphate (2.5 g l^{-1})

Copper II Sulphate 1.25 g

Deionised water

Add the Copper II Sulphate to 400 ml of deionised water in a volumetric flask. Make sure it has completely dissolved and make up to 500 ml. Store at room temperature in a polypropylene container. This reagent is only required for activating the OTCR (see Section 7)

6.3.3 Stock Sulphanilamide

Sulphanilamide	5.0 g
Concentrated Hydrochloric Acid	50 ml
Deionised water	

Carefully mix 50 ml of Hydrochloric acid with 350 ml of deionised water in a 500 ml volumetric flask. Add the Sulphanilamide and dissolve. Make the solution up to 500 ml with deionised water. Store in a polypropylene container for up to one week.

6.3.4 Brij 35

Brij 35	30 g
Deionised water	100 ml

Add the brij to 75 ml of deionised water in a beaker and mix until it is completely dissolved. It may be necessary to warm the solution gently. Make up to 100 ml and store in a plastic container. This solution deteriorates after 2-3 days and reduces the efficiency of the OCTR.

6.3.5 Working Imidazole Buffer

Stock Imidazole Buffer	50 ml
Brij 35 (30% W/V)	1 ml
Deionised water	

Mix 50 ml of deionised water with 50 ml of stock Imidazole Buffer in a 100 ml measuring cylinder. Transfer the solution to a polypropylene bottle and add 1 ml of Brij 35.

6.3.6 Working Sulphanilamide

Stock Sulphanilamide	100 ml
Brij 35 (30% W/V)	1 ml

Add the Brij 35 to 50 ml of stock Sulphanilamide. Change the reagent daily.

6.3.7 1-Naphthyl Ethylene Diamine Dihydrochloride (NED) (1 g l^{-1})

1-Naphthyl Ethylene Diamine Dihydrochloride	0.25 g
Brij 35 (30% W/V)	0.5 ml
Deionised water	

Dissolve the NED in 200 ml of deionised water in a 250 ml volumetric flask and add 0.5 ml of Brij. Make up to 250 ml with deionised water. Store the reagent in a polypropylene container, and refrigerate when not in use. The solution is only stable for a few days.

6.4 Nitrate and nitrite standards

6.4.1 Stock nitrate standard 10 mmol l^{-1}

Potassium Nitrate	0.5050 g *
Deionised water	500 ml

Add exactly 0.5050 g* of Potassium Nitrate to 300 ml of deionised water in a 500 ml volumetric flask. Dissolve completely and make up to 500 ml. Store the standard in a sealed **plastic** container. This will give a stable 10 mmol l^{-1} solution.

***NB This quantity depends on the assay of the chemical. Adjust according to the manufacturers specification.**

6.4.2 Stock nitrite standard 10 mmol l^{-1}

Sodium Nitrite	0.3450 g *
Deionised water	500 ml

Add exactly 0.3450 g* of Sodium Nitrite to 300 ml of deionised water in a 500 ml volumetric flask. Dissolve completely and make up to 500 ml. Store the standard in a sealed **plastic** container. This will give a stable 10 mmol l^{-1} solution. This standard is only required to check the activity of the OTCR (see Section 7)

***NB This quantity depends on the assay of the chemical. Adjust according to the manufacturers specification.**

6.4.2 Working nitrate standards

Nitrate concentrations in the ocean do not normally exceed 50 µmol l^{-1}. Working standards are therefore normally prepared to cover the 0-25 or 0-50 µmol l-1 ranges using the same dilutions outlined in Section 6.2.2.

6.5 Phosphate reagents

6.5.1 Antimonyl Potassium Tartrate (3 g l^{-1})

Antimonyl Potassium Tartrate	1.5 g
Deionised water	

Add the Antimonyl Potassium Tartrate to 400 ml of deionised water in a 500 ml volumetric flask. Dissolve completely (it can be slow to dissolve) and make up to 500 ml. Store the reagent in a plastic container indefinitely.

6.5.2 Stock Molybdate/Antimony

Concentrated Sulphuric Acid	35 ml
Ammonium Molybdate	3 g
Antimonyl Potassium Tartrate stock	25 ml
Deionised water	

Slowly add the Sulphuric Acid to 300 ml of deionised water in a 500 ml volumetric flask. Add the Ammonium Molybdate and 50 ml of the stock Antimonyl Potassium Tartrate solution, mixing between additions. Mix thoroughly and make up to 500 ml with deionised water. Store the reagent in a polypropylene container at room temperature. It will keep for up to one week.

6.5.3 Stock Ascorbic Acid

Ascorbic Acid	6 g
Acetone	200 ml
Deionised water	

Add the Acetone to 200 ml of deionised water and transfer to a plastic reagent container. Add the Ascorbic Acid and mix until fully dissolved. Store the reagent at 4°C for up to one week.

6.5.4 Working Molybdate/Antimony

Molybdate/Antimony	100 ml
Sodium Dodecyl Sulphate (15%W/V)	4 ml

Add 2 ml of Sodium Dodecyl Sulphate (see Section 6.1.1) to 100 ml of stock Molybdate/Antimony reagent and mix thoroughly. Renew the reagent daily.

6.5.5 Working Ascorbic Acid

Stock Ascorbic Acid	10 ml
Deionised water	

Mix together the stock Ascorbic Acid reagent with 50 ml of deionised water. Renew the reagent daily.

6.6 Phosphate standards

6.6.1 Stock phosphate standard 10 mmol l^{-1}

Potassium Dihydrogen Phosphate	0.681 g *
Deionised water	500 ml

Add exactly 0.681 g *of Potassium Dihydrogen Phosphate to 300 ml of deionised water in a 500 ml volumetric flask. Dissolve completely and make up to 500 ml. Store the solution in a **glass** container. This will give a stable 10 mmol l^{-1} solution.

***NB This quantity depends on the assay of the chemical. Adjust according to the manufacturers specification.**

6.6.2 Working phosphate standards

The concentration of phosphate in seawater is very low, normally less than 3 µmol l^{-1} and so very low working standards are required. An initial dilution of the stock standard is required. Working standards are normally prepared to cover the 0-2.5 µmol l^{-1} concentration range.

