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Automated Analysis of Nutrients in Seawater

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AUTOMATED ANALYSIS OF NUTRIENTS IN SEAWATER

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Abstract

This report describes a continuous-flow autoanalyser and its use to determine the concentrations of the following dissolved nutrients in seawater: ammonia nitrogen, nitrate nitrogen, nitrite nitrogen, organic phosphate, total phosphate and reactive silicate.
Details are given on (i) sampling and storage of seawater prior to analysis; (ii) comparisons of nutrient concentrations determined by automated methods and manual methods with the use of standard addition techniques for validation; (iii) problem-solving procedures to maintain optimum instrument operation.

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ABBREVIATIONS AND NOTATIONS

AR	Analytic reagent
PW	Distilled water that has been dionized and filtered through a MILLI-Q triple bed unit
SW	Seawater
LNSW	Low-nutrient oceanic surface water from a 20 l plastic container, and agitated daily to aerate. This enhances algal growth which removes the nutrients. The water is then filtered.
NFSW	Nutrient-free seawater prepared by passing through Zirconium hyrodoxide to remove traces of silicate and phosphate
SI	Computer notation for silicate
SiO_4^{3-}-Si	Chemical notation for silicate
PO	Computer notation for phosphate
PO_4^{3-}-P	Chemical notation for phosphate
NH	Computer notation for ammonia
NH_3-N	Chemical notation for ammonia
N2	Computer notation for nitrite
NO_2^--N	Chemical notation for nitrite
N3	Computer notation for nitrate
NO_3^--N	Chemical notation for nitrate
SP	RV "Sprightly"
ID	Internal diameter
OD	Outside diameter

DF Dilution factor: the volume of sample divided by the final volume of diluted sample

RSD Relative standard deviation

Common Units:

l - litre; µ l - microlitre; ml - millilitre; m - metre; nm - nanometre; g - gram; µ g - micrograms; (µ g-atom) - microgram atom (to convert µ g to µ g-atom divide by atomic weight (N = 14, P = 31, Si = 28)); min - minute, oz - ounce.

PART I

THE NEED FOR AUTOMATED NUTRIENT ANALYSIS

1.1 INTRODUCTION

Automated procedures for the analysis of water samples for nutrients are preferable manual methods when large numbers of samples are to be processed; where continuous rather than batch sampling is required; and where samples have a reasonably consistent matrix and do not require individual handling during analysis, e.g. standard addition.

The advantages of an automated analyser in sea-going work are:

- Speed of analysis. Samples can be processed in real-time, either in batch mode or as a continuous flow.

- Small samples. The volume of water available for each analysis is limited by the size of the samplers and the number of analyses to be done. As automated methods require less water than manual methods, more water remains for other purposes.

- Precision of analysis. Human error is minimised, provided the operator fills the sample cups correctly.

- Collection of a new type of data. Discrete samples provide discrete data points and interpolation is necessary. Only large oceanographic features can be examined from these data. A continuous flow inlet and an autoanalyser provide continuous nutrient concentration data limited in resolution only by slurring of the sample stream in the intake line.

- Real-time data display. An autoanalyser linked to a microprocessor and visual display unit can provide real-time concentration maps or profiles of water bodies. This allows immediate recognition of significant hydrological features so that experiments can be done 'in situ'.

1.2 COMPARISON OF MANUAL AND AUTOMATED CHEMICAL ANALYSES

Intercalibration of manual and automated procedures is necessary to detect systematic bias in either method, and to ensure that data from automated analyses is compatible with data from manual analyses already stored in world data banks.

The automated procedures were checked for consistency by (i) randomly analysing duplicate samples by the method described here, and (ii) analysing water from the same sample using the automated method and a standard addition technique.

Inter-methodological comparisons were made by analysing (i) common standards made up of low-nutrient water (LNSW), distilled water, deionised water and artificial seawater, (ii) seawater samples collected from the same Niskin bottles at stations in the Tasman Sea but stored in different containers and preserved by previously tested preservation methods.

1.2.1 Comparison of duplicates

Two samples were bottled from each Niskin bottle and preserved. Figures 1-5 show the concentration of nutrient in the first sample plotted against that in the second sample for the five nutrients described in Section 2. The water samples were taken at each of ten stations from vertical profiles from the surface to $\leq$ 5000 m. Each symbol in the figures appears approximately 14 times (the number of depths sampled at each of the ten stations). Identical concentrations produce a straight line with a gradient of one and an intercept of zero (the 1:1 line). Analyses of duplicate samples by autoanalyser methods were found to agree within the accuracy of the method (see Table 1).

1.2.2 Inter-methodological comparison

Standards: Stock standards made up independently and prepared in distilled, deionized water, were used to calibrate the manual (Major *et al.* 1972) and the

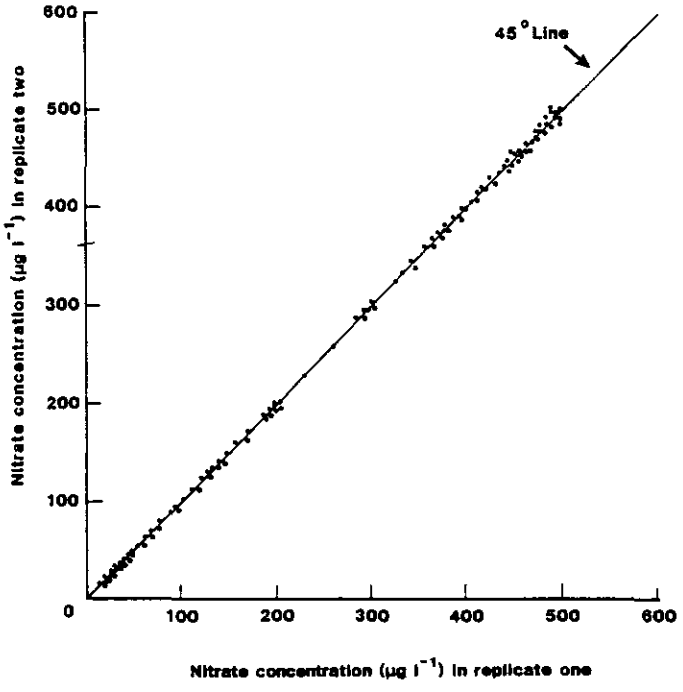

Figure 1. Comparison of duplicate ammonia determinations by autoanalysis ($\mu g\ l^{-1}$)

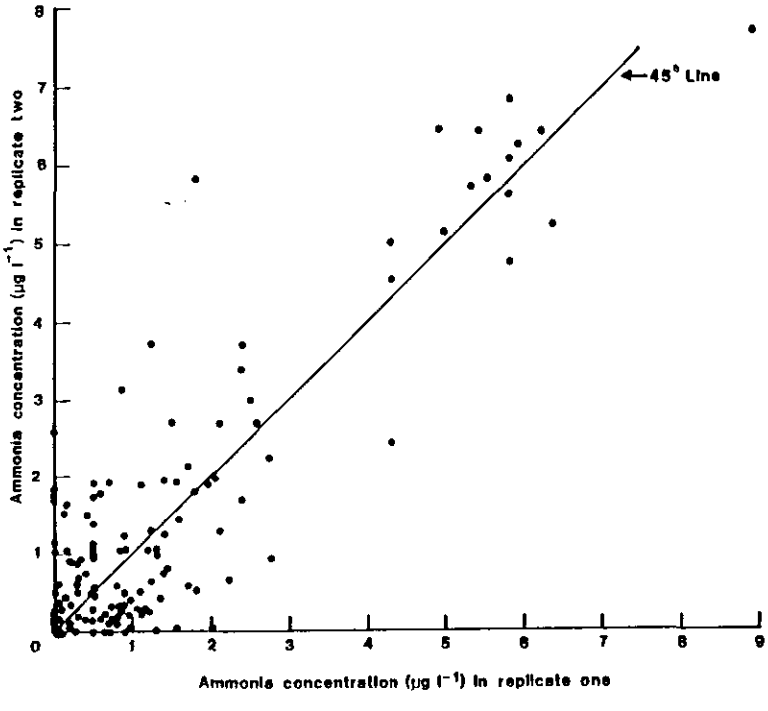

Figure 2. Comparison of duplicate nitrate determinations by autoanalysis ($\mu g\ l^{-1}$)

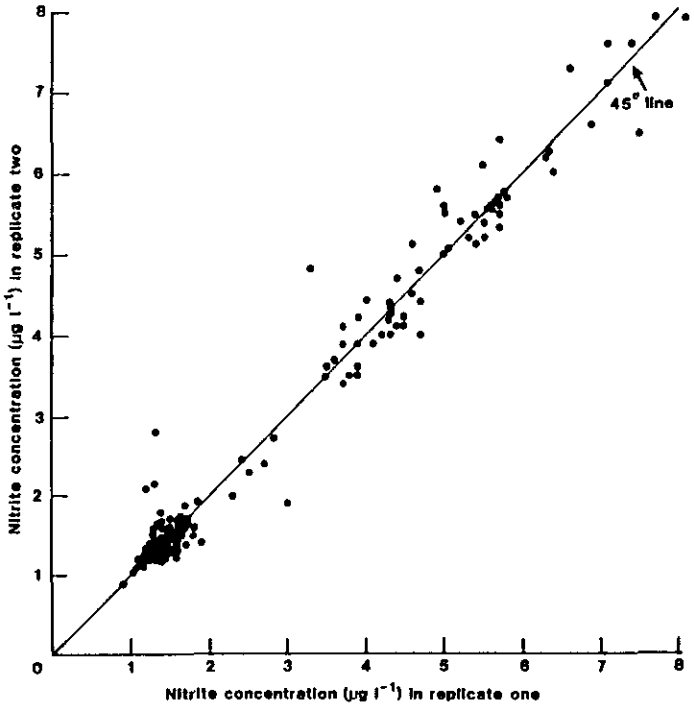

Figure 3. Comparison of duplicate nitrite determinations by autoanalysis ($\mu g\ l^{-1}$)

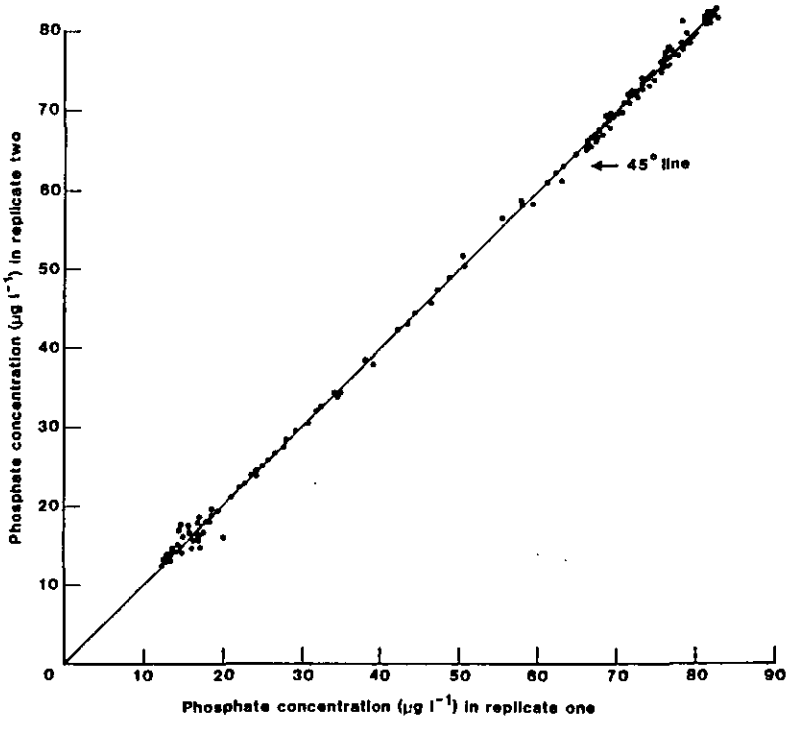

Figure 4. Comparison of duplicate phosphate determination by autoanalysis ($\mu g\ l^{-1}$)

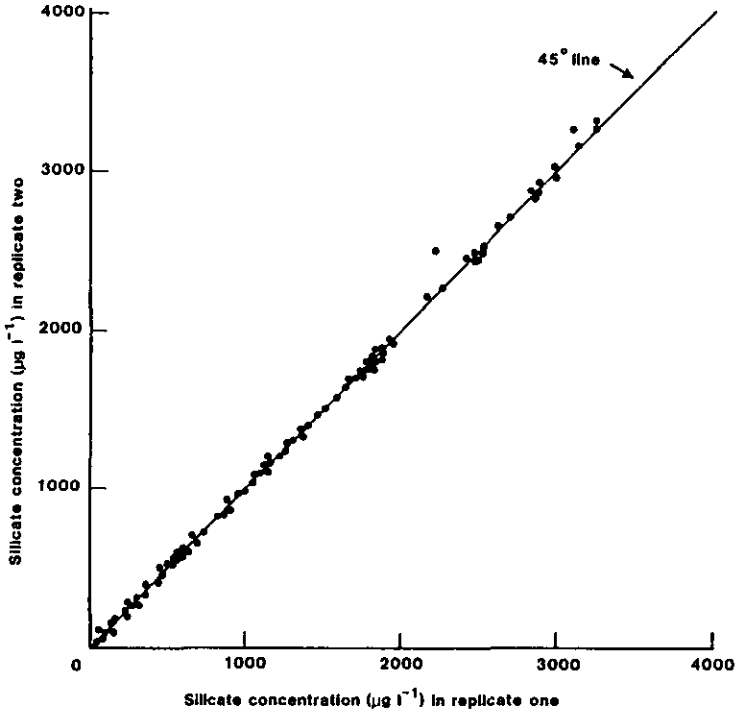

Figure 5. Comparison of duplicate silicate determination by autoanalysis (µg l^{-1})

Table 1. Comparison of duplicate sample concentrations (µg l^{-1}) determined by autoanalysis

Nutrient	Equation of plot of duplicate vs duplicate Y = mX+c	r^2	t Statistic[a]	Conclusion
Ammonia	Y = 0.927 X+0.220	0.762	-1.70	m = 1[b]
Nitrate	Y = 1.007 X+0.129	1.000	0.35	m = 1
Nitrite	Y = 0.994 X+0.038	0.975	-0.46	m = 1
Phosphate	Y = 1.008 X-0.490	0.989	-1.70	m = 1
Silicate	Y = 1.008 X-2.83	0.998	2.00	m = 1

[a] t is computed to test whether m = 1 at the 1% confidence level.

[b] m = 1 is interpreted to mean that duplicate samples are equivalent within the precision of the procedure for sampling, storage and analysis.

r = correlation coefficent.

automated methods. Cross-checks for consistency of these stock standards for silicate, phosphate and nitrate are shown in Figures 6-8. Stock standards normally used to calibrate manual methods were treated as unknowns and determined by comparing them against standards used to calibrate automated methods. Results are expressed as plots of expected concentration levels versus the corrected peak height on the autoanalyser recorder.

Sagami Chemical Company standards, prepared in artificial seawater of salinity 30.5‰, were analysed for comparison of silicate and nitrate concentrations using manual and automated methods. Results are shown in Table 2 and indicate good agreement of the two methods.

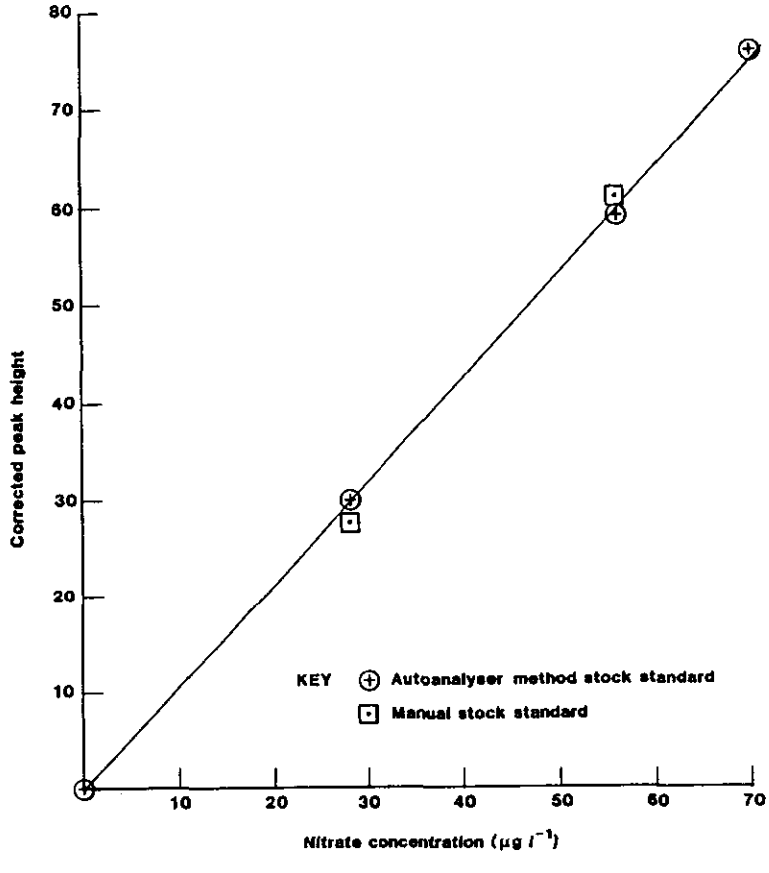

Figure 6. Expected nitrate concentration (µg l^{-1}) in stock standards used to standardize automated and manual methods versus corrected peak height when both are determined by autoanalysis