1 ml stock standard in 100 ml deionised water	100 μmol l^{-1}
50 μl 100 μmol l^{-1} standard in 100 ml deionised water	0.5 μmol l^{-1}
100 μl 100 μmol l^{-1} standard in 100 ml deionised water	1.0 μmol l^{-1}
150 μl 100 μmol l^{-1} standard in 100 ml deionised water	1.5 μmol l^{-1}
200 μl 100 μmol l^{-1} standard in 100 ml deionised water	2.0 μmol l^{-1}
250 μl 100 μmol l^{-1} standard in 100 ml deionised water	2.5 μmol l^{-1}

7. THE OPEN TUBE CADMIUM REACTOR (OTCR)

The Open Tube Cadmium Reactor (OTCR) is a 24 turn cadmium coil with the same internal dimensions as the glass mixing coils. Its function is to reduce the nitrate present in the sample to nitrite (see Section 5.2). It needs careful use and maintenance to ensure long life and efficiency of reduction.

When not in use, the OTCR must always be stored full of the stock Imidazole buffer solution, and the jump leads on each end sealed together using an appropriate piece of transmission tubing. At no time must the interior of the OTCR be exposed to air, since oxygen will react with the cadmium to form cadmium hydroxide and this will reduce the reduction capacity of the metal.

7.1 Preparation of the OTCR and fixing to the analytical cartridge

Prior to use the OTCR will need activation. This is achieved by forcing a 1:1 solution of stock Imidazole Buffer and stock Copper II Sulphate solution through the length of the coil using a syringe. The coil should be left filled for 5 minutes. In the meantime ensure that the analyser has been running on reagents for a period of at least 10 minutes and that the bubble injection port is supplied by the Nitrogen gas feed valve. Following the activation period clear the copper sulphate from the coil by drawing through only stock Imidazole. Remove the jump lead joining the first two glass mixing coils on the nitrate/nitrite line and inserting the OTCR between them. It is essential that Nitrogen is continuously fed along the nitrate/nitrite line from now on. Make sure the bubble pattern is re-established before commencing analysis.

7.2 Removal of the OTCR from the analytical cartridge

The OTCR can be removed form the cartridge once all the samples and standards have been run. It must be carried out whilst the system is still running on reagents. Disconnect the two jump leads on each side of the OTCR and reconnect the glass coils. Immediately upon removal, the OTCR must be refilled with stock Imidazole buffer and the ends resealed. Make sure that the jump leads are joined together using an appropriate length of transmission tubing.

7.3 Assessment of reduction

Clearly the accuracy of the method is heavily dependant upon the complete reduction of nitrate in the sample. If the coil is activated correctly the pH of the buffer

solution ensures that reduction stops at nitrite. If the coil is overactive the nitrite can be further reduced to nitric oxide and a false low result obtained. In contrast the coil may not be sufficiently activated causing only part reduction of the nitrate to nitrite; again producing a low result.

To ensure the OTCR is working correctly it is important to carry out the following procedure

1 Prepare a 25 μmol l^{-1} nitrite standard and run without the OTCR installed.

2 Install the OTCR and rerun the standard.

3 Prepare a 25 μmol l^{-1} nitrate standard and run with the OTCR installed.

All peak heights should be identical. If the nitrite peak is lower when the OTCR is installed then it is likely that the nitrite is being further reduced and the column is overactive. The coil can be deactivated by continually aspirating a high concentration nitrite standard, whilst watching changes in the chart recorder baseline. When it has increased to a stable level remove the OTCR and repeat 1 and 2.

If the nitrate standard peak is lower than the nitrite standard peak (2 and 3 above) then the reduction efficiency of the coil is in question. Reduction efficiency is defined as

$$\frac{\text{Nitrate peak height}}{\text{Nitrite peak height}} \times 100$$

For accurate analysis it should be 100% but in practise efficiencies over 95% are quite acceptable. If the efficiency is lower than 90% the coil should be reactivated as detailed in 7.1.

8. SAMPLE COLLECTION AND STORAGE

Seawater samples are collected from depth using General Oceanic 10 litre Niskin bottles fitted to a twenty four bottle CTD rosette package. The bottles are subsampled for nutrient analysis as soon as the CFC and oxygen subsamples have been taken.

The samples are drawn directly into transparent 30 ml Elkay polythene diluvials with press fit caps. Care must be taken to ensure that both the vial and its cap are thoroughly rinsed with the sample water at least three times before filling. Do not touch the inside of the diluvial or cap.

Samples should be analysed as soon as possible after collection. If more than a two hour delay is anticipated the samples should be stored refrigerated at 4°C; they will keep for up to 24 hours. **Seawater samples should not be frozen if high quality data are required.** Experiments have shown that once frozen sample stability cannot be guaranteed; up to 25% difference between analysis of duplicate pairs have been observed. Neither should samples be stored with preservative since they destroy the activity of the OTCR.

9. ASSEMBLY AND OPERATION

This section gives details of the procedures required to setup and run the JRC continuous flow analyser. The tasks are listed in an order which should aid the user to quick and efficient operational status.

9.1 General assembly

9.1.1 Setup

- Remove wooden lid from the continuous flow assembly and the front and rear panels from the recorder/interface box.
- Unpack the computer and the printer.
- Position all the components of the system as shown in Figure 1 and at sea fix all components securely to benches/bulk heads.

9.1.2 Electrical connections

- Connect the power unit to the appropriate connections on the rear panels of the pump unit, the analytical cartridge unit (heating baths) and the photometer units.
- Connect the sample unit, and the power unit through a plugboard and a 240 V-115 V transformer to the 240 V mains or directly to 110 V electrical supply.
- Connect the chart recorders to the mains power supply. Ensure the input voltage rating of the recorders is appropriate for the voltage supply.
- Using the recorder/photometer cables connect the recorder output from the photometer units to the positive and negative inputs on the top panels of the chart recorders.
- Connect the photometer input leads from the chart recorders to the photometer-computer interface.
- Connect the output socket (RS232 interface) of the photometer-computer interface to the DT2815 A/D expansion board connection at the rear of the PS/2 computer.
- Connect printer to PS/2 via the printer interface socket.
- Ensure the fibre optic light source is connected between the light source output on the back of the 304 unit and the flowcell housing (test and reference, there are 6 connections). Take great care while doing this, the fibre optic cables can easily be damaged if bent.