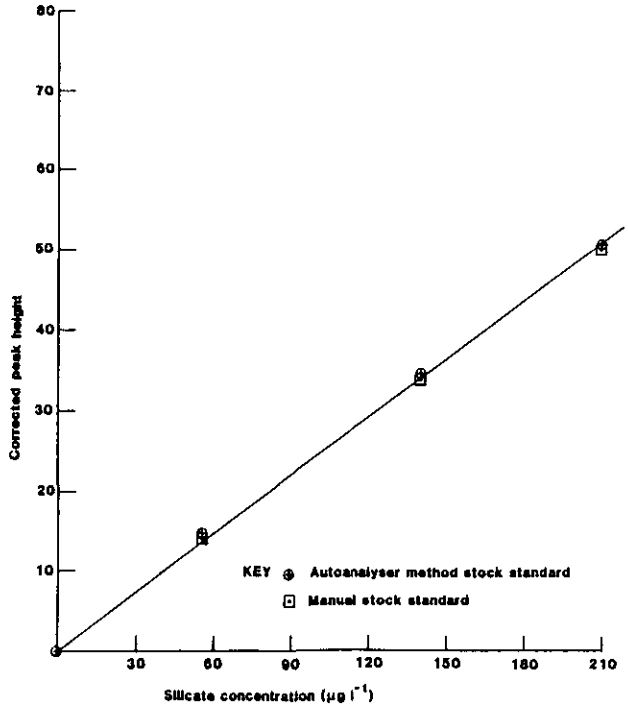

Figure 7. Expected phosphate concentration (µg l^{-1}) in stock standards used to standardize automated and manual methods versus corrected peak height when both are determined by autoanalysis

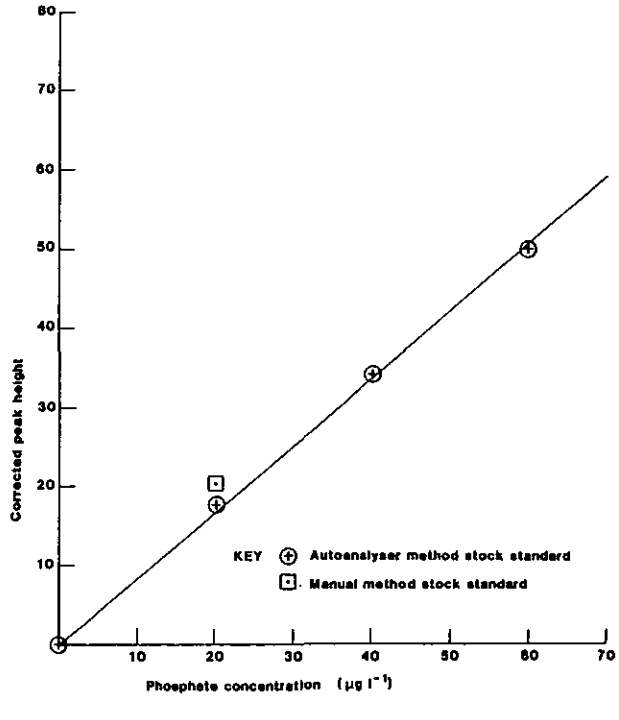

Figure 8. Expected silicate concentration (µg l^{-1}) in stock standards used to standardize automated and manual methods versus corrected peak height when both are determined by autoanalysis

Table 2. Test of agreement between automated and manual determinations of commercial standard solutions of nitrate and silicate (µg l^{-1})

Nutrient	Sagami standard (Expected levels)	Manual analysis (Determined levels)	Automated analysis
Nitrate µg NO_3^--N l^{-1}			
	0	0.0	0.0
	70	75.6	72.8
	140	141	136
	210	220	204
	420	452	411
Silicate µg SiO_4^{4-}-Si l^{-1}			
	0	0	0
	140	140	140
	280	273	266
	700	728	694
	2800	2790	2760

Oceanic samples: Two sets of samples taken at different depths from oceanic stations were stored separately for analyses. Figures 9-11 show nutrient concentrations determined by autoanalyser and plotted against concentrations determined by the manual methods. Student's t-tests for the significance of any deviation from the 1:1 line are shown in Table 3. The statistical tests show that the results of automated and manual analyses agree for phosphate within the accuracy of the methods but do not agree for nitrate and silicate.

Silicate discrepancy

A matrix error in either the automated or manual methods was suspected, but disproved when a sample of water from 1500 m was spiked and analysed by both methods with the same results (Fig. 12). The silicate discrepancy was caused by the reagent blank being made up in artificial seawater (which contains substantial amounts of silicate) and subtracted from the sample values; the instructions ae to use silicate-free distilled water. The instructions say a reagent blank should be made up in silicate free distilled water and

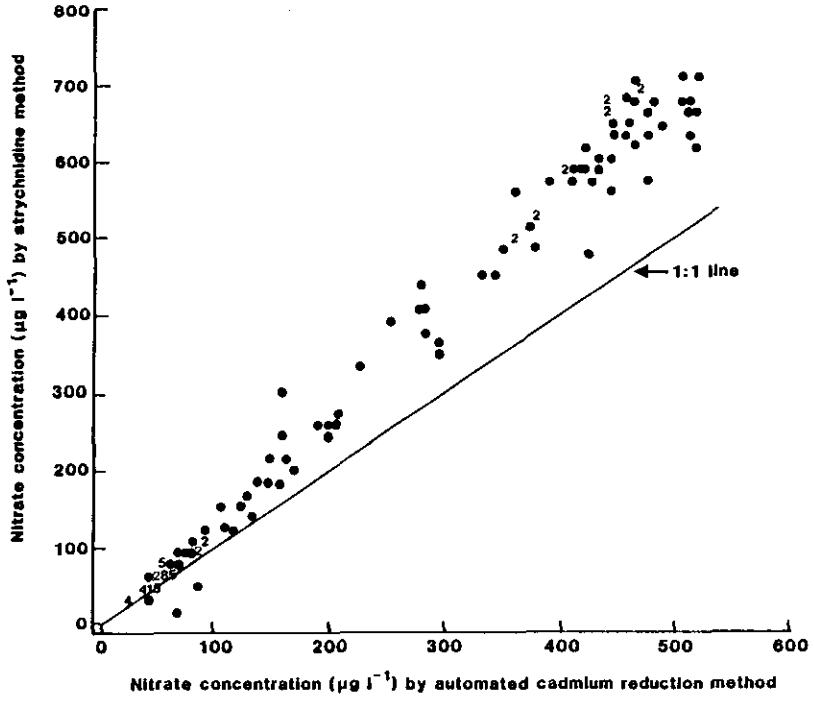

Figure 9. Comparison of nitrate results from automated cadmium reduction with the strychnidine method

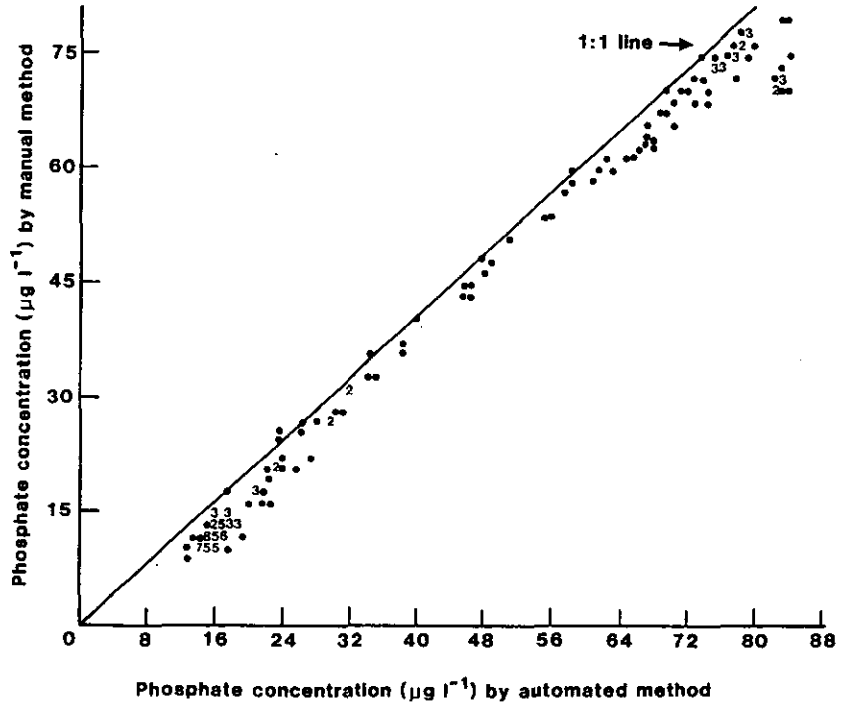

Figure 10. Comparison of automated and manual sample analyses - phosphate

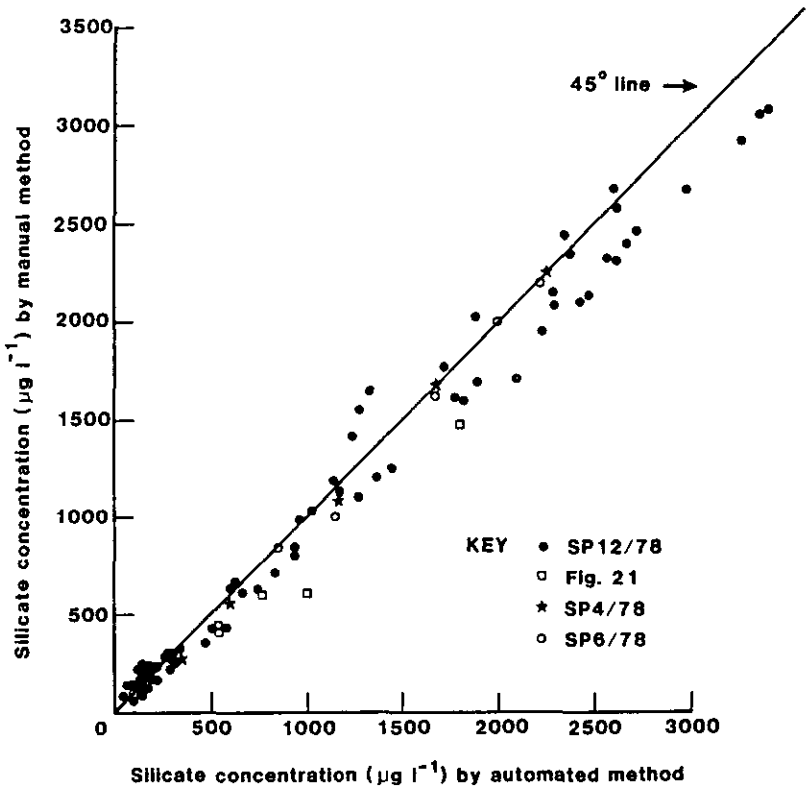

Figure 11. Comparison of automated and manual sample analyses - silicate

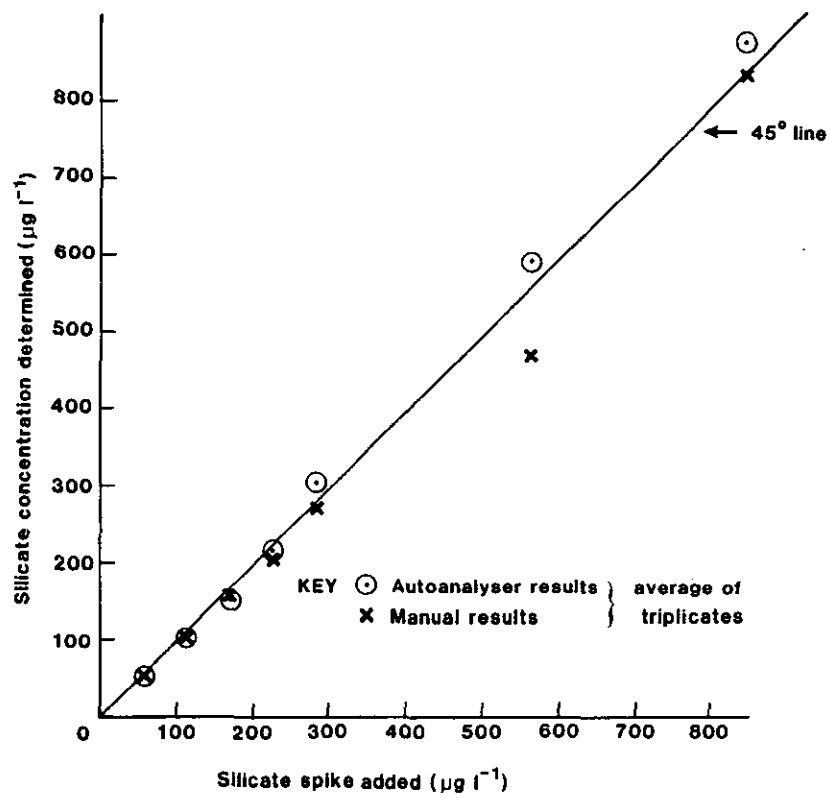

Figure 12. Measured silicate concentrations minus the original concentration versus spike added to a water sample from 1500 m for manual and automated methods

Table 3. Test of agreement between automated and manual analyses of oceanic samples

Nutrient	Equation of plot of automated (X) vs manual (Y) analysis Y = mX+c	r^2	t Statistic[a]	Conclusion[b]
Nitrate	Y = 1.418 X-14.22	0.988	45.651	$m \neq 1$
Phosphate	Y = 0.990 X-4.35	0.990	-1.24	$m = 1$
Silicate	Y = 0.924 X+13.04	0.989	-8.79	$m \neq 1$

[a] t is computed to test whether m = 1 at the 1% confidence level.

[b] m = 1 is interpreted to mean that the results obtained by the two methods are equivalent within the precision of the procedures for sampling, storage and analysis; $m \neq 1$ means the results are not equivalent.

r = correlation coefficent.

subtracted from sample values. At low concentrations, the percentage error is high. When the correct blank is subtracted concentrations determined by manual and automated methods agree well (see Figure 13). The average difference between the correct blank value ($\simeq$ 6 µg l^{-1}) and the incorrect blank value (range 15-130 µg l^{-1}, depending on the degree of contamination of the artificial seawater) is 9-124 µg l^{-1}.

The incorrect method was followed until February 1984. Data analysed before this date can be used unaltered for qualitative work, especially for deep waters where the percentage error is less (Airey and Storey 1985). If comparisons of nutrient concentrations determined before this date are to be made with the new data, the values can be adjusted as follows:

- For recent data get (from the ocean monitoring service group) the artificial seawater blank, and the standard curve for that batch of samples. Determine the concentration of silicate in the blank, add it to the computer listed value then subtract 6 µg l^{-1} (an approximate blank value determined by averaging recent values).

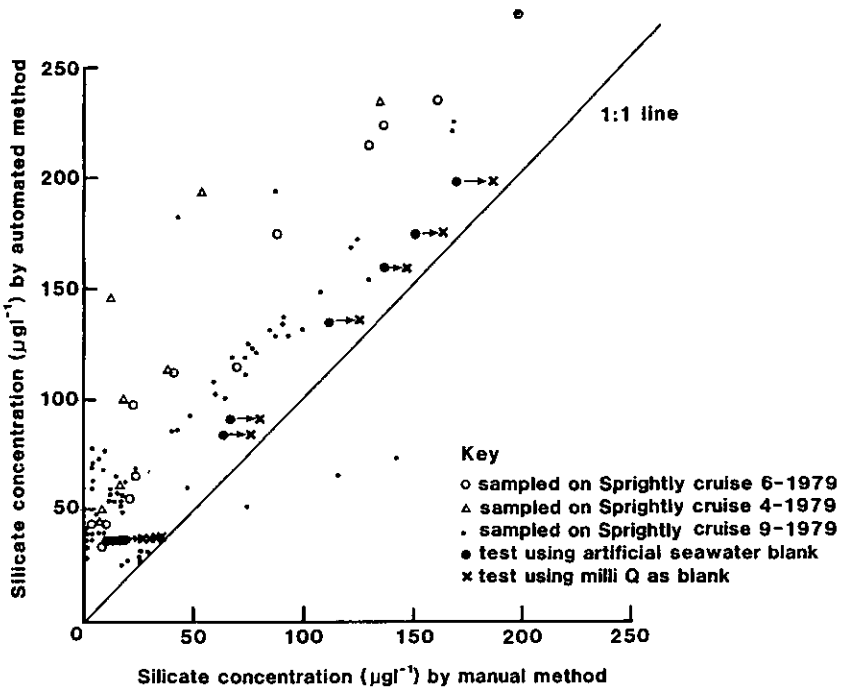

Figure 13. **Silicate concentrations in shallow waters, by manual method versus those determined by automated method. Crosses show the agreement when correct blank values are subtracted.**

. If no laboratory data can be found for a particular cruise, estimate how much silicate (between 9-124 6 µg l^{-1}) should be added to bring the data to the same order of magnitude as data measured by the automated method for recent samples taken from the same geographical area.

Nitrate discrepancy

As the discrepancy between the results from each method increased with increasing concentration, a matrix error was suspected. Figure 14 shows measured concentrations minus the original concentration for a series of standards mixed with water from 1500 m. The autoanalyser values lie on the 45° line but the strychnidine values are high, which seems to indicate a matrix effect in the strychnidine method. However, we have investigated the strychnidine method extensively (Airey and Storey 1985) and have discovered that incorrect use of the standard curve gives the errors. It is not possible to say whether there is also a matrix error.

The error in the data has occurred since 1975. This means that all data prior to June 1984, when the autoanalyser was commissioned for divisional use, will not be compatible with data after that date. Due to variability in the strychnidine reagent the discrepancy varies and only a rough adjustment is possible (see Airey and Storey 1985).