9.1.3 Plumbing

- Connect 3/8" id tubing to the wash reservoir barbed fitting on the rear of the sample unit and extend to waste. Ensure the tubing is kept to a minimum to allow adequate drainage.
- Connect 3/8" id tubing to the drain port of the analytical cartridge unit and extend to waste.
- Connect the sample wash pump tube to the sample unit wash reservoir inlet port using 1/16" id tubing.

9.1.4 Turntable adjustment

- Make sure that the turn table is locked in position and that there is no play during rotation. To lock the turntable lift off the dics which holds the sample cups and tighten the screws on the side of the rotor with the appropriate allen key.
- Centre the sample probe in the wash reservoir ensuring it is positioned vertically. It can be unlocked, repositioned and relocked by turning the knurled barrel at the base of the probe. Turn clockwise to unlock and anticlockwise to tighten.

9.2 System operation checks

- Turn on the mains power to the power module.
- Check the sample unit is functioning correctly, by setting the sample and wash times to 5 seconds and the stop count to 3. Fill the first 3 sample cups with deionised water. Turn the power switch on, press reset and then start. Ensure that the sample unit follows through a five second sample, a five second wash and a sample tray advance cycle for each sample. The cycle should automatically stop after sampling the third cup and the unit sound three times. During this period ensure that the flow is even and at the expected rate. Any inconsistencies will indicate debris in the sample/wash flowlines.
- Check the sample unit rotate switch causes the sample tray to rotate clockwise and the advance switch moves the probe one place toward the centre of the sample ring.
- Ensure the peck cycle switch is off.
- Turn on the pump module and place all reagent and wash lines in deionised water. Ensure all waste lines on the cartridges are connected to the waste outlets.

- Latch all four pump platens down and observe the flow through the system. Ensure that it is even. Pumping will only begin when all 4 platens are latched. Air valve clicks should be heard every 2 to 3 seconds.
- Observe the flowcell for even flow and presence of debris.

9.3 Routine operation

9.3.1 System on

- Turn on the power supply to the 304 power unit.
- Turn on the heat bath control. The red LED light will flash when the heating bath temperature has stabilised. Rotate the temperature potentiometer (small screw to the side of the heat bath control) clockwise to increase the temperature.
- Ensure the chart switch is in the '<0>' position on the 310 chart recorder units. Turn the power on.

9.3.2 Bubble pattern

- Verify that the bubble gate switch on the side of the photometer is in the on position.
- Place all the reagent lines in deionised water containing 20 ml l^{-1}, 15% w/v sodium dodecyl sulphate.
- Place the sample and wash lines in deionised water.
- Turn on the power to the 302 pump module and 301 sampler unit.
- Ensure all waste lines on the cartridge module are secured inside the drainage ports and latch on all four pump platens.
- Leave system to stabilise for 10 minutes. During this period the system will pump distilled water with surfactant through the cartridge.
- Observe the bubble injectors. Bubbles should be introduced in phase with the pump and the pattern throughout the cartridge should be consistent and even.
- Observe the cartridge for evidence of surging.
- Ensure all bubbles are removed at the appropriate place by the debubbler fittings.

9.3.3 Photometer adjustment

- The standard calibration on the 305A photometer unit should be adjusted to the setting as recommended by the particular flow diagram. This will be variable depending upon the concentration of the highest standard anticipated for the analytical run. Final adjustment to the setting can be made at the start of the sampling procedure.
- For each photometer unit carry out the next 11 steps.
- Set the photometer damp switch to 1.
- Turn the function switch to the sample mode
- Turn the sample coarse gain to to 0.1.
- The liquid display diode on the top of the photometer box should display a three digit number. Rotate the sample fine adjust control until the liquid crystal diode reads marginally below 5.00 V. If this cannot be reached it will be necessary to increase the sample coarse gain control. Adjust it using the lowest possible coarse gain setting in order to avoid distorting the photometer signal. It is essential that almost 5.00 V is reached to obtain the maximum sensitivity from the photometers.
- Turn the function switch to reference mode.
- Adjust the reference coarse and fine gain controls in order to set the reference voltage to marginally above 5.00 V.
- Turn the function switch to the absorbance mode. The display should read 0.00 V ±0.02 V. However this is not always the case with the absorbance mode often showing a negative reading causing errors in the analysis and problems with the software. To alleviate this further adjust the sample voltage below 5.00 V and the reference voltages above 5.00 V so that the absorbance blank reading is +0.1 V.
- Using the recorder '<0>' knob on the 310 recorder unit, adjust the recorder pen to 5 graduations on the chart recorder paper.
- Turn the function switch to the sample mode. Readjust to 5.00 V if necessary. Using the variable input knob on the 310 recorder unit, adjust the position of the pen to 100 graduations on the chart recorder paper.
- Turn the function switch back to the absorbance mode and readjust the baseline if necessary.
- The photometer damp switch may now be corrected to the level indicated by the flow diagram for the particular method.

9.3.4 Baseline check

- Turn both recorders on and observe the baseline recording. Do not proceed until a steady baseline is achieved at this stage. This will ensure correct hydraulic and electronic function of the analyser.

9.3.5 OTCR installation

- Place all the reagent lines in their respective reagents, place the sample and wash lines in artificial sea water and allow the system to stabilise for 10 minutes. Turn on the nitrogen regulator to give gas flow (approximately 100 bubbles per minute measured by aspiration of a beaker of water) to the nitrate valve and ensure consistent positive pressure.
- Install the OTCR as outlined in Section 7.1 and readjust the photometer unit by working again through the steps given above.
- Readjust the sample and reference voltages as necessary to compensate for absorbance changes due to the change of wash solution matrix.
- Readjust the baselines with the recorder zero knob and observe the recorder for a steady baseline.

9.3.6 Analysis

- Load the sample tray observing the loading criteria necessary for the operation of the Softpac software (see Section 10.2).
- Check the stop count corresponds to the number of samples, and the sample and wash times are set to 40 secs.
- Begin the sample run by pressing **reset** and then **start**.
- Collect data as detailed in Section 10.3.
- At the end of the run, the probe will return to the wash solution and remain there. Press **reset**.
- Make sure the baseline is re-established so as to allow for any drift during the analytical run.

9.3.7 Shut down

- Remove the OTCR as detailed in Section 7.2.

- Remove the reagent tubes from the reagents and the wash tubes from the sodium chloride solution and place in a wash solution containing only distilled water. The tubes should remain in the wash for a period of at least 20 minutes to allow the cartridge to clear completely.
- Unlatch the pump platens and turn off the power switches in reverse order.