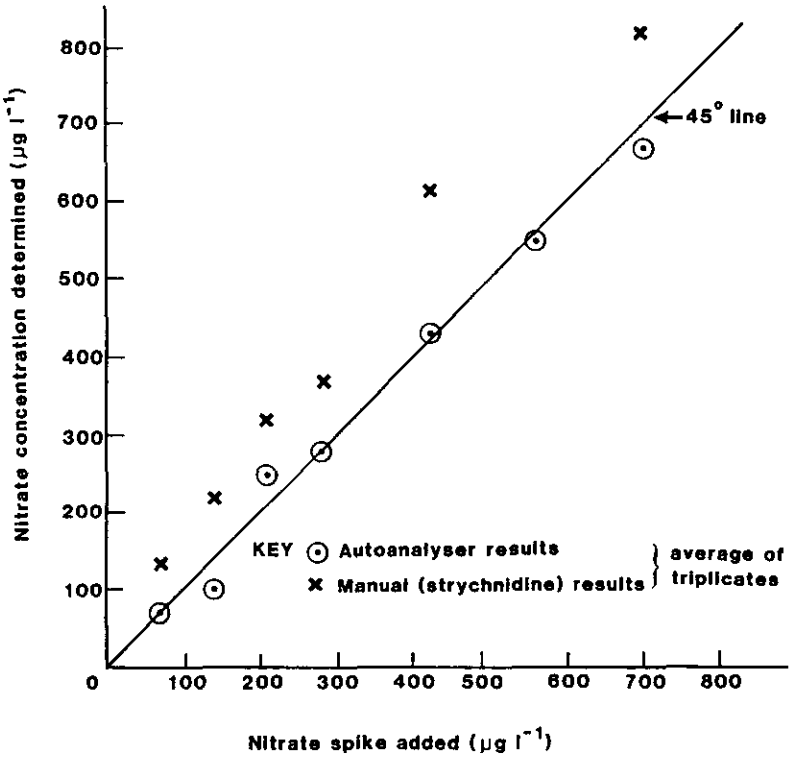

Figure 14. **Measured nitrate concentrations minus the original concentrations versus spike added to a water sample from 1500 m for manual and automated methods**

1.3 FORMAT OF RESULTS FROM AUTOANALYSER

A detailed account of the data storage and retrieval is given in Airey *et al.* (in prep.).

The scope of the system In the laboratory, nutrients in batches of preserved or unpreserved samples can be analysed automatically.

At sea, the autoanalyser may be used in two ways : to process water samples in batches as they are taken from Niskin bottles; and to process a continuous stream of water, pumped from the surface while underway, or from a vertical profiling pump while on station. These features are discussed in Section 2.

Form of results Peak heights indicative of nutrient concentrations are recorded initially on chart paper for both discrete and continuous samples. The chart is electronically digitized and the sample concentration values are computed from peak heights of a range of standards. The data is finally stored in a masterfile that lists the nutrient concentration with date, time, position, depth, salinity, oxygen, temperature and cruise number. The record is kept on a disc pack and is retrievable on paper.

Computer display of data in masterfiles When data has been stored in masterfile format, it can be manipulated to provide graphical output in the following forms :

- x-y plots of any variables (e.g. duplicate samples, which may be checked for agreement, or correlation of nutrient levels with other parameters)
- contour plots of surface nutrient levels
- contour plots of vertical profiles of water masses.

PART II

SAMPLING AND AUTOANALYSIS

2.1 COLLECTION AND PRE-TREATMENT OF SAMPLES

2.1.1 Sample collection

The sampling method depends on the type of information required.

(i) Hydrocasts Nansen, Niskin and NIO hydrology bottles have all been used successfully. If samples are to be analysed immediately at sea, they may be sampled directly into autoanalyser sample cups (Kayline polypropylene centrifuge tubes) and loaded into the sampler tray of the autoanalyser (see Section 2.2.). If samples are to be stored for later analysis in the laboratory, they should be collected in uncontaminated receptacles (e.g. Nasco Whirlpak bags), from which they are filtered. They should then be sealed in sample cups with a preservative and/or frozen (see Section 2.1.2 and 2.1.3).

(ii) Continuous sampling surface waters A continuous stream of water can be pumped from the surface, filtered and supplied to an autoanalyser flow-through sample holder, from which it is drawn for nutrient analysis. The inlet system is described below, and the sample holder is described in Section 2.2.

Pump: For continuous surface sampling, a Mono pump is used. The sample is drawn from 5 m below the water line, and pumped through a 1/2 inch diameter nylon hose at a rate of 50 l min^{-1}.

Header tank: Pumped water enters the 10-l header tank and resides there for 12 s. The tank is located 4 m above the level of the analyser, providing gravity feed through the nylon hose to a filter unit.

Filter unit: The unit removes over 91% of particulate material of greater than 8 µm diameter from the water from the header tank. The filter holder (Whatman Gamma 12) contains a Whatman 12/80 filter, which needs to be renewed about every 12 h of use, depending upon the particulate load of the water.

Flow regulator: A regulator (Series 3135, Fischer and Porter) is positioned after the filter to monitor and control the flow rate. The meter operates over the range 0-240 ml min^{-1} and is graduated in percentage of the maximum flow rate.

(iii) Continuous sampling of a concentration profile A continuous sampler is used to investigate small-scale processes that cannot be resolved by extrapolating data from water bottles set large distances apart. The system (Fig. 15) is designed to sample with minimum disturbance to the water column. To minimise slurring in the intake hose, air bubbles are injected at the intake port, and the distance between the top of the intake hose and the sample holder connected to the autoanalyser is made as short as possible (see Section 2.2.). Results using this system can be found elsewhere (Airey *et al.* 1983).

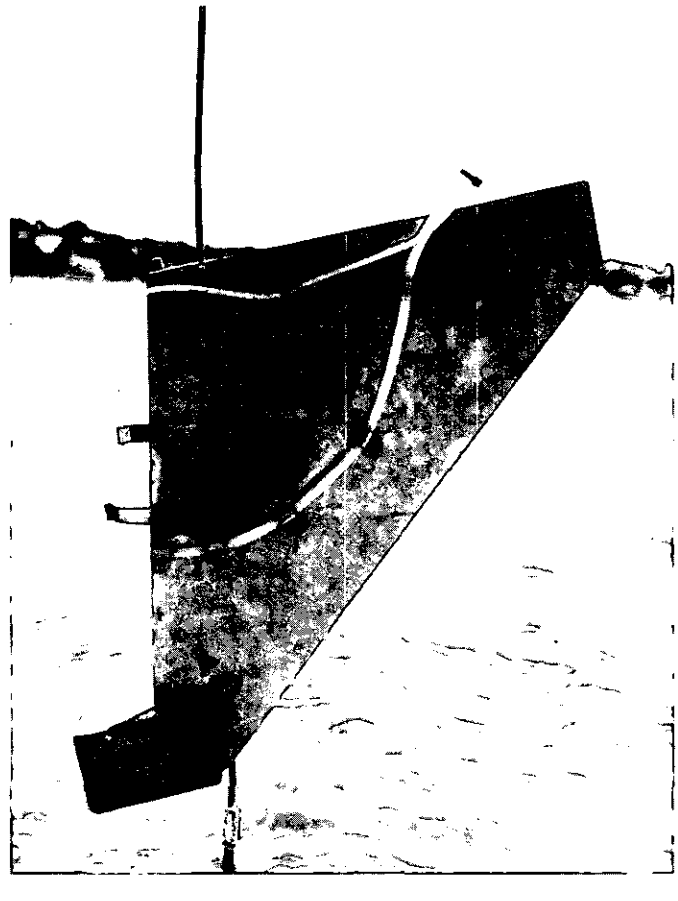

Figure 15. Continuous profiling water supply - intake point

Pump: For continuous profile sampling to depths of 60 m, a peristaltic pump (Watson-Marlow Model HRSV) is used on deck. The pump is connected on one side to an intake hose (see below) and, on the other side, to the sample holder described in Section 2.2. (ii).

Intake line: The sample intake hose is a polyethylene tube of I.D. 1/8 inch, O.D. 1/4 inch (Cole-Parmer Cat. No. 6407-02). It is attached at its inlet point to a plate aligned with the water currents at the sampling depth (Fig. 15). The mixing of successive samples as the sample is pumped to the surface is reduced by injecting air at the intake point through a second polypropylene tube of I.D. 1/20 inch and O.D. 1/8 inch (Technicon Cat. No. 562-5001-01). Air pressure builds up in the air line until it exceeds the external air pressure and a bubble is liberated into the intake line. The sample intake line then contains a stream of alternating segments of water and air in an approximate volume ratio of 4:1, which can be varied by adjusting air and/or water flow rates. (Note: the continuous profiling supply has not required a filter system in waters tested to date. A filter system (Morris *et al.* 1978) is used for turbid estuarine conditions.)

2.1.2 Sample filtration

In oceanography the division between dissolved species and particulate species is set arbitrarily at 0.45 µm. Filtration of samples before storage has the advantages that the result may be expressed as the dissolved nutrient level if 0.45 µm filters are used, and some of the living organisms are removed. A disadvantage is that the sample may become contaminated. Some filters contaminate samples more than others. Whatman GF/F or GF/C 0.45 µm filters are recommended (see Table 4). As the GF/F filter is thicker than the GF/C, it will filter a larger volume of water before becoming blocked.

Either of the following units may be used for filtration: a Millipore manual filter holder with 20 ml disposable syringe (in this case, separate filter units must be kept for each sample depth to avoid cross-contamination); or a Millipore batch filtration unit for processing of up to 30 samples concurrently. See Appendix A for instructions on use.

2.1.3 Sample storage

It is frequently necessary to store samples before analysis, although this is less satisfactory than immediate analysis because errors are introduced when preservatives are added and the container wall affects the samples in time.

Table 4. Nutrient concentrations before and after filtration through Whatman GF/C (0.45 µm)

Nutrient	Conc. before filtration ($\mu g\ l^{-1}$)	Conc. after filtration ($\mu g\ l^{-1}$)
Ammonia	0	0
	7	9
	14	6
	28	30
Nitrate	0	0
	56	60
	84	82
Nitrite	0	0
	2.8	2.4
	5.6	5.2
	14.0	14.0
Phosphate	0	0
	10	8
	20	20
	30	32
Silicate	0	0
	56	58
	84	84
	210	210

An acceptable storage method must

- preserve the sample for long periods (months)
- use containers and preservatives that do not contribute to or remove any of the nutrients
- use a preservative that does not interfere with the reagents in the colorimetric analysis

- preserve as many nutrients as possible.

A range of storage methods was tested (see Appendix B). The selected method for preservation of nitrate, nitrite, phosphate and silicate is to add 20 µl of 5% $HgCl_2$ to a Kayline polypropylene centrifuge tube containing 17 ml of filtered seawater sample. Ammonia can be stored but many replicates of each sample must be taken because the samples are easily contaminated.

After storage, samples are processed in batches (see Part 3).

2.2 THE AUTOANALYSER

The autoanalyser used at CSIRO Marine Laboratories is shown schematically in Fig. 16. Components are listed in Appendix H, and the operation of each component is described below.

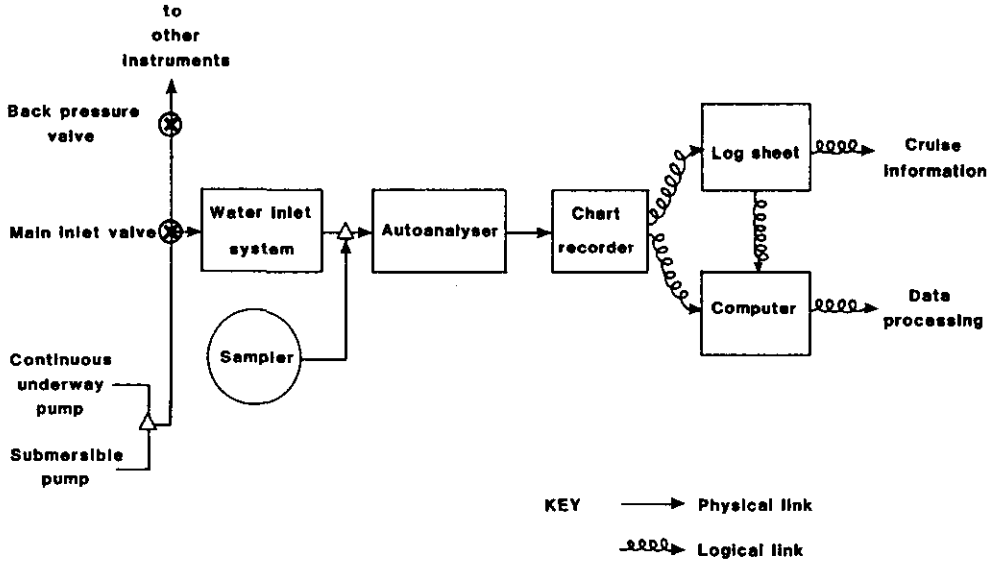

Figure 16. Autoanalyser configuration - schematic representation

2.2.1 Sampler

The 40-cup batch-sampler is a Technicon Sampler IV with the probe arm drilled out to accommodate up to five probes (one for each nutrient) in each probe holder. One or two wash pots can be fitted.

One washpot: This contains low-nutrient seawater (LNSW) or nutrient-free seawater (NFSW) (see Section 2.2.4 for definitions). All the probes are in the holder at the extremity of the sample arm. The pure water (distilled deionized water PW) baseline is set by pumping PW through a three-way valve (e.g. Omnifit, Yopharmacia). Once the baseline is set, the valve is opened so that (a) LNSW from the washpot or (b) samples, subdivided by LNSW from the washpot, enters the manifolds.

LNSW is held in a 4-l, acid-washed, pre-rinsed polyethylene container. It is pumped using flow-rated tubing through the autoanalyser peristaltic pump to the sampler washpot at 7.3 ml min^{-1} [see 2.2.5). The excess LNSW and air are pumped back to the LNSW container at 7.8 ml min^{-1}. To prevent overflow in the washpot, the withdrawal rate should exceed the input rate. LNSW can be recycled without contamination.

Two washpots: If silicate contamination in the LNSW cannot be removed as described in Appendix C, PW can be used as an intersample wash for the silicate channel only by putting the silicate probe in the holder next to the other holder. This arrangement can result in a small interface of sample and wash peaks.

(Note: The flow rates shown have been balanced for the sampling rates in the manifolds for ammonia, nitrate, nitrite, phosphate and silicate analyses. Should these change, the LNSW pumping rates may need alteration.)

To operate all five channels simultaneously using the minimum volume of sample, a cam that allows analysis of 20 samples per hour with a sample-to-wash ratio of 11 to 5 is required. The analysis rate and sample-to-wash ratio, together with the length of the manifolds, determines whether colour will reach a plateau value. The plateau value is the most reproducible point at which to read absorbance, so for any new system, empirical tests should be made to determine the optimum values of the rate and sample-to-wash ratio. In the present configuration, the nitrate manifold is the rate limiting channel -- analyses may be run at faster rates on the remaining channels. Cams are available with rates of analysis ranging from 20 to 120 samples per hour, and with sample-to-wash ratios determined by fixed intervals on a single cam or by variable intervals on a double cam.

2.2.2 Continuous sampling

A constant-level flow-through reservoir interfaces continuous-flow samples and the autoanalyser. Large volumes of seawater flush out connecting tubing and filters to ensure that the sample is representative of the seawater at the hose inlet. The five sample probes sit in the reservoir during continuous-stream sampling.

2.2.3 Sample-baseline switching valves

In both batch mode and continuous-stream sampling, pure water is used as a reference level. To allow the operator to switch between seawater sample (SW) and pure water baseline (PW), a four-way valve has been placed in each line.

In the position marked "SW", the probes in the reservoir feed directly into the autoanalyser sample line, and the baseline wash (PW) runs to a waste line that is clamped to prevent wastage of PW.

In the position "PW", the baseline wash (PW) feeds directly into the autoanalyser, and the seawater sample from the probes in the reservoir runs to waste (i.e. the clamp on the waste line is left open).

2.2.4 Reagents, reagent containers and inlet system

Reagents and standards for automated colorimetric analyses should be prepared in a room that is free of contamination. It is essential that ammonia and nitric acid vapours are excluded if ammonia or nitrate assays are to be done.

Bulk supplies of the following liquids should be maintained in a contamination free environment :

PW -- Distilled deionized water (distilled water is prepared in bulk and, immediately before use, passed through a Milli-Q triple bed filtration system).

LNSW -- Low-nutrient seawater is prepared from oceanic surface seawater collected in bulk and stored in 20 l plastic containers. With exposure to sunlight and agitation twice daily, nutrient levels fall to detection limits in 3-4 days. If the seawater is turbid or still contains active algal populations after this period, it is filtered by being passed from a plastic container through an all-glass Millipore filter unit (Millipore Cat. No. YY 42 14200) containing a 142 mm diameter 0.45 µm pore size filter (Millipore Cat. No. HAWP 14250). The filtered LNSW is collected in a 5 l side arm flask and transferred to a pre-cleaned 20 l polyethylene jerrycan (Cole-Parmer Cat. No. 6060) with a polyethylene faucet (Cole-Parmer Cat. No. 6078). Alternatively, offshore surface Coral Sea water could be shipped in bulk to the autoanalyser.

NFSW -- nutrient-free seawater is prepared by passing filtered NLSW through a zirconium hydroxide column to remove any silicates and any remaining phosphate (see Appendix C). Even filtered LNSW contains measurable quantities of silicate which should be removed when preparing standards (to avoid having to subtract a large blank).

All chemicals used in the preparation of reagents and standards should be A.R. grade unless otherwise stated.

Reagents are stored in Nalgene hedpak containers (Cole-Parmer Cat. No. 6061). They are dispensed from the containers through transparent drip bags (Solution Administration Set 30-500) which visually indicate reagent flow as a drip. Frequent examination is necessary to check if a reservoir is empty or if the reagent line is not being pumped properly.

The drip bags connect to a reagent/wash changeover unit (Chemlab) that allows the reagent lines to be flushed without disconnecting the fittings.

2.2.5 Pumps

Both Chemlab CP-30 pumps and Technicon pumps have been used for ammonia silicate, nitrate, nitrite and phosphate channels. An air bar is used on the Technicon pump to regulate the flow in the air delivery line, but regular air injection is possible on both pumps without the use of the bar.

For all channels, flow-rated peristaltic pump tubing is used (Elkay 116-0549 series) and, for connection, standard autoanalyser tubing (Elkay 0.025 inch I.D.) is used (see manifold diagrams for sizes used).

2.2.6 Manifolds

The sample and reagents flow through and react in the manifold to form a colour that is measured in the flow cell of the colorimeter.

Manifold housings, trays and covers are Technicon (Cat Nos. 170-0316, 170-0, 170-0233 respectively), and Technicon AA11 heating baths are used to assist colour development in phosphate and ammonia analyses.

Glassware used to construct the manifolds is from the 116 and 170 series (Elkay) (see Figures 17-21).

2.2.7 Colorimeter

Both Technicon and Chemlab colorimeters with interference filters and 50 mm flow cells have been used for detection on all channels. As the latter gives

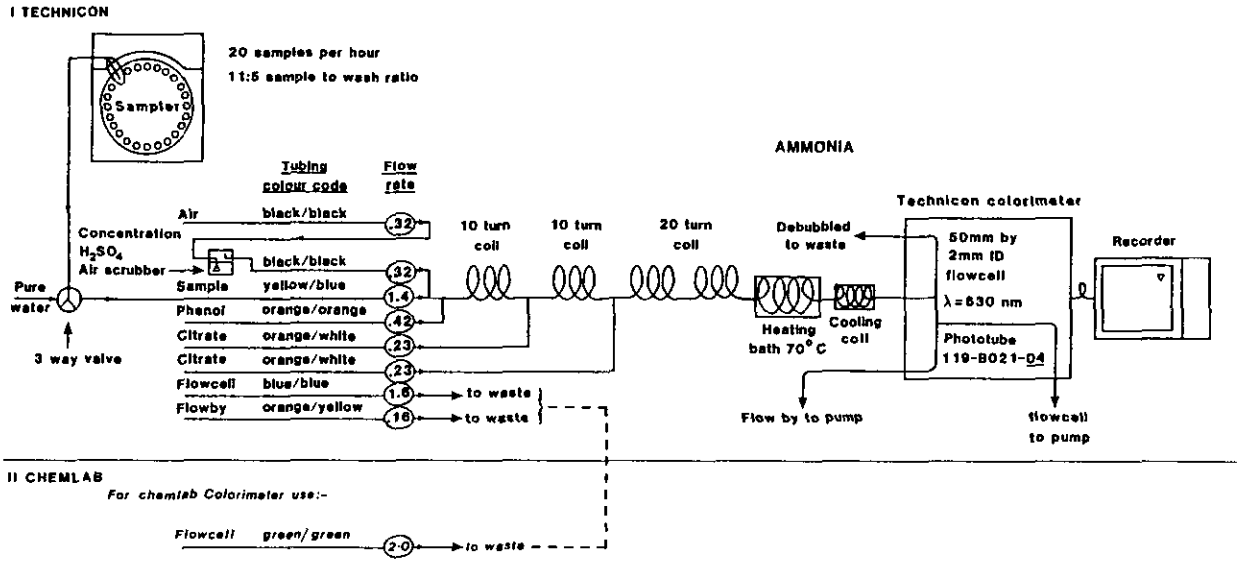

Figure 17. Manifold diagram - dissolved ammonia nitrogen

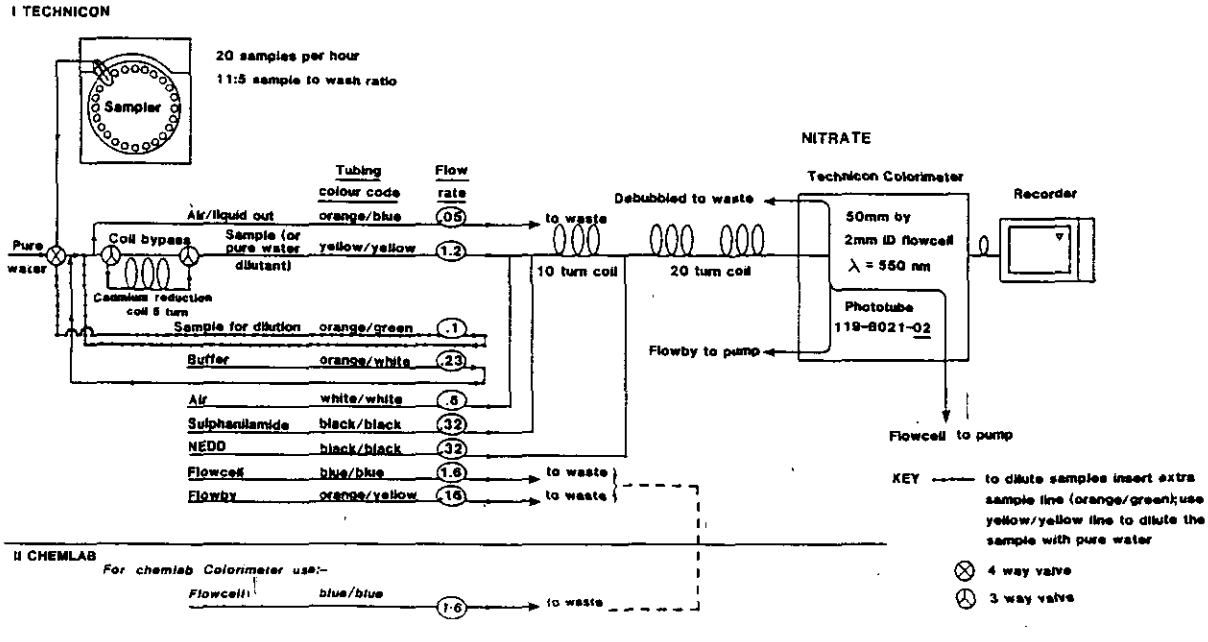

Figure 18. Manifold diagram - dissolved nitrate nitrogen

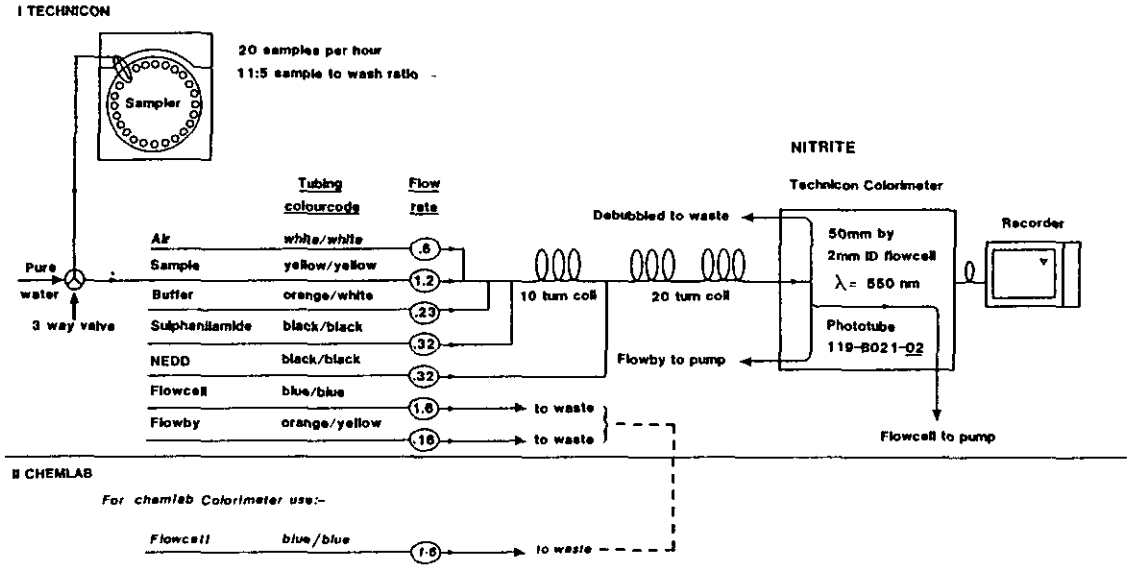

Figure 19. Manifold diagram - dissolved nitrite nitrogen

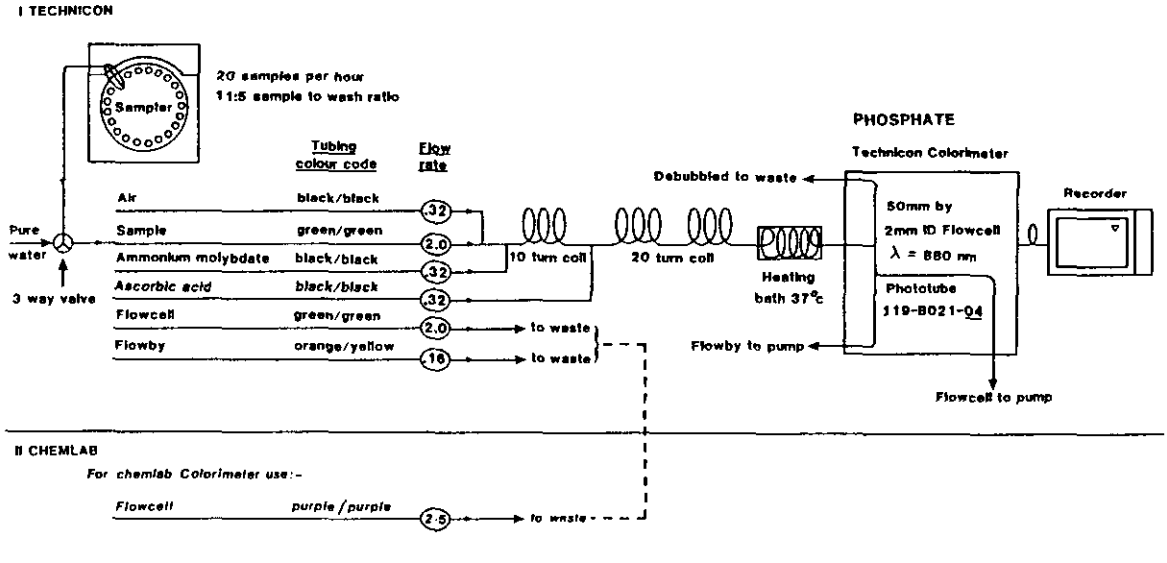

Figure 20. Manifold diagram - dissolved inorganic phosphate

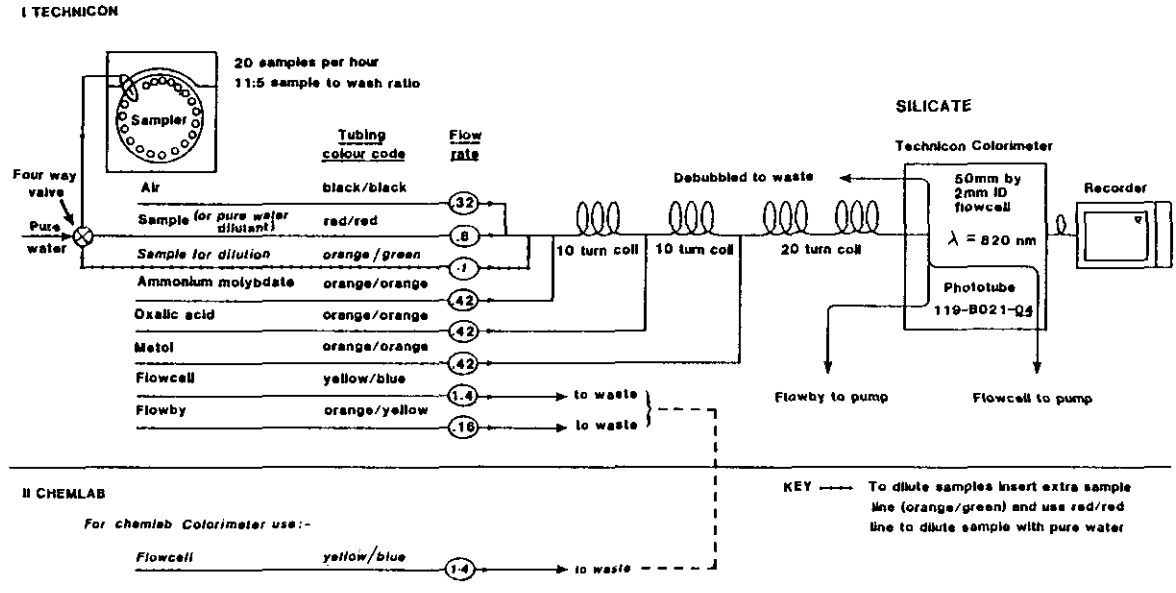

Figure 21. Manifold diagram - reactive silicate

better peak shapes, it should be used where possible on all channels. To prevent pressure build-up, waste lines from the colorimeter must be at least 2 mm ID and should run into larger diameter tubing as soon as practicable.

2.2.8 Recorders

Hewlett-Packard 7123-A chart recorders have been used to display colorimeter output. However, YEW Type 3056 Vertical Pen recorders (with 1, 2 or 3 channel options) are used now. The characteristics of the YEW recorders are described in the manufacturer's manual (YEW, 1979).

2.3 METHODS

2.3.1 Ammonia

(i) *Principle*

The automated determination of ammonia is based on the Berthelot reaction --

the reaction of ammonia and ammonium ion with alkaline phenol and reactive chlorine in the presence of a catalyst, in this instance, nitroprusside. The product is a coloured compound closely related to indophenol blue. The absorbance of this product is measured at 630 nm.

(ii) Sampling handling

To avoid contamination of samples with ammonia, contact with air be minimised during collection and processing of samples. Smoking in the room where samples are handled should be prohibited and smokers should not collect samples.

Filters do not need changing after each station but must be thoroughly rinsed with portions of the sample water before a sample is filtered and collected. Degradation of particulate matter on the filter produces ammonia which contaminates the first water passing over them.

Samples may be stored at room temperature in glass or Kayline polypropylene containers using 20 µl of 5% mercuric chloride preservative solution per 15 ml of sample. However, immediate analysis is preferable. As no storage method yet gives a precision of 5-10% at the concentrations (1-10 µg NH_3-N l^{-1}) found in ocean waters, any contamination gives serious errors.

(iii) Ammonia manifold

The manifold for dissolved ammonia determination is shown in Fig. 17. The air to be injected into the manifold is scrubbed by passing it through dilute sulphuric acid to remove ammonia. To prevent precipitation of magnesium and calcium hydroxides from seawater (which occurs when the pH rises). Citrate buffer is added to the reaction stream before any other reagent To accelerate colour production, heat the reaction mixture to 70°C.

The baseline for the system is set using PW in the sample line. If the baseline is very high, contamination has occurred in the PW, the air lines or the manifold tubes, or hydroxides have precipitated in the lines and it may be necessary to run 50% hydrochloric acid through the sample line to clean the manifold.

To avoid contamination of samples in the sample tray, the waste lines from all manifolds must run to an external reservoir. For example, Grasshoff buffer from the nitrate analyses contains ammonia, which must not be released into the laboratory atmosphere.

The performance of the manifold is :
Rate: 20 samples per hour
Sensitivity (at 7 µg NH_3-N l^{-1}) : 1 µg NH_3-N l^{-1}
Relative standard deviation: see Table 5
Detection limit: 1µg NH_3-N l^{-1}

Table 5. Dissolved ammonia nitrogen - relative standard deviation (R.S.D.)

Manifold only	
Concentration $\mu g\ NH_3\text{-}N\ l^{-1}$	R.S.D.* %
7.0	2
13.6	1
28.0	1

* Calculated from 10 standards analysed at each concentration level.

(iv) Reagents

Phenol -- Dissolve 9.37 g phenol and 0.125 g sodium nitroprusside in 1 l PW. Store in light-proof polyethylene container. Discard when reagent becomes greenish.

Buffer -- Add 6.18 g sodium hydroxide and 112 g tri-sodium citrate to 1.5 l PW. Heat gently to remove carbonate, as CO_2, till the volume is less than 1 l. Cover to protect from air. After cooling, add PW to make the volume 1 l. Store in a tightly sealed, light-proof glass container.

Oxidant -- Add 1.5 ml sodium hypochlorite (13% available chlorine) solution to 400 ml buffer. Add more buffer to make 500 ml and store in a light-proof glass container at 4°C when not in use. The reagent will last for a maximum of two days.

(v) Standards

Stock standard: 140,000 $\mu g\ NH_3\text{-}N\ l^{-1}$. Dissolve 0.6607 g of ammonium sulphate (AR) in 1 l PW. Store in a tightly stoppered glass flask, preferably in cool, dark surroundings.

Working standards: In offshore oceanic waters from 0-4000 m, the usual concentrations will be 0-7 µg NH_3-N l^{-1}. Inshore waters receiving waste or areas with zooplankton activity may have values up to 56 µg l^{-1}. In anoxic sediment pore waters, up to 560 µg l^{-1} may be found.

Standards are prepared by dispensing the appropriate volume of stock solution from a Hamilton Digital Diluter into 15 ml LNSW in a Kayline polypropylene centrifuge tube (see Appendix D).

(vi) Notes

Contamination is very difficult to avoid. It is better to analyse from an on-stream than a batch system if possible. If not, analyse the samples as soon as they are collected, and limit the number of samples exposed to air in the sampler.

To avoid contamination the following should be kept away from the samples: *smokers; persons or clothing* that have been in contact with fish; ammonia-based floor cleaners and brass cleaners; air circulating from toilets; dirty, greasy or sweaty hands.

During batch analysis, an interface mixing signal is recorded if the salinities of the samples vary by more than 10°/$_{oo}$ from that of the intersample LNSW wash. It is desirable to match the sample and wash salinities within these limits using PW to dilute the LNSW.

In oceanic samples, no interferences from other naturally occurring components should be encountered.

2.3.2 Nitrate nitrogen

The automated determination of dissolved nitrate nitrogen is based upon the reduction of nitrate ion to nitrite ion. The nitrite ion is reacted with sulphanilimide to form a diazonium ion, which reacts with N-1-naphthylethylenediamine dihydrochloride to form a coloured azo dye.

The concentration recorded is the level of nitrate plus nitrite species in the sample. The nitrite level is determined separately and subtracted from the total concentration to obtain the nitrate concentration.

Samples should be filtered (see Appendix A). Samples may be stored at room temperature in propylene containers using mercuric chloride (20 µl of 5% mercuric chloride solution per 17 ml tube) as a preservative.

(iii) Nitrate manifold

The manifold for dissolved nitrate nitrogen determinations is shown in Fig. 18. Initially in the manifold, nitrate ion is reduced to nitrite ion by passing the sample through a coil packed with copper-coated cadmium granules. The sample then enters the second stage, similar to the manifold for nitrite determinations, where colour-producing reagents are added.