9.4 Common operational problems

9.4.1 Uneven or broken bubble injection pattern

If this is a problem first listen to the solenoid click and assess whether or not the valve is firing at a regular spaced interval. Erratic firing may simply be due to a borderline setting of the phasing potentiometer which if adjusted very slightly by a half rotation will correct the problem. Erratic firing due to a board fault will require the replacement of the air phaser.

If the board is firing correctly, the problem is likely to be due to back pressure in the cartridge. Back pressure may be caused by a blockage in the flow line, stickiness due to insufficient wetting agent in the reagents, or the use of transmission tubing which is too narrow and subsequently affects the flow. Transmission tubing is available in a variety of bore sizes and whenever a tube is removed it must be replaced with one of exactly the same type. It is possible that a badly designed joint allowing large dead space to develop might be the cause. Ensure that all glass joints butt properly.

9.4.2 Baseline drifting / peaks unable to return to baseline between samples

This is seen mainly with phosphate analysis and very occasionally with silicate. Often it is caused by the complex formed during the analysis adhering to the side of flowcells and transmission tubing. It subsequently desorbs causing curves to become erratic and baselines to rise and fall at unpredictable times.

The coating effects can be reduced to a minimum by periodic washing with weak sodium hydroxide solution coupled with about two hours wash with distilled water between analytical runs. Constant removal and washing of the flow cell is also helpful. It must be remembered that new tubing will coat more quickly and this effect can be reduced by passing an acidic solution of iodine through the system prior to analysis. But take care since excess iodine will stain the transmission tubing. In all instances PVC transmission tubing which coats very readily should be kept to an absolute minimum.

9.4.3 Uneven peak forms

Uneven peak forms are usually caused by stretched pump tubing, blockages and old reagents.

Pump tubing becomes progressively stretched and flattened during its lifetime resulting in variable volumes being added to the sample stream. Since all the pump tubes are flow rated this can have a considerable effect on the proportioning of sample to reagent and can subsequently affect the accuracy of the analyses. Pump tubing should be changed regularly if the system is in constant use; establishing and maintaining a regular change is good practise. Any sign of peak inconsistency which cannot be attributed to blockages or intersample air compression should be corrected by changing the pump tubing for the entire channel.

Blockages usually occur at the cartridge connectors and are often difficult to see. Small bubbles emerging from a connector usually indicate a blockage. The connector should be removed and forcibly flushed through with distilled water using a plastic syringe and appropriate tube sleeving. An uneven flow through the sample aspirator is also an indication of a blockage early in the analysis line.

Caution

Do not pinch transmission tubing to clear blockages in this system. This can cause the tubing to kink and subsequently impede the flow through the system. This method of clearing tubing is only appropriate for larger bore systems. To clear the RFA microbore tubes remove the offending tube from the system and force distilled water through using a syringe.

9.4.4 Peak notching

Notched peaks are a result of proportioning errors introduced by the intersample air bubble. These types of error are never eliminated completely from the system; even when the peaks are problem free, the error due to the disproportionation is still present but is moved to the base area of the peak where it will have no effect on the analyses. If notched peaks are present they should present themselves at the same point of each successive sample peak. Their effect can be overcome by adjusting the length of the transmission tubing from the three way sample splitter to the sample injection point so that the time taken by a bubble to move from the centre of the pump to the injection site is equal to the sample plus wash time. The problem usually only affects the analysis using the largest volume of sample; this is phosphate in the JRC system. If it persists try a slightly larger sample tube.

9.4.5 Steady baseline rise (no chemical reasons)

This is characterised by a steady increase in the baseline which is often very stable, unlike the fluctuating rise noticed with increases due to chemical complexing. It results from faulty or diminishing output from the lamp source supplying the six fibre optic cables to the photometers. It can easily be corrected by replacing the lamp (take care the lamp becomes extremely hot during normal operation).

9.4.6 Erratic signal with no apparent cartridge fault

Erratic signals can occur due to bubble break up just before the sample stream enters the flow cell, thereby causing the photometer to read the bubble signal. The flow cell inlet is at right angles to the direction of flow, and particulates may accumulate at the base of the arm causing the air bubble to split. Additional complications may arise if the feed tubing to the flow cell is kinked in any way. The problem can easily be alleviated by cleaning the flow cell by aspirating with distilled water using a syringe and appropriate transmission tubing.

9.4.7 Regular spiking on an otherwise stable baseline

This is due to the photometer signal being read at the wrong time. The signal is electronically gated so that updating only occurs when there is no bubble in the flow cell. The problem can be solved by adjusting the bubble gate set screw until the spiking disappears. Adjustments should be made by only half a turn at a time.

9.4.8 Untidy peak characteristics and poor precision

A common cause of poor precision coupled with untidy peak characteristics is degradation of the pump tubes. Consequently make sure pump tubes have not become stretched and flattened. If the problem persists check that the transmission tubes are in good order. Also make sure tube connections are tight and clear of debris. Tube connectors become discoloured after time and should be changed prior to a cruise or after any period of prolonged use.

9.4.9 Non-zero reagent blank

Sometimes it is necessary to reduce the photometer signal to zero at the start of an analytical run. This is due to degradation of reagents with time. It is particularly noticeable

on the silicate line, where the blue colour of the silicomolybdate complex is sometimes visible in the working molybdate reagent. This increases the signal from the reagent blank. The result is that there is a gradual loss of sensitivity over a period of time which may not be immediately apparent. Always check on reagent stability. Some reagents are stable for many months others need replacement daily, particularly the working molybdate reagents for silicate and phosphate.

9.4.10 Reagents precipitating out of solution

This occurs if the proportion of wetting agents added to the reagents is too high and as a result of inhomogeneity variable concentrations are delivered to the analytical cartridge over a period of time resulting in poor reproducibility. There is also some evidence that the precipitate may absorb light at the wavelength of analysis resulting in overestimation of colour.