The copper-cadmium reduction coil operates effectively (>95% reduction of nitrate ion) for about 24 h of continuous operation or 20 h of intermittent use. However, its performance should be monitored continually by passing a nitrite standard, comparable in concentration to the nitrate standards being used, through the column whenever standardization is taking place. If the reduction coil is operating efficiently, the peak height of the nitrate standard should be 95% or more of the peak height of the equivalent nitrite standard. When reduction efficiency falls below 95%, it is best to switch the stream through a bypass line and to replace the exhausted coil. The standards should be repeated before samples are analysed. Whenever the manifold is not in operation, the reduction coil should be filled with buffer and clamped closed.

If it is necessary to clean the manifold, 50% hydrochloric acid may be passed through the lines after the reduction coil. The passage of acid through the reduction coil will destroy the reducing capacity of the coil.

If ammonia nitrogen determinations are being made simultaneously, the wastes from the nitrate manifold must be collected externally to the analytical area otherwise the Grasshoff buffer waste will contaminate the ammonia line.

For samples of concentrations greater than 200 µg NO_3^--N l^{-1}, a dilution circuit is inserted as shown if it is desired to process such samples as a batch. Otherwise, it is more useful to dilute the samples manually (see Appendix E).

The performance of the manifold is:
Rate: 20 samples per hour
Sensitivity (at 28 µg NO_3^--N l^{-1}): 1 µg NO_3^--N l^{-1}
Relative standard deviation: see Table 6
Detection limit: 0.04 µg NO_3^--N l^{-1}

Table 6. Dissolved nitrate nitrogen - relative standard deviation (R.S.D.)

Manifold only		*Sampling, storage and manifold*	
Concentration $\mu g\ NO_3^-\text{-}N^{-1}$	R.S.D.* %	Concentration $\mu g\ NO_3^-\text{-}N\ 1^{-1}$	R.S.D.+ %
27.9	1	118	2
56.7	1	340	5
69.9	1	420	4
		478	2

* Calculated from 10 standards analysed at each concentration level.

+ Calculated from 10 samples, individually sampled, stored and analysed, at each concentration level.

(iv) Reagents

Reduction coil: Sieve cadmium granules through 700 µm and 300 µm meshes and keep the middle fraction. Rinse this fraction in 50% hydrochloric acid and wash thoroughly with PW. To plate the granules with copper, add 3% copper sulphate solution to the washed granules and shake gently so that all granules contact the solution. If all the copper has been removed from solution (i.e. the blue colour has disappeared), add more copper sulphate solution and repeat the process until all the granules are copper-coated (i.e. the solution remains blue). Decant the excess copper sulphate and rinse the copper-coated cadmium granules with PW, leaving the last rinse water in the container to protect the cadmium granules from the atmosphere.

To hold the granules, block the exit end of a five-turn glass autoanalyser coil of 2 mm ID (Technicon No. 170-0103-01) with glass wool. Connect a piece of 3 mm ID plastic tubing to a syringe and draw the granules up into the tubing. Connect the tubing to the coil and eject the granules into it. Shake the coil to move the granules around until they form a loosely-packed column. Note that the column should not be packed firmly; otherwise channelling of the reagent stream will occur and the column will be exhausted rapidly.

When the coil has been packed to the desired length, attach the coil to the system described in step 1 below, run working buffer solution through the coil

for 10 minutes, then condition the coil by passing a nitrate standard of around 100 µg NO_3^--N l^{-1} in working buffer solution for 10 minutes. The reducing capacity of the coil must be pre-tested the following procedure is rapid and convenient, especially if many coils are prepared together.

1. Set up a peristaltic pump with flow tubing delivering around 2.0 ml min^{-1} and attach the reduction coils to the tubing.

2. Prepare a working buffer solution containing around 100 µg NO_2^--N l^{-1} and take a 10 ml aliquot of this solution (Aliquot 1).

3. Pass the working buffer containing 100 µg NO_3^--N l^{-1} through the reduction coils and collect a 10 ml aliquot (Aliquot 2) from each coil.

4. Prepare a working buffer solution containing around 100 µg NO_3^--N l^{-1}, pass this through the reduction coils and collect a 10 ml aliquot (Aliquot 3) from each coil.

5. To each of the 10 ml aliquots, add 0.5 ml of 1% sulphanilamide solution in 10% hydrochloric acid.

6. Between 2 and 8 minutes after adding sulphanilamide, add to each aliquot 0.5 ml of 1.0% N-1-naphthylethylenediamine dihydrochloride.

7. Allow the aliquots to stand for 15 minutes to develop colour, then read the absorbance of each solution in a static cell spectrophotometer at 543 nm, using 1 cm cells.

8. For each coil, compare aliquots 1, 2 and 3 in the following manner : If the absorbances of aliquots 1 and 2 are not identical, the coil is over-reducing and destroying nitrite as well as nitrate. Condition the coil further using the 100 µg NO_3^--N l^{-1} in working buffer solution. If the absorbance of aliquot 3 is not greater than or equal to 95% of the absorbances of aliquot 2, the coil is not reducing sufficiently and must be repacked.

Stock buffer solution: Dissolve 10 g ammonium chloride, 20 g sodium tetraborate decahydrate, and 1 g sodium ethylenediaminetetra-acetic acid in 1l PW. Store in a polyethylene container so fumes cannot escape into the atmosphere to contaminate sampler and reagent for the ammonia line.

Working buffer solution: Dilute the buffer stock solution 1:10 with PW.

NEDD: Dissolve 0.31 g napthylethylenediamine dihydrochloride in 1 l PW. Store in a polyethylene container. Discard when it becomes brownish.

Sulphanilamide: Dissolve 3.12 g sulphanilamide and 31 ml concentrated hydrochloric acid in 1 l PW. Store in a polyethylene container.

(v) Standards

Stock standard: 28,000 µg NO_3^--N l^{-1} Dissolve 0.2020 g potassium nitrate in 1 l PW. Store in a tightly stoppered glass flask in dark, cool surroundings.

Working standards: Standards are prepared by dispensing the appropriate volume of stock solution from a Hamilton Digital Diluter into 15 ml LNSW in a Kayline polypropylene centrifuge tube (see Appendix D and Table 13). Expected levels: in surface oceanic waters, up to 56 µg NO_3^--N l^{-1} may be found, with the higher levels at upwelling fronts. From 0-300 m, up to 100 µg l^{-1} may be found, as nitrate is released by protein degradation. Levels increase with depth and at > 300 m, concentrations of 100-550 µg l^{-1} are found. In this range sample dilution is required (Appendix E).

(vi) Notes

During batch analysis, an interface mixing signal is recorded if the salinity of the sample varies by more than 10°/$_{oo}$ from the salinity of the intersample LNSW wash. It is desirable to match the sample and wash salinities within these limits.

In oceanic samples, no interferences from other naturally occurring components should be encountered.

2.3.3 Nitrite nitrogen

(i) Principle

The automated determination of dissolved nitrite nitrogen is based on the reaction, under acidic conditions, of nitrite ion with sulphanilamide to form a diazonium ion, which then reacts with N-1-naphthylethylenediamine dihydrochloride to form an intensely coloured azo dye. The absorbance of the reaction product is measured at 550 nm. The filter has a 30 nm band pass.

(ii) Sample handling

Samples should be filtered. Samples may be stored in Kayline propylene tubes with 20 µl of 5% mercuric chloride preservative solution in each 17 ml sample (see Appendix A).

(iii) Nitrite manifold

The manifold for dissolved nitrite nitrogen determination is shown in Fig. 19. If ammonia nitrogen determinations are being made simultaneously, the wastes from the nitrite manifold must be collected outside the analytical area, or in a sealed container from which gases emitted are acid-scrubbed prior to release into the laboratory, otherwise the Grasshoff buffer waste will contaminate the ammonia determinations.

The performance of the manifold is described :
Rate: 20 samples per hour
Sensitivity (at 5 µg NO_2^--N l^{-1}): 0.5 µg NO_2^--N l^{-1}
Relative standard deviation: see Table 7
Detection limit: 0.04 µg NO_2^--N l^{-1}

Table 7. Dissolved nitrite nitrogen - relative standard deviation (R.S.D.)

Manifold only		*Sampling, storage and manifold*	
Concentration µg NO_2^--N^{-1}	R.S.D.* %	Concentration µg NO_2^--N l^{-1}	R.S.D.+ %
3.72	2	2.20	22
5.66	1	2.19	15
11.3	0.2	2.55	30

* Calculated from 10 standards analysed at each concentration level.

\+ Calculated from 10 samples, individually sampled, stored and analysed, at each concentration level.

(iv) Reagents

Stock buffer solution: Dissolve 10 g ammonium chloride, 20 g sodium borate decahydrate and 1 g sodium ethylenediaminetetra-acetic acid in 1 l PW. Store in a polyethylene container so fumes cannot escape into the atmosphere to contaminate reagents and samples for the ammonia line.

Working buffer solution: Dilute the buffer stock solution 1:10 with PW.

NEDD: Dissolve 0.31 g naphthylethylenediamine dihydrochloride in 1 l PW. Store in a polyethylene container and discard when it becomes brownish.

Sulphanilamide: Dissolve 3.12 g sulphanilamide and 31 ml concentrated hydrochloric acid in 1 l PW. Store in a polyethylene container.

(v) Standards

Stock standard: 14,000 g NO_2^--N l^{-1}. Dissolve 0.0690 g sodium nitrite in 1 l PW. Store in a tightly stoppered glass flask in dark, cool surroundings.

Working standards: Standards are prepared by dispensing the appropriate volume of stock solution from a Hamilton Digital Diluter into 15 ml LNSW in a Kayline polypropylene centrifuge tube (see Appendix D). Expected levels : From 0-4000 m; 0-14 µg NO_2^--N l^{-1}. The highest concentrations will be at the pycnocline. High values up to 200 µg l^{-1} is found in anoxic sediment pore waters.

(vi) Notes

During batch analysis, an interface mixing signal is recorded if the salinity of the sample differs by more than 10‰ from the intersample LNSW wash. It is desirable to match the salinities within these limits.

In oceanic samples, no interferences from other naturally occurring components should be encountered.

2.3.4 Dissolved inorganic phosphate

(i) Principle

The automated determination of dissolved inorganic phosphate is based upon the reaction of orthophosphate and molybdate to form a dodecaheteropoly acid, which is subsequently reduced to an intense blue compound.

The introduction of antimony ions with the molybdate reagent provides a catalyst for the formation of the heteropoly complex. This complex is reduced by ascorbic acid, the reduction being speeded by heating the reaction coil to 70°C.

The absorbance of the product is measured at 880 nm.

(ii) Sample handling

Samples should be filtered. Samples may be stored in Kayline polypropylene tubes with 20 µl of 5% mercuric chloride preservative solution per 17 ml sample; or in Pyrex glass vials with Teflon-lined caps containing a drop of chloroform. (See Appendix A).

(iii) Description and performance of manifold

The manifold for dissolved inorganic phosphate determinations is shown in Fig. 20. The performance of the manifold is:

Rate: 20 samples per hour
Sensitivity (at 5µg PO_4^{3-} l^{-1}): 1 µg PO_4^{3-}-P l^{-1}
Relative standard deviation: see Table 8
Detection limit: 0.2 µg PO_4^{3-}-P l^{-1}

(iv) Reagents

Acid molybdate: Dissolve 6 g ammonium molybdate, 500 ml 5N sulphuric acid and 50 ml 0.3% potassium antimony tartrate solution in 450 ml of PW. Store in a light-proof polyethylene container. Discard when blue platelets form.

Potassium antimony tartrate solution: Dissolve 3 g potassium antimony tartrate in 1 l of PW. Store in a polyethylene container.

5 N sulphuric acid: Dissolve 140 ml sulphuric acid in 860 ml PW.

Table 8. Dissolved inorganic phosphate - relative standard deviation (R.S.D.)

Manifold only		*Sampling, storage and manifold*	
Concentration µg PO_4^{3-}-P l^{-1}	R.S.D. %	Concentration µg PO_4^{3-}-P l^{-1}	R.S.D. %
22.6	2	25.3	2
36.0	3	55.7	2
61.3	2	68.4	0.4
		83.7	1.5

Ascorbic acid: Dissolve 2 g ascorbic acid and 1 g hydrazine sulphate in 500 ml PW. Store in a light-proof glass container. Refrigerate at 4°C when not in use. The reagent should be stable for two days.

(v) Standards

Stock standard: 40,000 µg PO_4^{3-}-P l^{-1}. Dissolve 0.1757 g of potassium dihydrogen phosphate in 1 l PW. Store in a tightly stoppered glass flask in dark, cool surroundings.

Working standards: Standards are prepared by dispensing the appropriate volume of stock solution from a Hamilton Digital Diluter into 15 ml NFSW in a Kayline polypropylene centrifuge tube (see Appendix D). From 0-4000 m, the range will be 0-80 µg PO_4^{3-}-P l^{-1}. The levels gradually increase with depth and may reach a maximum near the oxygen minimum.

(vi) Notes

Containers for samples and standards may be made of polyethylene or glass.

During batch analysis, an interface mixing signal is recorded if the salinity of the sample varies by more than 10‰ from the salinity of the intersample LNSW wash. It is desirable to match the salinities within these limits.

It has been reported that silicate can interfere with phosphate analysis. However, in oceanic samples, no interference is encountered from silicate at naturally occurring levels.

2.3.5 Dissolved total phosphorus

(i) Principle

Organically bound phosphorus cannot be determined colorimetrically until the carbon-oxygen-phosphorus bonds are broken using ultraviolet irradiation from a high-powered ultraviolet source. Total phosphorus is determined after conversion of organic bound phosphorus species to inorganic phosphate, which may then be determined as in Section 2.3.4. Dissolved total phosphorus (i.e. dissolved inorganic and organically bound phosphorus) minus inorganic phosphorus, equals dissolved organically bound phosphorus.

(ii) Sample handling

Samples are stored in 50 ml glass vials with Teflon-lined caps. Several drops of chloroform are used to preserve the sample, which is then frozen for storage.

When processing, the sample should be slowly, but completely thawed to room temperature and mixed thoroughly before irradiation.

(iii) UV irradiation and the phosphate manifolds

The procedure presently used is not completely automated. Samples are dispensed into 15 ml quartz vials, a few drops of hydrogen peroxide (30%) are added, and a loose Teflon cap placed on the vial. The vials are then placed in a rack surrounding a 550W ultraviolet lamp (Hanovia). After irradiation for 90 minutes, the samples are allowed to cool, and are then batch-processed in the same fashion as the dissolved inorganic phosphate samples in Section

2.3.4. Only 16 samples per hour can be irradiated. These are then processed using the manifold for dissolved inorganic phosphate determinations.

(iv) Reagents

Reagents are those used for the determination of dissolved inorganic phosphate (see Section 2.3.4.).

(v) Standards

Stock and working standards are those used for dissolved inorganic phosphate determinations. At 0-4000 m, the expected levels are 0-100 µg PO_4^{3-}-P l^{-1}.

(vi) Notes

Refer to Section 2.3.4 (dissolved inorganic phosphate).

If chloride is oxidized to chlorine during the UV irradiation step, the chlorine will interfere with the colour-producing reaction in the manifold. To avoid this interference, irradiation should be performed on a neutral or weakly basic sample, that is, a seawater sample with natural pH around 8.3 may be heated with a few drops of hydrogen peroxide without altering the pH enough to produce chlorine during irradiation.

The optimum irradiation time of 90 minutes was found by irradiating a naturally enriched seawater sample until further irradiation produced no greater yield of phosphate. We assumed that glucose-1-phosphoric acid contains carbon-to-phosphorus bonds representative of naturally occurring organic phosphorus compounds. The digestion process was estimated to be 100% efficient by comparing a solution containing 60 µg l^{-1} of inorganic phosphate with a solution (irradiated for 90 minutes) glucose-1-phosphoric acid containing 60 µg l^{-1} of organic phosphate.

2.3.6 Reactive silicate

(i) Principle

Reactive silicate is combined with acid molybdate to form β-1:12 silicomolybdic acid. Isomerisation of the β-1:12 silicomolybdic acid is optimised at pH 2, although at this pH, phosphomolybdate complex forms

interfere in the silicate estimation. This can be prevented by the addition of oxalic acid, which reacts with excess molybdate. The β-isomer is then reduced by metol (p-methylaminophenol) to form a blue heteropoly acid, the absorbance of which is measured at 820 nm.

(ii) Sample handling

Samples should be filtered. Samples may be stored in Kayline polypropylene tubes with 20 µl of 5% mercuric chloride preservative solution per 17 ml sample (see Appendix A).

(iii) Silicate manifold

The manifold for silicate determinations is shown in Fig. 21. Note the difference in the waste lines for Technicon and Chemlab colorimeters. For samples of concentration greater than 200 µg SiO_4^{4-}-Si l^{-1}, a dilution circuit is inserted as shown if samples are processed as a batch. For the occasional high value it is possible to dilute such samples manually (see Appendix E). For example, during shipboard work, when water from each of fourteen bottle cast is analysed immediately, only a few samples may require dilution. If the deep samples are to be analysed as a batch, 20 µl of the $HgCl_2$ (5%) must be added to preserve the samples until there are sufficient to run using the dilution circuit.

The performance of the manifold is:
Rate: 20 samples per hour
Sensitivity (at 10 µg SiO_4^{4-}-Si l^{-1}) : 1 µg SiO_4^{4-}-Si l^{-1}
Relative standard deviation: see Table 9
Detection limit: 0.5 µg SiO_4^{4-}-Si l^{-1}

(iv) Reagents

Ammonium molybdate: Dissolve 10 g ammonium molybdate and 40 ml of 5N sulphuric acid in 960 ml of PW. Store in a light-proof polyethylene container. Discard when blue platelets form in the reagent (approximately one week).

Oxalic acid: Dissolve 7 g oxalic acid and 100 ml 50% sulphuric acid in 900 ml of PW. Store in a polyethylene container.

Metol-sulphite: Dissolve 12 g sodium sulphite and 10 g metol in 1 l of PW. Store in a light-proof plastic container. Discard when the reagent becomes brownish.

Table 9. Dissolved reactive silicate - relative standard deviation (R.S.D.)