10. ANALYTICAL SOFTWARE

The commercially available Softpac plus software used with JRC system is a fully automated data collection package specifically designed to collect and process the raw data received from the photodetector outputs of the RFA 300 continuous flow system. It enables the user to view real time display of the analytical peaks, to produce calibration curves, to calculate the final concentrations and where necessary to recover the initial data and rework as required. This section contains information which will enable the operator to use the software for routine analysis. It is not intended to give comprehensive coverage of all the functions available and so the reader will be refered to the RFA Softpac operations manual where appropriate. The software is based on a series of menus and the initial set up of a number of default tables. The final data are produced in a spreadsheet format compatible with commercially available software such as Lotus 123. Many of the parameters in these tables will only require setup once and in general the software is easy to use. The package also contains further functions which although not essential to normal operation, may be of some use for data manipulation. Details of these other functions may be found in the RFA PC Softpac operation manual.

10.1 Features of the software

The program starts automatically when the PS/2 is switched on, initially displaying the main menu. The menu has eight commands (plus help) which can be accessed by pressing the appropriate function key, these are as follows

F1 - Help

F2	No function
F3 - Method Table	Sets the general run parameters such as collection rate, channel name etc.
F4 - Sample Table.	Stores the position of sample and standard numbers.
F5 - Display Analog Signal	Allows the operator to see the analog signal display continuously and consequently monitor the stability of the analyses of all three channels simultaneously.
F6 - Spreadsheet.	Display of all calculated data. This allows the user to manipulate the data, generate reports, export data and save to disk.
F7 - Standards table	Stores the value of the standards indicated on the sample table; up to 20 can be defined.

F8 - Calculations	Allows calculations, analog peak editing, standard curve determinations and report printouts.
F9 - Instrument control	Controls the grouping of channels and optional password protection.
F10 - Escape	Terminates the program but not the data collection if a sample batch is running.

10.2 Software setup

- Call up the method table - **F3** and enter 1 at the channel prompt.
- Press **esc**
- Call up the sample table - **F4** and enter the sample numbers remembering that the table fills left to right and not down each column. Move from one location to another using the **RTN** (return) function. The first sample must be identified as SYNC -1 as this is used by the program to identify the position of the first peak and so allow reading of the sample peaks at the same point. To compliment the first sample cup of the analytical run the SYNC cup must be filled with a high concentration standard, preferably the highest standard to be measured. The second sample is the blank. The third is a second SYNC peak with the highest standard concentration. The fourth sample is identified as CO and is used in the calculation of the carryover. This sample must be the same concentration as the sample which follows it, routinely this is a blank. The fifth sample is the blank and this is followed by six standards identified as S1-S6. The standards increment upwards in concentration to the maximum anticipated concentration, which will be S6. To check for drift an appropriate standard is placed in the run after every six samples. The final sample is identified as D2 - drift correction cup and is a sample with the same concentration as S2. An example of the sample table is given in Table 2, the position of standards and blank solutions are common to every run.
- Whilst still in the sample table press **F3** to overlay the sample table with the method screen.
- Fill in the method table by placing the cursor on the appropriate parameter and typing in the relevant value. An example of the method table for routine silicate analysis is given in Table 3. The methods table for the other two parameters are entered by changing the first two parameters only. The JRC system has been set up so that the table names default to Method 1 - silicate; Method 2 - nitrate/nitrite; Method 3 - phosphate.

TABLE 2

AN EXAMPLE OF THE SAMPLE TABLE

	ID	Dil	Wgt		ID	Dil	Wgt
1	SYNC	1	1	2	B	1	1
3	SYNC	1	1	4	CO	1	1
5	B	1	1	6	S1	1	1
7	S2	1	1	8	S3	1	1
9	S4	1	1	10	S5	1	1
11	S6	1	1	12	samp	1	1
13	samp	1	1	14	samp	1	1
15	D2	1	1	16	samp	1	1

...../con

last sample = D2 (Drift correction cup = S2)

TABLE 3

AN EXAMPLE OF THE METHODS TABLE

CHANNEL #= [1]

CHANNEL NAME = SILICATE

START IGNORE TIME = [0]

INITIAL BASELINE LEAD TIME = [80]

FINAL BASELINE LAG TIME = [80]

CORRECTIONS CODE Y/N [Y]

CYCLE TIME = [80]

COLLECTION RATE = [1] POINTS/SEC

- Press **F7** to call up the standards table.
- Fill in the standards table by placing the cursor in the relevant position and typing in the required value. An example of the standards table is given in Table 4. Such

a table is set as the default in the JRC system. The dil (dilution) and wgt (weight) columns are not used for routine JRC work.

TABLE 4

AN EXAMPLE OF THE STANDARDS TABLE

Calibration Code: 1

Units: umol /l

Name: silicate

S1	0	S11	0
S2	10	S12	0
S3	20	S13	0
S4	30	S14	0
S5	40	S15	0
S6	50	S16	0
S7	0	S17	0
S8	0	S18	0
S9	0	S19	0
S10	0	S20	0

- Now press **esc** twice to leave only the sample table displayed.
- This table must now be named by pressing **ALT N** . The system will prompt for a table name. Enter the table name eg the Station number with the suffix S (for silicate, P or N for phosphate and nitrate).
- The table must now be saved. The sample table, method table and standard table are saved together. Press **ALT S** and enter the name of the table. This stores the table in a file with the extension .**TBL**., in the directory **SOFTOPAC\DATA**. The table can then be loaded at any time to examine a particular run.
- Repeat the above procedure for all three channels. The only alterations required are the channel number and name in the method table and the standard values in the

standard table. The tables can then be stored with a similar name i.e station number suffixed with either N or P.

(**ALT.C** is a useful command which allows the contents of table 1 to be copied to table 2 and so save time retyping all the sample numbers. Press **ALT C** and the screen will prompt for a start and end cup. It is only necessary to change the table number to 2 in the second column and press **ctrl-Enter**. All entries will then be copied from the first table to the second.)

- Press **F9** from the main menu and set up the software as shown below to collect data from all three photometer outputs simultaneously when Group 1 is activated. This is done by positioning the cursor with the arrow keys and then marking the point with the space bar. Once set up, the markers will not need to be moved unless one of the channels is out of service.

Group	Channel			
	1	2	3	4
1	X	X	X	
2				X
3				
4				

- Press **F5** from the main menu to display the analog signal on the screen.
- Ensure that the display ON/OFF indicators are set to **On** for the first three channels.
- Set the chart speed to 60 cm/hr, and the screen/chart to SCR. All other settings should be zero.

10.3 Data Collection

Having set the variables as described above, the software is ready for data collection. It is possible to set up the software whilst the analyser is stablizing on reagents. To initialize data collection

- Press **RESET** and then **START** on the sampling tray.
- When the sample probe enters the first sample, initiate data collection by escaping to the main menu.

- Press **ALT 1** and the screen will prompt for a file name into which the data will be stored. Enter the **station number** (no more than 8 digits) and press **RETURN**. The first three channel indicators at the top of the screen will change to **ON**.
- Press **F5** followed by **F3** to view the signal from the three photometers. The screen should be left on during the run.
- At the end of the run, press **ESC** twice to exit to the main menu.
- Press **ALT 1**. The screen will ask whether to pause or stop.
- Once a satisfactory baseline has been achieved press **S RETURN** to stop data collection.

During operation, the screen will display data held in the buffer memory. Periodically, the data will be transfered to the hard disk, and the screen will be cleared. It is possible to exit the software program during data collection by escaping to the main menu and pressing **F10** followed by **S**. This will exit the program to **DOS**. To return to the Softpac program simply type **EXIT** at the prompt. Data collection will not be affected.

At the end of the analytical run, three data files will have been produced and stored in the subdirectory **C:\SOFTPAC\DATA**. The files will have the following extensions.

filename. **DAT**	= channel 1	=	SILICATE
filename. **D12**	= channel 2	=	NITRATE + NITRITE
filename. **D13**	= channel 3	=	PHOSPHATE

10.4 Peak editing and calculation of results

The calculations submenu is accessed by pressing **F8**. The menu contains six functions. **F3**, **F4** and **F7** which allow the operator to change the method, sample and standard parameters as previously described in the setup. This is especially useful for calling up old data files which may have been analysed with different parameters. Normally however the set parameters used during the sample run are used and no alterations are required.

The **F6** function key is used after the calculation process to load the spreadsheet and allow results to be printed.

- Press the **F8** function key to begin the calculations. It is normal to calculate the results from the raw data files generated by the sample run. The calculations

submenu also allows the operator to calculate corrections, plot a standard curve or generate a report.

- Press **F8** again and highlight the **FROM RAW DATA** selection and press **enter**. The system will prompt for the filename. To list the data files stored in the program, type *.* and press enter. A file can then be selected from the list and loaded.
- Setup the calculation parameters displayed on the screen as follows

Invert raw data	N
Plot curve	N
Auto/Interactive	INTER
Decimal places	2
First sample	1
Peak height/area	HEIGHT
Threshold	10
Ascending slope	1
Apex	10
Descending slope	1
Plateau points	3
Integration points	3
Execute	Y

From the information above, the peak heights are matched with the sample id's in the sample table, and a raw data curve is plotted and displayed on the screen together with a number of function keys which are used for editing. The peaks are also marked to indicate the position at which the peak height is read. If the sample or standard table has not been loaded correctly a series of error messages will occur. These can be dealt with by escaping from the calculations window and making the necessary changes. This must be done in order to obtain a full spreadsheet of results.

- Use the **F3** (move back) and **F4** (move forward) functions to step through the data one peak at a time allowing editing of a selected peak.
- **Press F5** to edit a peak. The position of the peak height reading can be moved by using the cursor arrows and pressing **F2** to re-mark the point. The **F5** submenu will

also allow close inspection of the peak characteristics by using the **F4** and **F5** zoom functions.

- Press **F10** to escape to the main peak editing display.
- Press either **F7** or **F8** to delete or insert peaks respectively. Beware that deletion of peaks in this way will cause complications with the sample table and is not recommended; it is best to highlight any errors on the final spreadsheet.
- Press **F9** upon completion of all editing and the calculation process will continue. At this point the program will apply the corrections as specified in the sample table. i.e baseline, carryover and drift correction.

10.5 Standard calibration curves

After the above functions have been completed, the standard curve will be calculated and displayed on the screen. The standard curve is printed with a function menu to allow editing.

- The calibration code is set to 1 for a linear curve.
- To edit the data points, press **F3** and move the X with the direction keys.
- Press **F2** to delete a standard point from the curve.
- Press **F5** to replot the point.
- Complete all changes (a curve with correlation 0.999 or better should be achieved) and press **F9** to continue. The report of the individual sample data will then be displayed. The display will include sample numbers, peak heights, calculated results and error flags (see Section 10.8).
- Press **F9** to generate a spreadsheet from the report sheet. When the report screen is exited, the calculated results are added to the **CAL**. file.

Having pressed **F9** the procedure will be repeated for each of the other two channels starting with the calculations menu as described above. The program will display the datafile name with the extension **D12**. This indicates that it is ready to calculate the raw data collected from the second channel. All the calculations derived from the three sets of raw data will be stored in the spreadsheet initially generated for Channel 1.

10.6 Data files

The directory **C:\SOFTPAC\DATA** will now contain the following files.

station number S.TBL	Silicate table specific to station, containing details of the samples and standards, together with the method and standard tables.
station number N.TBL	Nitrate table specific to station containing details as above.
station number P.TBL	Phosphate table specific to station containing details as above.
station number.DAT	Raw data file containing raw digital output from the first channel (silicate).
station number.D12	Raw data file containing raw digital output from the second channel (nitrate).
station number.D13	Raw data file containing raw digital output from the third channel (phosphate).
station number.CAL	File containing calculated data.

10.7 Spreadsheets

When all data have been collected from all three channels, a spreadsheet file is generated and identified as the filename plus the extension .**LDT**. The spreadsheet is specifically designed for scientific applications. There are a number of quality control and statistical calculations which may be incorporated into specific method files. These allow manipulation of data within blocks, and the combination of spreadsheets. The reader is refered to the Softpac manual for the numerous functions available in the spreadsheet option.

- Press **F6** to access the spreadsheet file. The spreadsheet, which will load automatically, will contain the calculated data from all three channels.
- Press **ALT F** and scroll down to the save option to save a file. Press **enter** and the system will prompt for a filename, which can be entered as the station name. (i.e the same name as the original raw data file). Do not use the .**LTD** extension since it is best not to overwrite the original spreadsheet; it is useful to keep the peak heights resulting from the initial calculations from the raw data and any error codes.
- Press **ALT F** and scroll down to the print option to print the spreadsheet. Press **enter** and the system will ask if you require the information in 132 lines and with a border. Press **y** to print 132 lines and **n** to print border.