Manifold only		*Sampling, storage and manifold*	
Concentration $\mu g\ SiO_4^{4-}$-Si l^{-1}	R.S.D.* %	Concentration $\mu g\ SiO_4^{4-}$-Si l^{-1}	R.S.D.+ %
57.8	1	103	5
139	0	518	2
211	1	1319	2
		1353	1

* Calculated from 10 standards analysed at each concentration level.

\+ Calculated from 10 samples, individually sampled, stored and analysed, at each concentration level.

5N sulphuric acid: Dissolve 140 ml sulphuric acid in 860 ml PW. Store in polyethylene containers.

(v) Standards

Stock standard: 140,000 $\mu g\ SiO_4^{4-}$-Si l^{-1}. Dissolve 0.94 g of sodium hexafluorosilicate in 1 l PW. Store in a tightly capped polyethylene bottle, preferably in cool, dark surroundings. The purity of the sodium hexaflorosilicate is usually approximately 98%. Strickland and Parsons (1972) suggest using 0.96 g to give approximately 140,000 $\mu g\ SiO_4^{4-}$-Si l^{-1}. We have not needed to do this.

Working standards: Dispense the appropriate volume of stock solution from a Hamilton Digital Diluter into 15 ml of NFSW in a Kayline polypropylene centrifuge tube (see Appendix D). From 0-100 m, the usual concentration range is 0-150 $\mu g\ SiO_4^{4-}$-Si l^{-1}. Concentration increases with depth. Concentrations of 150-3500 $\mu g\ l^{-1}$ need to be diluted (see Appendix E).

(vi) Notes

Containers for samples and standards should be made of polyethylene or polypropylene to avoid leaching of silicate from glass.

When analysing a batch of samples, an interface mixing signal is sometimes recorded if the salinity of the sample varies by more than $10^{o}/oo$ from the salinity of the intersample wash. It is desirable to match the sample and wash salinities within these limits.

In oceanic samples, no interference is encountered from phosphate at naturally-occurring levels.

PART III

OPERATING PROCEDURES

3.1 INSTRUMENT OPERATION

3.1.1 Batch mode operation

(i) *Start-up procedure*

1. Empty all waste pots.

2. Check all reagents to ensure full packs and shake to mix (see 3.2.1).

3. Switch on power
   -- at power point
   -- at power boards
   -- at transformer
   -- at colorimeters.
   Allow 60 minutes for electronics to warm up.

4. Place tubing rack on the vertical rod supports of the pump -- note correct position for stretching tubes (see 3.2.2).

5. Insert starting time on tubing life log.

6. Push down platens.

7. Check that sampler probes are in pure water (PW).

8. Open low-nutrient seawater (LNSW), PW and Grasshoff buffer hedpacks at taps and at drip bags.

9. Ensure valves around reduction coil in NO_3^- manifold are open (see 2.3.2. (iii)).

10. Turn reagent/wash changeover unit to "flush" position.

11. Switch on pumps.

12. Allow PW to flush through all channels for 5 minutes.

13. Open all hedpacks at tap and at drip bag.

14. Insert any connectors into reagent bottles.

15. Turn reagent/wash changeover unit to "reagent" position.

16. Allow reagents to flush thrcugh all channels for 10 minutes.

17. Check bubble patterns in manifolds (see 3.2.3).

18. Check GAIN settings on colorimeters. If necessary, alter these to appropriate settings for samples (see 3.2.4).

19. Check chart paper in recorders -- replace if necessary (see 3.2.5).

20. Label charts with colour code for nutrients and date.

21. Place pens in pen holders on recorders and switch pen holder to DOWN position.

22. Set the baseline for each pen on the recorder
    -- turn INPUT switch to ZERO position
    -- use POSITION switch to move pen to desired baseline
    -- turn INPUT switch to MEAS position.
    Note: If there are two or three pens on the recorder, offset the baselines, e.g. at 0, 10, 20 units.

23. On recorder :
    -- turn POWER switch to ON position
    -- turn CHART SPEED switch to 6 cm h^{-1}
    -- turn RANGE switch (outer knob) to 0.25 V cm^{-1}.
    Note: Do not adjust VERNIER switch (inner knob).

24. Turn CHART DRIVE switch to START and allow baseline to steady before beginning samples.

25. Prepare standards appropriate to GAIN settings (see 3.2.4).

26. Load sampler tray with:
    -- marker
    -- wash
    -- standards,
    and insert STOP peg in tray (see 3.2.6).

27. Enter sample tube description on log sheets (see 3.2.8).

28. When baseline is steady, place probes in sampler arm position A (LNSW wash) (see 3.2.7), and draw up LNSW for 2 minutes to allow time for any interface between PW and LNSW to be cleared.
29. Depress SAMPLER button and ALARM button on sampler to start sampler tray movement.

30. Read standard peaks from charts and enter peak heights in standards column on log sheet (see 3.2.9).

31. Plot standard curves.
    If satisfactory, start loading samples (see 3.2.9).

(ii) Running procedure

1. Check manifolds and charts at intervals to detect any irregularities in the system. If irregularities are found, remove the cam in the sampler and leave the probes in the wash pots until the problem is located and rectified. Then re-start the sampler by inserting the cam.

2. When a sample has been successfully processed (as indicated by its peak on the chart recorder), remove the sample cup and insert a new sample cup.

3. At end of sampler tray (40 samples)
    -- leave probes in LNSW wash for 2 minutes
    -- place probes into washpot B (PW) for 10 mins to establish baseline
    -- complete loading of tray with marker, wash and new samples and enter the details on the log sheet
    -- reinsert probes into A position of sampler and allow 1 minute flowthrough
    -- depress SAMPLER and ALARM buttons to restart tray
    -- mark new load number on chart.

4. Observe checklist attached to instrument for inspections to be conducted half-hourly, at commencement of each load, and daily.

(iii) Shutdown procedure

1. When last sample has been taken, allow 1 minute flowthrough of LNSW wash on probes.

2. Place probes in washpot B (PW) for 10 minutes to establish baseline.

3. Switch off sampler.

4. Check charts to ensure last sample and baseline are satisfactory.

5. Switch off recorders and remove pens and charts.

6. Switch off colorimeters.

7. Switch reagent/wash changeover unit to "flush" position and allow PW flowthrough for 15 minutues.

8. Close all hedpacks (except for buffer, LNSW and PW packs) at tap and at drip bag.

9. Remove reagent lines from reagent bottles and return appropriate reagents to refrigerators.

10. After 15 minutes, close buffer, LNSW and PW hedpacks at tap and at drip bag.

11. Switch off pumps.

12. Close valves around reduction coil in NO_3^- manifold to hold buffer in contact with coil.

13. Lift platens.

14. Release tubing holders to allow tubes to relax. Insert time of use in tubing log.

15. Switch off power
    -- at transformer,
    -- at power point.

16. Empty wastepots.

17. List reagents to be prepared before next run.

18. Place charts and logs together in storage box.

3.1.2 Continuous mode operation

(i) Start-up procedure

1. Follow the steps for start-up procedure in 'Batch mode operation' (Section 3.1.1 (i)).

2. Plot standard curves and check that these are acceptable.

3. Switch 4-way sample/baseline switching valves to PW to set the baseline for continuous flow records.

4. External, large volume pumps are used to bring a continuous stream of seawater onto the ships. There are different pumps for continuous surface sampling and continuous profiling (see Section 2.2.1).

   For continuous surface sampling: Switch on the external pump and open valves to admit water to the header tank. Check that the header tank is flushing properly. Ensure that the filter is clean, and adjust the flow regulator so that the sample holder is receiving a constant supply without splashing. Then proceed to Step 5.

   For continuous profiling sampling: Lower the sample intake line to the water surface, then switch on the external pump. Check that water is being admitted to the sample holder as above, and adjust the flow rate of the pump until the sample holder is being regularly flushed without splashing. Then proceed to Step 5.

5. Remove the probes from PW wash on the batch sampler. Place the probes in the continuous seawater supply in the continuous sample holder. Switch the sample baseline switching valves to SW to introduce continuous seawater supply to the autoanalyser.

(ii) Running procedure

1. Mark the charts with cruise and station details. Mark the time every hour for surface sampling. Mark depth and/or time for profiles. A PW baseline must be set at fixed intervals to allow compensation for instrumental drift. It is recommended that this baseline be set

   - every 2 hours for continuous underway sampling
   - at the start and finish of every profile for continuous vertical profiling
   - before and after every set of standards (see 3.2.9 (b)).

2. Check the manifolds and charts at intervals to detect any irregularities in the system. If irregularities are found, turn the sample/baseline switching valves to PW until the fault is rectified. Then reset the baseline before switching back to PW.

3. For continuous surface sampling, observe the exterior of the filter and, if the filter is overloaded (indicated by its appearance and/or by a falling flow rate on the flow regulator), turn the sample/baseline switching valves to PW and replace the filter (see Section 2.1.1).

(iii) Shutdown procedure

1. When sampling has finished, switch the sample/baseline switching valves to PW and determine the baseline.

2. Switch off the external pump and, if undertaking continuous profiling sampling, bring the intake line on board.

3. Continue from Section 3.1.1. (iii) Step 4.

3.2 OPERATIONAL CHECKS AND PROCEDURES

3.2.1 Hedpaks

Hedpaks should be checked regularly for leaks and to see that pumping has not created a vacuum, which distorts the pack.

3.2.2 Pump tubing

Pump tubing should be changed after 200 hours operation, or earlier if flow becomes irregular. When new, the tubing should be positioned with minimum stretching. Tension can be increased as the tubing ages.

3.2.3 Bubble pattern

The bubble pattern in manifold lines should be regular. If an excess of bubbles appears, check whether a reagent has run out, the line is out of the reagent, or a vacuum has developed in the hedpak. No bubbles in the manifold usually indicates the air line is disconnected.

3.2.4 Gain settings

Graphs showing the range of standards that covers 0-90% of the chart paper for each GAIN setting are reproduced in Figs 22-26. (Note - These figures are drawn for the colorimeters in use at present. If new phototubes are inserted or new equipment bought, check these graphs. If different, reconstruct for each gain setting). To select an appropriate GAIN setting for the samples to be processed:

- guess the approximate concentration of the nutrient in the samples
- set the GAIN so that standards will encompass this estimate
- run the PW baseline, then swap the probe to LNSW
- select a typical sample and run it
- adjust the GAIN setting for each channel so that the sample peak is approximately 40-50% of the width of the chart paper. (Note: if the signal is too noisy, select a lower GAIN setting and make do with a lower peak height)
- select standards appropriate for the chosen GAIN setting.

3.2.5 Chart paper

As the chart paper comes to the end, a red 40 cm RENEW CHART and 20 cm RENEW CHART will appear on the chart. There is sufficient paper to record 20 more samples, but the chart should then be changed.

To change the fold charts for Yew 3056 Recorder (type 3045-04 or XT127).

- Push down the chart compartment lock release lever, then pull the catch forward and draw out the chart paper compartment. Load the well-riffled chart into the chart compartment while bending the chart with figures.

- Push the chart to the right of the compartment. Chart driving holes should be square at the right and rectangular at the left. Thread chart behind chart guide roller shaft and mate the drive holes with the sprockets.

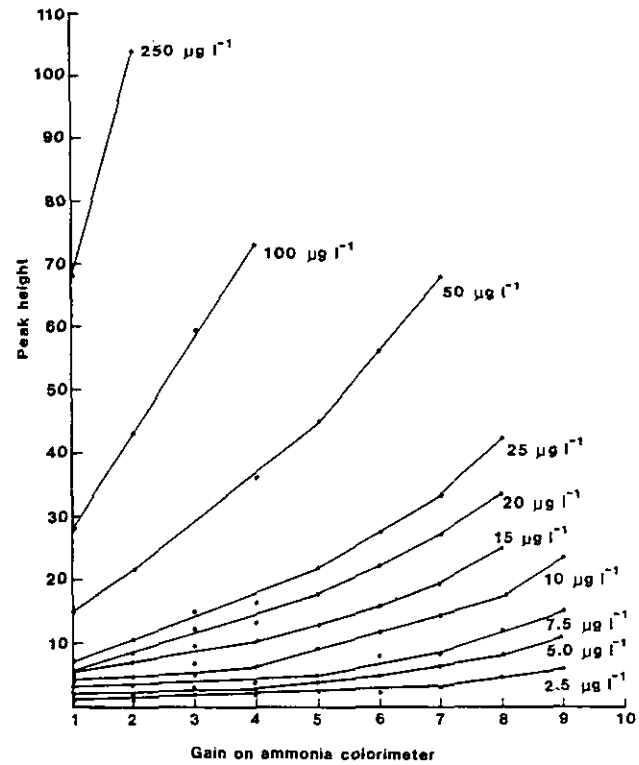

Figure 22. Expected peak heights at various gain settings for standards - ammonia

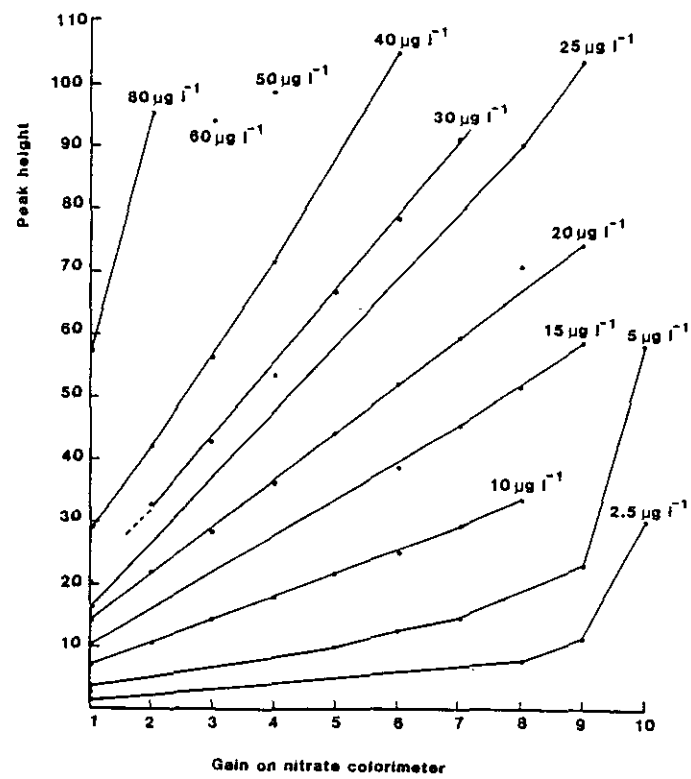

Figure 23a. Expected peak heights at various gain settings for standards - nitrate

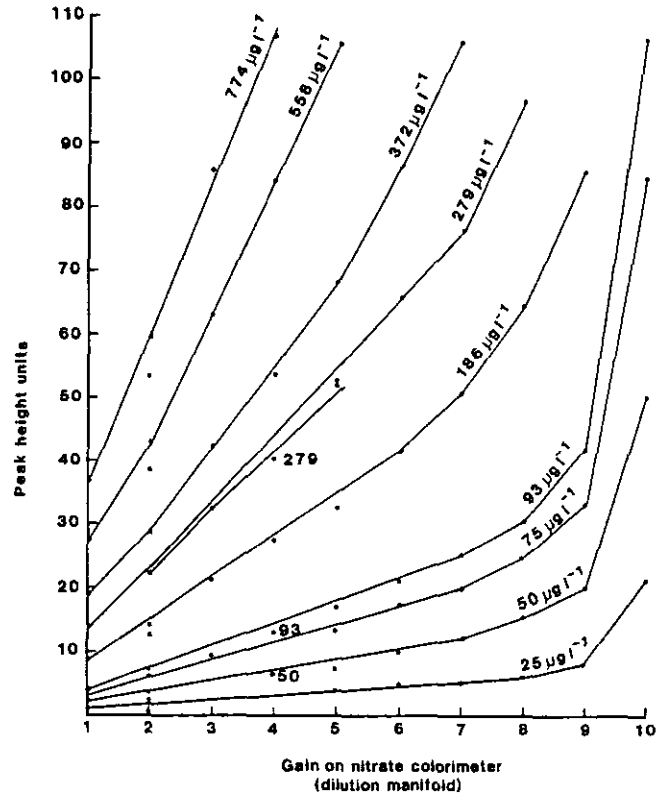

Figure 23b. Expected peak heights at various gain settings for standards - nitrate with dilution circuit

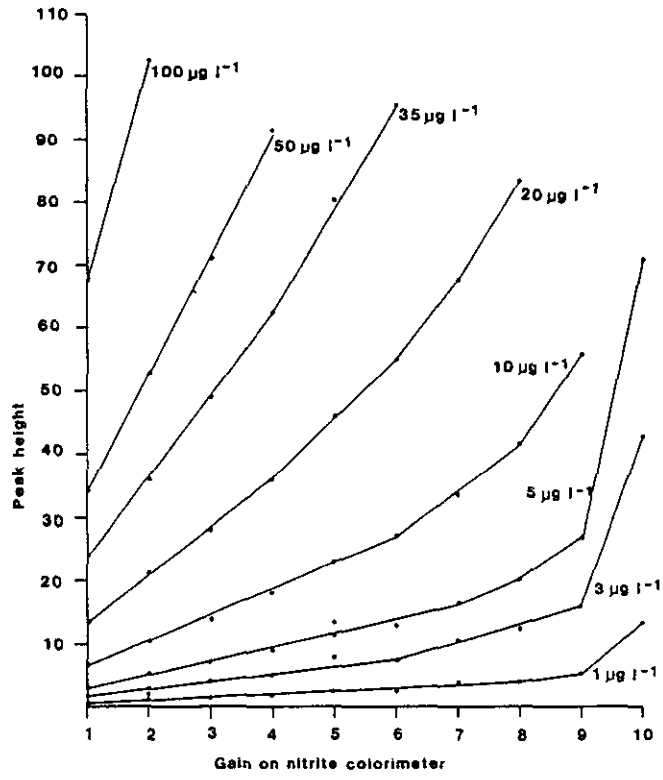

Figure 24. Expected peak heights at various gain settings for standards - nitrite

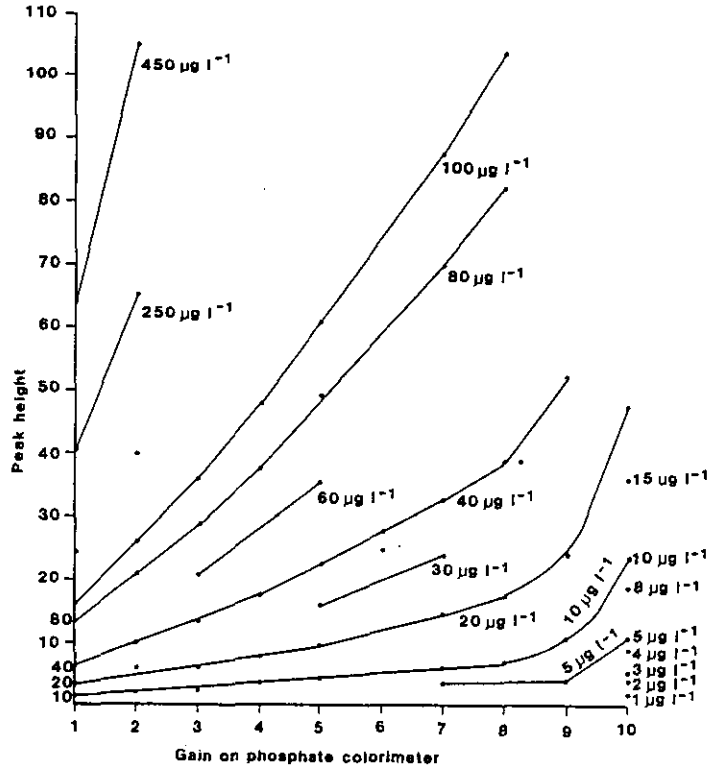

Figure 25. Expected peak heights at various gain settings for standards - phosphate

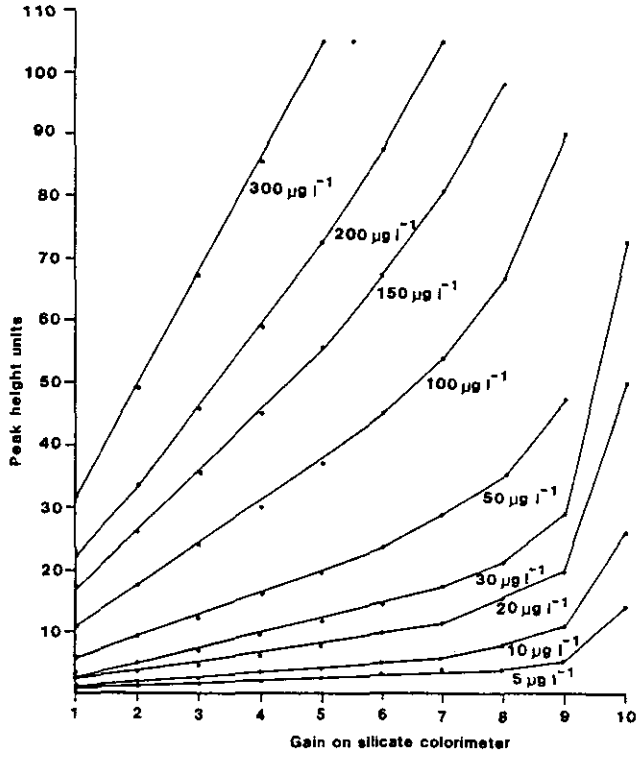

Figure 26a. Expected peak heights at various gain settings for standards - silicate

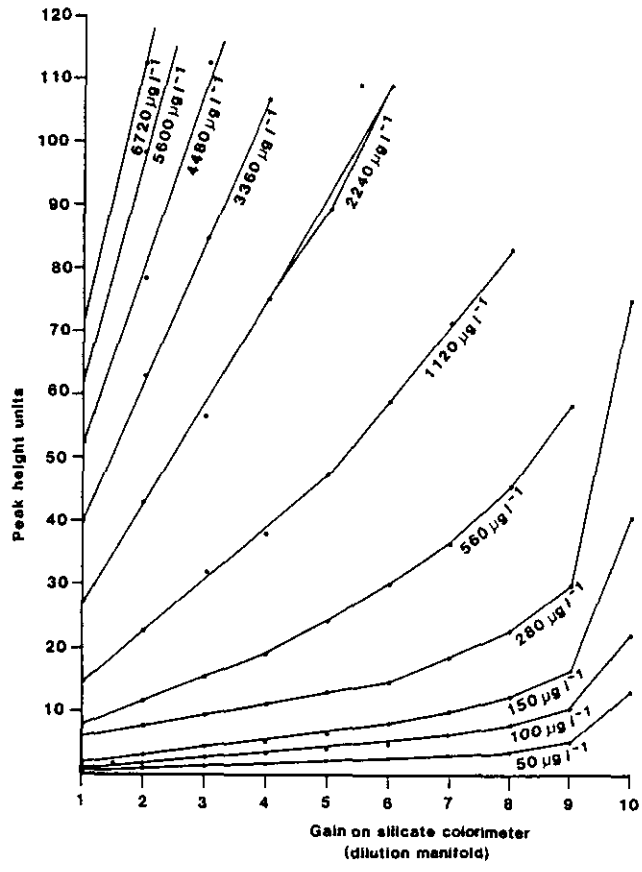

Figure 26b. Expected peak heights at various gain settings for standards - silicate with dilution circuit

- Push the catch forward and return the chart compartment to the original position.

- Advance the chart downwards (by turning the chart advance wheel manually) until two or three folds of the chart drop into chart compartment. Be sure the chart drive sprocket teeth are engaged in chart perforations. The chart can be folded inside the instrument, or allowed to hang vertically outside the instrument. Generally, chart paper expands and contracts as ambient humidity varies, so store chart paper carefully.

3.2.6 Sampler tray

The sample tray should be loaded with sample tubes. All loads should begin with a marker to indicate start of load, followed by a wash to flush sample lines.

3.2.7 Sampler arm

Two positions are available for inserting probes in the sampler arm. The A position uses an LNSW wash, and the B position uses a PW wash. Two probe holders are available to allow probes to be used in both positions.

3.2.8 Log sheets

An example of a log sheet and instructions for its use are included in Appendix F.

3.2.9 Standard curves

(i) Definitions

The standard curves are plots of concentration values of standards versus peak heights recorded on the chart recorder. The preparation of standards is discussed in Appendix D and the peak heights recorded for standards at various gain settings on the colorimeter are shown in Figs 22-26. The computation of standard peak heights and the calibration curve is described in Appendix F (F.3 Standards).

(ii) Operating procedure

(a) Setting optimum standards: When running the autoanalyser after a substantial break (e.g. between cruises), all reagents and tubing should be renewed so that sets of standards can be run under optimum conditions. For each nutrient standard, the corrected peak height (standard peak height minus blank peak height) for each standard should be compared with the expected corrected peak height at that gain setting (Figs 22-26). If the difference between observed and expected corrected peak heights is less than 5%, the results can be accepted. These standard peak heights are referred to below as "reference standard peak heights". If the results are not acceptable, follow the trouble-shooting procedures.

(b) Quality control of standards: Continuous quality control of standards is required to ensure that results are comparable between runs.

The frequency of running of standards should be as follows :

- whenever conditions are altered in a manifold (e.g. new reagents, new tubing, new run after a long break) the first tray of samples should contain a blank and four standards of different concentration for each nutrient (chosen for the selected GAIN setting). Such standards and blanks must be in a separate set of tubes for each nutrient, as interaction between standards causes errors. The curve must be drawn immediately and any dubious result not fitting the curve must be rerun. Blank values are extremely important; ensure they are not contaminated, run several for accuracy.

- for sequences of 40 samples per batch, the second and third trays should contain a blank and top standard for each nutrient. Check the corrected peak height against the original standard curve. Every fourth tray should contain a blank and four standards. Check with the results from the first tray.

- for Niskin cast sampling, about 14 samples per tray will be generated. Each tray should contain a blank and top standard for each nutrient; if trays are more than four hours apart, they should contain a blank and four standards;

- for continuous surface sampling, a blank and four standards should be run every four hours for each nutrient of interest.

The criteria for acceptability of standard calibrations are :

- first, the blank is important and should be consistent. Do several blanks if necessary. If the peak height corresponding to a new blank differs by more than two chart units from the reference blank peak height, the blank should be re-run. Fluctuations in the peak heights of blanks usually mean that the LNSW used as the blank is contaminated, has algal growth in it, or is a new batch with different properties from the previous one.

- second, the standards must be comparable between runs. If the peak heights generated by a new standard differ by more than 5% from the peak height for the corresponding reference standard peak height, the standard should be repeated. If they are still different, follow the trouble-shooting procedure.

- third, the standards must be comparable from day to day and week to week at a selected GAIN. If deviations occur, remake the standards and check the stock against a commercial standard.

(iii) Possible problems with standard curves:

Standard curves may not turn out as expected, even if the flow characteristics

at the manifold is correct. The problems and probable causes are shown below:

Problem: Scatter of standard points labelled X. (See Fig. 27.1).

Probable causes:
-- standards have been poorly prepared - renew them and shake well. Re-read Appendix D
-- irregular turbidity in LNSW matrix (replace)
-- old pump tubing causing irregularities in volumes

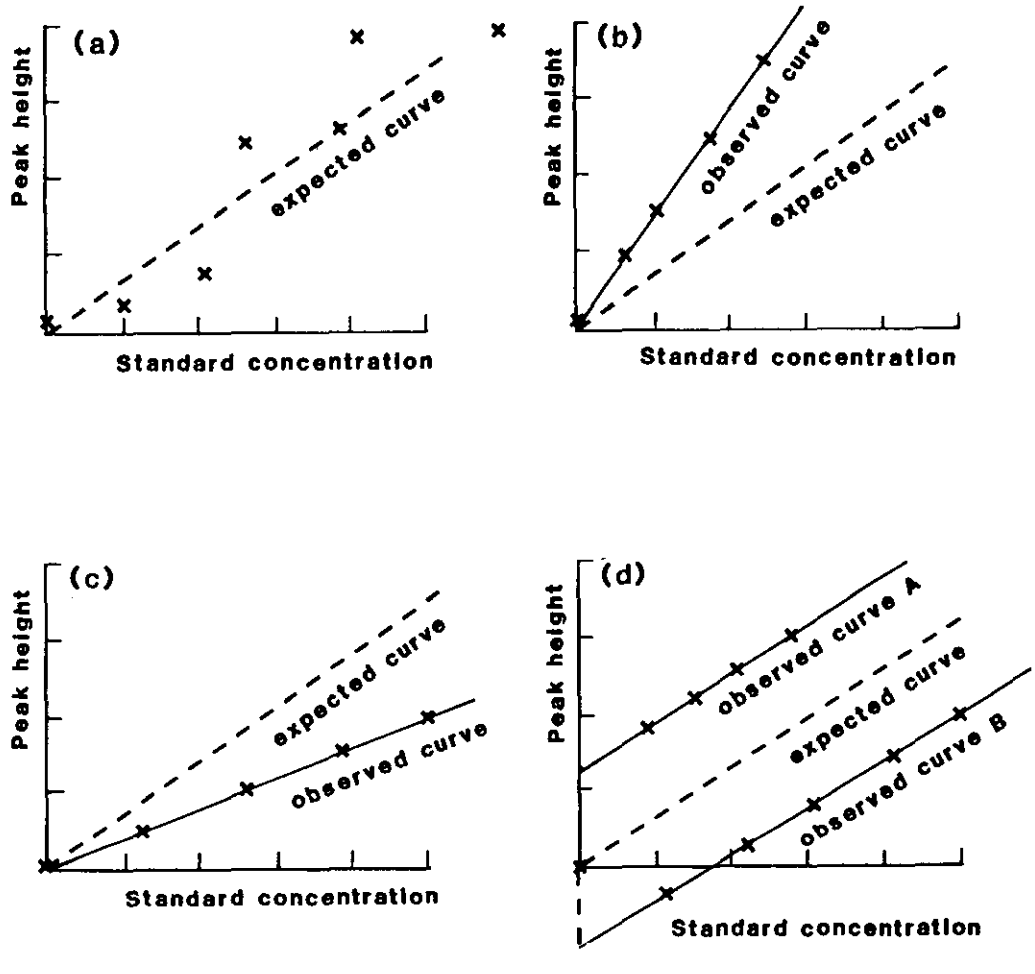

Figure 27. Peak height versus standard concentration (i) Scattered points (ii) Gradient too high (iii) Gradient too low (iv) Curve parallel, but not through zero

Problem: Standard curve is linear but gradient is greater than expected. (See Figure 27.2).

Probable causes:
-- contamination of standards - renew them
-- gain too high

Problem: Standard curve is linear but gradient is lower than expected. (See Figure 27.3).

Probable causes:
-- standards have deteriorated - renew them
-- reagents have deteriorated
-- gain too low
-- reagents may not be pumping in the correct ratios; check pump tubes
-- pump tubing has aged - replace tubes
-- the wavelength of the selected interface filter is incorrect

Problem: Standard curve is parallel to expected line but does not go through zero (Figure 27.4).

Probable causes:
Curve A: Blank value not large enough or standards too high

-- blank matrix is not same as standards (repeat several blanks)
-- digital diluter is delivering too much standard

Curve B : Standards are turbid (repeat). Blank is :

-- contaminated
-- is not prepared from the same matrix as the standards
-- micro-organisms from the LNSW used have extracted nutrient (if standards are left for a long period before analysis). Rerun standards or replace blank and run in triplicate.
-- digital dilutor delivering constantly low volume of standard. Check this against Gilmont pipettes.

3.2.10 Trouble-shooting procedure

If the chart recorder shows a major irregularity that will affect the processing of all subsequent samples, it is necessary to :

1. place the probes in the washpot LNSW for 3 minutes so that no further samples are lost
2. allow the system to continue flowing till the most recent sample has

passed through the colorimeter, then put probes in PW wash pot to get a PW baseline on the chart output

3. only then interrupt the flow to rectify the fault, (e.g. stop the pump to replace a tube).

If the fault can be rectified without disturbing the flow (e.g. cleaning a blocked sample tube, or straightening a waste line), it is not necessary to wait for the most recent sample to appear on the chart output before acting. (See also Section 3.3)

3.3 TROUBLE SHOOTING

The Technicon manufacturer's manual gives the procedures for rectifying common faults, which will not be repeated here. Only problems most frequently encountered with the present system will be discussed.

In most cases, a problem will be detected because of irregularities on the chart output, e.g. irregular spikes or off-scale readings. Hence, the trouble-shooting guide shows chart irregularities, the probable causes of these irregularities and the remedial measures. Sample chart outputs are included in Appendix G. Only instrumental problems are discussed here. Problems associated with particular methods are discussed in Section 2.3.

PROBLEM OBSERVED ON CHART	*POSSIBLE CAUSES AND REMEDIES*
PW baseline too high	1. Contamination of PW. Prepare a new batch. If baseline is still unsatisfactory, the Milli-Q water system should be examined.
	2. Dirty flow lines or flow cell. Place probes in 50% HCl to clean the lines. If this fails, clean the flow cell by disconnecting at the inlet and passing KOH solution through it.
	3. Turbidity in the flow line. Collect the solution after the colorimeter and inspect visually. If turbid, prepare new reagents.
LNSW baseline too high	1. Any of the three problems mentioned above may be a cause.
	2. LNSW has, in fact, high nutrient levels. (see Section 2.2.4). Inspect the LNSW for obvious signs of decaying material. If there are any, discard the LNSW. Otherwise agitate vigorously and leave in sunlight for several days for nutrient levels to fall as they are removed by micro-organisms.
Baseline ragged	1. Dirty flow cell. Clean with 50% HCl or KOH as described above.
	2. Reagents not feeding properly. Check the manifold connection for each reagent, the valve connection for any blockages, and the reagent lines at the hedpaks.
Chart pen moves rapidly and intermittently at the top of the chart	This problem is always linked to the passage of air bubbles through the colorimeter flow cell. The de-bubbler prior to the flow cell will be effective only if the flow is regular. The cause may be any of the following:
	1. Insufficient sample in the sample cup, and the probe has introduced air for part of its cycle.
	2. Probe has stuck on the edge of the sample cup. Check alignment of the probe holder.

	3. Reagent lines are not all switched on; in addition to irregular air bubbles, a "surging" motion may be noticed in the flow lines.
	4. Manifold has a leak and air is entering the system.
	5. Debubbler not correctly positioned in the colorimeter.
	6. Waste lines from the debubbler and the flow cell are not vertical, have become submerged in the liquid in the waste receptacle, or are too narrow. Violent ship movements can cause the air and water in narrow waste lines to build up a back pressure. With wide diameter waste lines this should not happen.
	7. Pump tubes have aged and are not pumping correct quantities.
Standard peaks are too small	1. Standards incorrectly prepared. Check pipettes or digital diluter and remake standards if necessary.
	2. Reagents not feeding correctly. Check all connections at manifold, valves and hedpacks.
	3. Reagents deteriorated. Prepare new reagents and compare results.
	4. Tubing aged. Replace with new flow-calibrated tubing and check.
	5. GAIN setting incorrect. Check.
	6. Primary standard deteriorated. Prepare a new stock and standard and compare.
	7. Wrong filter inserted in the colorimeter. Check.
Standard peaks are abnormal	1. Wrong cam inserted. Check and change if necessary.
	2. Probe partly blocked or insufficient sample in the sample cup. Run a wire down the probe to check.
	3. Reagent feed lines obstructed. Check.