- A file can be loaded from memory by pressing **ALT F** and selecting the load option. Press **enter** and answer **n** (no) to the default request to overlay. The program will then ask for a filename and the name should be entered without the .**LDT** extension.
- Press **ALT F** and scroll down to the print option to copy spreadsheet files directly to a disc. Press **enter** and when prompted for a filename to print to, enter the target disk plus filename.e.g **A:\filename**. The spreadsheet will be copied onto a disk in the A diskdrive.
- Press **F10** to exit from the spreadsheet.

10.8 Error flags

The data point error flags are listed below

O	Peak off scale. A dilution factor will be reported
I	No peak found. The peak position is interpolated and the concentration reported
–	Calculated value is below zero
b	Baseline correction flag
d	Sensitivity drift cup
c	Carryover correction cup
M	More than one peak found
s	SYNC cup
N	Noisy peak due to unsteady signal

10.9 Additional software functions

The following is a list of functions available in the software package which although not essential to routine operation, may be useful for data manipulation. The various functions available are listed according to the major function they come under from the main menu screen.

10.9.1 Within **F4** - sample table

The functions are listed at the top of the sample table. To access any one of the them press **ALT** followed by the first letter of the required function. The functions which are available are as follows

ALT N - Name — Assigns a name to the sample table. Input the table name at the prompt and press **enter**.

ALT E - Erase — Deletes a set of samples identified in the sample table. The samples must be sequential. At the prompt, input the start and end cup number and press **ctrl-enter**.

ALT P - Print — Allows the operator to print all or part of the sample table. Input the start and end cup number and press **ctrl-enter**.

ALT L - Load — Loads a sample table which has previously been named and stored. All sample tables are stored in the directory **SOFTPAC\DATA** with the extension .**TBL**. Input the name of the sample table to load the table itself plus details of the standard table and method table stored along with it.

ALT S - Save — Saves a sample table along with details contained in the method table and standard table. Input the table name and press **enter**.

LT MA - Move — Moves a portion of the sample table to another part of the same table or to another table.

ALT C - Copy — Copies a sample table from one table to another. Type in the start and end cup numbers and press **enter**.

ALT F - Fill — Increments the numerical value of a cup.

10.9.2 Within **F6** - spreadsheet

At the top of the spreadsheet there are a number of functions which are accessed by pressing **ALT** followed by the first letter of the required function.

ALT M - Method — Allows the operator to create, save, delete or run a spreadsheet method. For example a spreadsheet method may be set up to process all data files in order to remove or rearrange data to produce a consistent form of report.

ALT F - Files	Allows access to the spreadsheet files. The submenu allows files to be loaded, saved. imported, exported, printed and cleared.
ALT C - Calculate	Allows recalculation of all cells in a spreadsheet when a value or formula has changed.
ALT S - Sort	Allows data to be sorted into groups, eg numerical or alphabetical order.
ALT E - Edit	This gives routines which allow inserting, deleting, moving, copying, reformatting, squeezing, filling and adjustment of column widths.

10.10 Exiting Softpac and backing up data

All softpac files must be backed up to disc daily.

- Press **F10** and the **E** to exit. The prompt **C:\SOFTPAC>** appears.
- Type **cd\softpac\data**.
- Type **DIR** to list the raw data files.
- Use the **copy *.* a**: command to copy files to disc.
- To continue using softpac type **Softpac** to reload
- Type **Parkheads** if you wish to turn off the IBM PS2

11. ROUTINE CLEANING AND MAINTENANCE

This section contains information regarding the routine cleaning and maintenance of the RFA system together with an indication of frequency. For normal operation, no maintenance is required on the 301 Sampler unit, 304 Power unit, 305A Photometers, 310 Recorder units or the 406 Photometer PC interface.

11.1 General.

- Empty all waste containers.
- Clean the exterior of all units.
- Dispose of all waste reagents, sample cups and standard solutions.

11.2 302 Pump unit

- Clean the pump platen surfaces using a tissue wetted with propan-2-ol (daily)
- Remove the pump tube shoulders from the pump unit and clean the rollers on both of the pump bodies using a tissue wetted with propan-2-ol (weekly).
- Remove the pump bodies and clean the roller cage (monthly).
- Lubricate the gears using a few drops of general purpose oil (monthly).

11.3 303 Cartridge unit

- Pump the phosphate line through with 0.1N Sodium Hydroxide for a period of twenty minutes, followed by deionised water for a further half hour (weekly).
- Replace all pump tubing (every 10 days). When replacing pump tubes, it is important to make sure that the new tube is cut to exactly the same length as the old. In this way the total analysis time will not be affected. Generally, tubes should be cut as close to the shoulder as possible and transmission tubing used to carry the sample or reagent stream onto the cartridge.
- Replace all transmission tubing (2-3 monthly). There are a number of similar types of tubing and it is essential that the correct diameter tubing is used and it is replaced with exactly the same length to maintain the correct flowrate.

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APPENDIX 1 SPARES AND ACCESSORIES FOR THE RFA 300

Sample unit

Part number	Description
0036-A301-0223-01	Probe retaining shaft
0037-A301-0224-02	Probe holder
0038-A301-0232-00	Probe, stainless steel
0041-A301-B231-00	Probe coupler assembly
0042-A301-B302-00	Tray assembly, sampler 301
0043-A301-B304-05	Tray cover assembly small cup

Tubing

Part number	Description
0097-A116-0536P06	0.025 Trans tubing (10ft)
0098-A116-0536P09	0.040 Trans tubing (10ft)
0100-A116-0536P17	0.100 Trans tubing (10ft)
0088-A303-2015P05	Trans tubing, 0.015 polyethlene
0089-A303-2020P03	Trans tubing, 0.020 silicone
0090-A303-2030P05	Trans tubing, 0.030 polyethylene
0091-A303-2034P05	Trans tubing, 0.034 polyethylene
0118-A303-1010P01	Pump tube orange / blue 37 µl
0107-A303-1015P01	Pump tube orange / green 74 µl
0108-A303-1020P01	Pump tube orange / yellow 118 µl
0109-A303-1025P01	Pump tube orange / white 166 µl
0110-A303-1030P01	Pump tube black / black 226 µl
0111-A303-1035P01	Pump tube orange / orange 287 µl
0112-A303-1040P01	Pump tube white / white 385 µl
0113-A303-1045P01	Pump tube red / red 482 µl
0114-A303-1051P01	Pump tube grey / grey 568 µl
0115-A303-1056P01	Pump tube yellow / yellow 642 µl
0116-A303-1060P01	Pump tube yellow / blue 722 µl
0117-A303-1072P01	Pump tube green / green 947 µl

Coils

Part number	Description
0072-A303-0205-00	5 turn coil clockwise
0073-A303-0210-00	10 turn coil clockwise
0074-A303-0215-00	15 turn coil clockwise
0075-A303-0225-00	25 turn coil clockwise
0076-A303-0305-00	5 turn coil anticlockwise
0077-A303-0310-00	10 turn coil anticlockwise
0078-A303-0315-00	15 turn coil anticlockwise
0079-A303-0325-00	25 turn coil anticlockwise

Fittings

Part number	Description
0095-A116-0003P01	Nipple N-8
0096-A116-0061P01	Nipple N-13

0057-A303-0100-00	Injection fitting, single 0.8 mm id
0058-A303-0101-00	Injection fitting, double 0.8 mm id
0065-A303-0108-00	Injection fitting, single 1 mm id
0064-A303-0107-00	Injection fitting, double 1 mm id
0060-A303-0103-00	Debubbler
0061-A303-0104-00	Debubbler - rebubbler
0067-A303-0111-00	Sample stream splitter, 4 pt
0062-A303-0105-00	Glass T
0059-A303-0102-00	Reagent addition tee
0063-A303-0106-00	Resample injection tee 0.5 mm id
0000-A383-0304-00	1/16 id male luer, poly

Flowcells

Part number	Description
0092-A305-0110-00	10 mm flowcell
0000-A305-0115-00	15mm flowcell
0000-A305-0130-00	30 mm flowcell
0000-A305-0704-00	Flowcell holder

Filters

Part number	Description
0000-A305-1540-00	Filter 540 nm
0000-A305-1820-00	Filter 820 nm
0000-A305-1880-00	Filter 880 nm
0000-A305-0705-01	Filter holder

Cartridge unit

Part number	Description
0000-A303-0705-00	Analytical cartridge tray
0000-A303-0706-00	Tray cover plexiglass
0000-A303-A001-01	Cartridge base , module
0083-A303-0708-00	Coil clip
0084-A303-0709-00	Fitting clip
0000-A303-0707-00	Drip tray assembly

OTCR

Part number	Description
0079-A303-0500-24	Open tube cadmium reactor 24"

Air valves

Part number	Description
0000-A373-0300-00	Air valve , 2 way

Heating baths

Part number	Description
0000-A303-B420-00	Heat bath , 2 ml
0034-A157-0283-08	Heat bath thermometer (36°C - 60°C)
0000-A381-0202-00	Heater , flexible 50 Watt

Recorders and Printer

Part number	Description
0102-A011-0970-01	Chart paper Recorder 310
0103-A011-9414P05	Recorder pens blue long
0104-A011-9414P06	Recorder pens red short
0000-A384-B006-00	Printer ribbon , okidata 193 plus

Electronic circuit boards

Part number	Description
0000-A304-B002-00	Temp controller PCB complete
0000-A305-B002-03	Photodiode PCB 305 and 305A
0000-A305-B003-09	Bubblegate PCB 305A
0000-A305-B004-01	Log ratio PCB 305A
0000-A305-B005-09	Display PCB 305A
0000-A305-B006-06	Switch PCB
0000-A305-B007-01	Gate adj PCB

Cables and switches

Part number	Description
0000-A304-0501-03	Hex fibre assy with lamp holder
0000-A304-0503-00	Qtz hex fibre assy short 304 module
0000-A381-0100-00	Lamp socket/fibre optic coupler
0000-A381-4000-00	Universal AC power cable
0000-A381-4001-00	305 DC power cable 1
0000-A381-4002-00	305 DC power cable 2
0000-A381-4003-00	305 DC power cable 3
0000-A381-4004-00	303 Heat bath power cable
0000-A381-4005-00	302 DC power cable
0000-A381-4008-00	Recorder cable 1
0000-A381-4009-00	Recorder cable 2
0000-A381-4010-00	Recorder cable 3
0000-A381-4011-02	Cable , 404 interface to recorder 6 ft
0000-A381-4014-00	Cable , RFAC rec to 404 int 6 ft
0000-A381-4017-01	Cable , RFAC samp to 404 int 6 ft
0000-A381-4020-01	Cable , samp iv to 404 int 6 ft
0000-A381-4023-00	Cable , AS175 to 404 int 6 ft
0000-A381-4029-00	Cable , RFAC/305 6ft
0000-A381-4032-00	Cable , RFAC/SCIC 6 ft
0000-A381-4035-00	Cable , RFAC/printer 6 ft
0000-A381-4041-00	Cable , RFAC/CRT 6 ft
0000-A381-4051-00	Cable , 240 samp to 404 int 6 ft
0000-A381-4052-02	Cable , 301 samp to 404 int 6 ft
0000-A381-4054-00	Cable , 404 int to Bristol rec 6 ft

0121-A381-0047-01	Lamp source button end w/o leads
0000-A381-2000-00	Switch , safety interlock
0000-A381-2019-00	BCD thumbswitch

Tools and assessories

Part number	Description
0000-A373-0601-00	Fan RFAC
0000-A381-8000-00	Finger guard , fan RFA
0000-A381-8001-00	Finger guard , fan RFAC
0000-A382-1001-CG	2/56 x 1/16 setscrew
0000-A382-2G02-CG	4/40 x 1/8 setscrew
0000-A382-3G04-CG	6/32 x 1/4 SS setscrew
0000-A383-B900-01	Allen wrench set
0000-A383-0200-00	Rubber bumper
0000-A383-0300-00	Grommet , 0.5 X 1.0
0000-A383-0303-00	Grommet , small
0000-A383-0400-00	Grommet , universal
0000-A383-0305-00	Luer lock ring black , nylon
0000-A382-2J04-SJ	4/40 x 1/4 black button head
0000-A538-0003-01	Silicone oil
0000-A538-0006-01	Lubriplate
0087-A303-0811-00	Weight , reagent line

Pump

There are numerous components to the pump, its body, base and outer assembly. These are given comprehensive coverage in the ALPKEM manual and so are not repeated here.