	4. Flow line or cell dirty. Clean with acid or alkali as described previously.
	5. LNSW used to prepare the standard contains particulate matter. Prepare a new standard with another batch of LNSW and compare.
	6. Salinity of the LNSW used to prepare the standard differs from the salinity of the wash, which may occur if one is an old batch.
Sample peaks are abnormal	1. Any of the factors that affect the shape of standard peaks may also affect the shape of sample peaks. However, a salinity difference between the sample and the wash is more likely. In this case, large, ragged spikes may be observed where mixing of sample and wash or wash and sample occurs.
	2. Interference from particulate matter is causing irregularly-shaped peaks. This occurs when the sample has not been properly filtered. If duplicates of the sample are available, these should be run instead.
Chart not rolling out correctly	1. Chart roll not properly engaged on the drive shaft.
	2. Chart perforations not correctly positioned on the drive sprockets.

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APPENDICES

APPENDIX A

FILTRATION METHODS FOR ANALYSIS OF DISCRETE SAMPLES

Filtration to remove turbidity is generally not required in oceanic samples. However, since only low levels of mercuric chloride preservative can be used in order not to poison the cadmium reduction column in the nitrate manifold, it is necessary to filter samples as part of the preservation technique. Filtration procedures for both individual batches of samples are described below.

A.1 Individual samples

Sample filtration has been satisfactorily performed using a 20 ml Millipore plastic disposable syringe (Cat. No. XX1102012) and a Millipore filter head (Cat. No. SXHA 02508) containing a Whatman GFF filter (0.45 µm, 25 mm diam.).

Procedure:

1. Collect the sample from the water sampler (e.g. Nansen or Niskin) in an 18 oz Nasco Whirlpak bag, or equivalent sterilized bag (check other brands for contamination - the Nasco Whirlpacks can be used without rinsing).

2. Rinse with the sample water the exterior part of the syringe that is likely to contact the sample.

3. Draw some of the sample water into the syringe and rinse the inside thoroughly.

4. Repeat 3.

5. Refill the syringe, add the filter head containing the Whatman filter and discharge through the filter.

6. Repeat step 5 twice, being sure to remove the filter head before filling the syringe. Note: The filter will break if water is drawn IN through the filter head.

7. Refill the syringe, attach the head and discharge the sample into the chosen sample container.

WARNING: Allocate a syringe and filter head to be used for a small range of depths and do not interchange syringes from different depths, otherwise cross-contamination will occur.

A.2 Batch filtration

The filtration process is expedited by the use of a Millipore batch filtration system (Cat. No. XX2702540).

Procedure:

1. Prepare the Millipore unit prior to each station thus :

   - wrap silicon rubber O-rings in Teflon tape to reduce contamination with silicate or ammonia

   - using forceps, place a Whatman GFF 0.45-m filter in each filter space to be used

   - place the filter tray cover on top of the unit and seal the filters that will not be used with non-contaminating stoppers. (NOTE: Rubber bungs should be covered with Parafilm or some similar silicon free and ammonia-free wrapping.)

   - place a cover over the entire filter assembly.

2. Collect the samples from the water sampler (Nansen or Niskin) in 18 oz Whirlpak bags and seal the bags until ready to process.

3. Remove the cover and apply the vacuum to the filter assembly.

4. Dispense 5 ml of each sample into its respective filter holder and reseal the sample bags.

5. When filtration is complete, switch off the vacuum and allow several minutes for pressure increase inside the filter assembly, then remove a stopper from an unused filter position to allow the pressure to equalize.

6. Remove the filter lid and filter tray and discard the filter washings from the base of the unit.

7. Replace the filter lid and tray and all stoppers in unused filter positions. Repeat steps 4-6, then proceed to step 8.

8. Place an acid-washed dried Kayline polypropylene centrifuge tube containing 20 √1 of 5% mercuric chloride solution in the receiving vessel rack under each of the filters to be used.

9. Repeat steps 4 and 5, using 17 ml instead of 5 ml of sample. Proceed to step 10.

10. Remove the filter lid and filter tray, and cap the centrifuge tubes as quickly as possible.

11. Remove the centrifuge tubes, tape the lids, invert several times to mix the mercuric chloride preservative with the solution, and label and store the samples.

12. Inspect the filter tray and replace any broken filters before preparing the Millipore unit again as in step 1.

APPENDIX B

STORAGE OF SAMPLES FOR NUTRIENT ANALYSES

B.1 Containers

For simultaneous storage of ammonia, nitrate and nitrite, inorganic phosphate and reactive silicate, 18 ml polypropylene centrifuge tubes (Kayline) have been used. For storage of total phosphate samples, 50 ml glass vials (Pyrex) have been used.

To prepare the containers :

- soak overnight in 10% hydrochloric acid
- rinse three times with distilled, deionized water (PW)
- oven dry at 40°C
- add preservative and seal the tube immediately with a cap and masking tape.

If containers are not prepared, they may be used at sea after rinsing thoroughly with the sample about to be collected. Preservative should be added as soon as the sample is collected.

B.2 Preservatives

A range of preservatives were tested (Table 10) and mercuric chloride chosen for simultaneous preservation of ammonia, nitrate, nitrite, inorganic phosphate and reactive silicate. The concentration of mercuric ion in the final sample was 43 mg Hg^{2+} l^{-1} (20 µl of 5% (w/v) $HgCl_2$ (A.R.) per 17 ml of sample). This is the most that should be used, as more will foul the cadmium reduction column in the nitrate manifold. Less will work, but we have not determined the minimum to prevent biological activity for several months. Total phosphate samples were preserved in glass containers with a few drops of chloroform. The worst performance of these methods is shown in Table 11.

Table 10. Preservation methods tested

Code	Sample size	Container	Preservative
GCF/GCU	50 ml	Pyrex 50 ml vial, teflon-lined cap	Three drops chloroform
PTF/PTU	17 ml	Polypropylene centrifuge tube and cap	20 µl of 5% w/v $HgCl_2$
CGF/CGU	50 ml	Polyethylene bottle and cap	0.05 µg Cd granules
CWF/CWU	50 ml	Polyethylene bottle and cap	0.5 µg Cd wire
AFF/AFU	50 ml	Polyethylene bottle and cap	Drop of anti-fouling paint pre-painted on bottle
GBF/GBU	50 ml	Polyethylene bottle and cap	1 ml Grasshoff buffer
GFF/GFU	15 ml	Pyrex vial, teflon-lined cap	Frozen
WPF/WPU	6 oz	Whirlpak ® sterile	Frozen bag
NTF/NTU	15 ml	Polypropylene	None

Key

GC	Glass container	U	Unfiltered
PT	Pink top (centrifuge tube)	CG	Cadmium granules
CW	Cadmium wire	AF	Anti-fouling paint
GB	Grasshoff buffer	GF	Glass container, frozen
WP	Whirlpak	NT	No treatment
F	Filtered through 0.45-µm Whatman GFC filter		

Table 11. Performance of selected storage methods

Nutrient	Container†	Days of storage	Depth of sample (m) 0	100	300
Nitrate	PTF	34	12%	10%	18%
Nitrite ≠	PTF	29	30%	36%	-
Inorganic Phosphate	PTF	34	16%	13%	10%
Total Phosphorus	GCF	34	5%	24%	31%
Silicate *	PTF	29	8%	6%	7%

* Maximum deviation from the initial nutrient concentration of samples analysed in duplicate on days 0, 1, 3, 5, 7, 9, 13, 29 and 34 days after storage.

† See Table 10 for details.

≠ Concentration for day 34 not available. Nitrites were determined at the detection limit of the method.

APPENDIX C

PREPARATION OF SILICATE-FREE AND PHOSPHATE-FREE SEAWATER

Granulated zirconium hydroxide has been shown to remove silicate and phosphate without significantly altering the major ion composition of seawater passed through it (Novoselev et al. 1977). The seawater is used as the matrix in which standards are prepared and as the wash between samples. The low blank values obtained by this procedure have two advantages. Firstly, a greater range of standard values can be fitted onto the chart for a given colorimeter gain, which is important for low concentrations (high gain). Secondly, if the standard curve is not linear, the precision of low-level estimations at high absorbances will be improved if the absorbance values recorded for low silicate and phosphate levels in standards are in the same range as those recorded in samples.

C.1 Preparation of granulated zirconium hydroxide

Zirconium hydroxide is precipitated with concentrated ammonia from 1 l of a 0.25 M solution of zirconium oxychloride ($ZrOCl_2.8H_2O$) at pH 8-8.3. The resulting suspension is washed partly free of chloride ions by decanting (2-3 washings with 200 ml distilled water). It is then frozen at -10°C until the entire mass has solidified (16-20 h). The granular precipitate is filtered and washed clear of chloride ions on the filter, and dried at room temperature. The yield is about 150 g of the air-dried hydroxide.

C.2 Purification of seawater

Place 16-18 g of zirconium hydroxide in a Bel-Art plastic column (a drying tube) of 2 cm diameter; the height of the sorbent layer is 11-12 cm. The ends of the column are attached to a peristaltic pump and seawater is pumped through a filter from the bottom of the column to the top. The system can be used to purify seawater for storage in plastic Nalgene hedpaks and subsequent use in the preparation of standards or for supplying inter-sample wash.

APPENDIX D

SELECTION AND PREPARATION OF STANDARDS

D.1 Selection of Standards

The type of samples, (e.g. surface or deep water) determines the choice of gain setting on the colorimeter. The gain should produce a maximum sample signal of 50-70% of full-scale deflection on the chart recorder to avoid non-linearity at higher responses.

Note: sample responses can be kept within the range on the chart by dilution of the sample (see Appendix E).

Having selected the appropriate gain setting from the concentration range to be determined, to choose a set of standards from Figs 22-26 that will encompass that range for each nutrient.

D.2 Preparation of Standards

Standards may be prepared in the laboratory using volumetrics and pipettes capable of dispensing 10-200 µl. However, operation at sea requires automation to avoid the problems of operator variability.

(i) Laboratory use

Working stock standards are prepared using Gilmont pipettes to dispense the stock solution into low-nutrient seawater (LNSW) in 100 ml volumetric flasks. Standards are prepared fresh from the working stocks. Pyrex glass is used for all nutrients except silicate, for which polypropylene is used.

The appropriate volumes to be dispensed are shown in Table 12.

(ii) Sea-going use

A Hamilton Digital Diluter is preferred for dispensing standards both in the laboratory and at sea. The stock solution is dispensed into a Kayline polyethylene centrifuge tube and diluted with 15 ml of LNSW. The appropriate volumes of stock solution to be dispensed for the standards are shown in Table 13. The general procedure is:

- insert the appropriate syringes into the Diluter (always 10 ml for the diluent syringe, and one of 50 µl, 250 µl or 1 ml for the stock syringe)
- use the thumbwheels to select the percentage of full range required (always 75% = 7.5 ml for the diluent)
- take up the diluent and stock, then discharge into a polyethylene centrifuge tube
- reset the stock solution pipette thumbwheel to 0%
- take up another 7.5 ml of diluent (LNSW) and discharge into the same centrifuge tube
- cap the centrifuge tube and mix the contents thoroughly.

Table 12. Preparation of standards using Gilmont pipettes

Nutrient	Stock Concentration	Standard Concentrations	Volume of stock (µl) required in 100 ml
Ammonia	140 µg NH_3-N ml^{-1}	0 µg NH_3-N l^{-1}	0
		7	5
		14	10
		28	20
Nitrate	28 µg NO_3^--N ml^{-1}	0 µg NO_3^--N l^{-1}	0
		2.8	0
		5.6	20
		8.4	30
		11.2	40
		14.0	50
		28.0	100
		56.0	200
		112.0	400
Nitrite	14 µg NO_2^--N ml^{-1}	0 µg NO_2^--N l^{-1}	0
		1.4	10
		2.8	20
		5.6	40
		14.0	100
Phosphate	40 µg PO_4^{3-}P ml^{-1}	0 µg PO_4^{3-} P l^{-1}	0
		10	25
		20	50
		30	75
		40	100
Silicate	140µg SiO_4^{4-}-Si ml^{-1}	0 µg SiO_4^{4-}-Si l^{-1}	
		14	10
		28	20
		42	30
		56	40
		70	50
		84	60
		112	80
		140	100

Table 13. Preparation of standards using a Hamilton digital diluter

Nutrient: AMMONIA

A. Stock Solutions

Stock = 140 µg NH_3-N ml^{-1}

Working stock = 7.5 µg NH_3-N ml^{-1}

Dilution of storage stock to produce working stock:

1. Mount 10 ml syringe in diluent position
   2.5 ml syringe in stock position.

2. Take up 9 ml of PW then 1.5 ml of stock (140 µg NH_3-N ml^{-1}) and discharge into a pre-cleaned dry glass tube of 30 ml minimum capacity.

3. Take up 9 ml of PW only and discharge into the same glass tube and mix well.

4. Take up 8.5 ml. of PW only and discharge into the same glass tube and mix well.

Computation: $\frac{1.5 \text{ ml}}{(1.5+9+9+8.5) \text{ ml}} \times 140 \text{ µg } NH_3\text{-N } ml^{-1} = 7.5 \text{ µg } NH_3\text{-N } ml^{-1}$

B. Standard solutions

Standard concentration (µ g NH_3-N l^{-1})	Volume of working stock (7.5 µ g NH_3-N ml^{-1}) in 15 ml (µ l)	Stock syringe (µ l)	Percentage of full stroke
0	0	50	0
2.5	5	50	10
5.0	10	50	20
7.5	15	50	30
10	20	50	40
15	30	50	60
20	40	50	80
25	50	50	100

Nutrient: NITRATE

A. Stock solutions

Stock = 28 µg NO_3^--N ml^{-1}

Working stock = 15 µg NO_3^--N ml^{-1}

Dilution of storage stock to produce working stock:

1. Mount 10 ml syringe in diluent position
   10 ml syringe in stock position.

2. Take up 6.5 ml of PW (SLOW speed), then 7.5 ml of stock (28 µg NO_3^--N ml^{-1}) (FAST speed) discharge into glass tube and mix well.

Computation: $\frac{7.5 \text{ ml}}{(7.5+6.5) \text{ ml}}$ x 28 µg NO_3^--N nl^{-1} = 15 µg NO_3^--N ml^{-1}

B. Standard solutions

Standard concentration (µg NO_3^--N l^{-1}) (l)	Volume of working stock (15 µg NO_3^--N ml^{-1}) (µl)	Stock syringe (µl)	Percentage of full stroke
0	0 in 15 ml	50	0
2.5	2.5 in 15 ml	50	5
5.0	5.0 in 15 ml	50	10
7.5	7.5 in 15 ml	50	15
10	10 in 15 ml	50	20
15	15 in 15 ml	50	30
20	20 in 15 ml	50	40
50	50 in 15 ml	50	100
100	100 in 14.9 ml †	500	20
150 *	150 in 14.9 ml †	500	30
200 *	200 in 14.8 ml †	500	40
300 *	300 in 14.7 ml †	500	60
400 *	400 in 14.6 ml †	500	80
500 *	500 in 14.5 ml †	500	100

* Required only if dilution is necessary (see Appendix E).

† Alter volume of diluent syringe.

Nutrient: NITRITE

A. Stock solutions

Stock = 14 µg NO_2^--N ml^{-1}

Working stock = 1.5 µg NO_2^--N ml^{-1}

Dilution of storage stock to produce working stock:

1. Mount 10 ml syringe in diluent position
   2.5 ml syringe in stock position

2. Take up 6.5 ml of PW, then 1.5 ml of stock (14 µg NO_2^--N ml^{-1}) and discharge into the same tube.

3. Take up 6.0 ml of PW only and discharge into the same glass tube and mix well.

Computation: $\frac{1.5 \text{ ml}}{(1.5+6.5+6) \text{ ml}}$ x 14 µg NO_2^--N ml^{-1} = 1.5 µg NO_2^--N ml^{-1}

B. Standard Solutions

Standard concentration (µg NO_2-N l^{-1})	Volume of working stock (1.5 µg NO_2^--N ml^{-1}) in 15 ml (µl)	Stock syringe (µl)	Percentage of full stroke
0	0	50	0
1	10	50	20
3	30	50	60
5	50	50	100

Nutrient: PHOSPHATE

A. Stock Solutions

Stock = 40 µg PO_4^{3-}-P ml^{-1}

Working stock = 15 µg PO_4^{3-}-P ml^{-1}

Dilution of storage stock to produce working stock:

1. Mount 10 ml syringe in diluent position
   10 ml syringe in stock position

2. Take up 5 ml of PW, then 6 ml of stock (40 µ g PO_4^{3-}-P ml^{-1}), discharge into the same tube and mix well.

3. Take up 5 ml of PW only, discharge into same tube and mix well.

Computation: $\frac{6 \text{ ml}}{(6+5+5) \text{ ml}}$ x 40 µg PO_4^{3-}-P ml l^{-1} = 15 µg PO_4^{3-}-P ml^{-1}

B. Standard Solutions

Standard concentration (µg PO_4^{3-}-P l^{-1})	Volume of working stock (15 µg PO_4^{3-}-P ml^{-1}) in 15 ml (µl)	Stock syringe (µl)	Percentage of full stroke
0	0	50	1
5	5	50	10
10	10	50	20
15	15	50	30
20	20	50	40
35	30	50	60
40	40	50	80
65	60	250	24
80	80	250	32

Nutrient: SILICATE

A. Stock Solutions

Stock = 140 µ g SiO_4^{4-}-Si ml^{-1}

Working stock = 30 µ g SiO_4^{4-}-Si ml^{-1}

Dilution of storage stock to produce working stock:

1. Mount 10 ml syringe in diluent position
   10 ml syringe in stock position

2. Take up 5.5 ml of PW, then 3.0 ml of stock (140 µg SiO_4^{4-}-Si ml^{-1}) and discharge into the same tube.

3. Take up 5.5 ml of PW only, discharge into the same tube and mix well.

Computation: $\frac{3 \text{ ml}}{(3+5.5+5.5) \text{ ml}}$ x 140 µg SiO_4^{4-}-Si nl^{-1} = 30 µg SiO_4^{4-}-Si ml^{-1}

B. Standard solutions

Standard concentration (µg SiO_4^{4-}-Si l^{-1}) (l)	Volume of working stock (30µ g SiO_4^{4-}-Si ml^{-1}) (µl)	Stock syringe (µl)	Percentage of full stroke
0	0 in 15 ml	50	0
10	5 in 15 ml	50	10
20	10 in 15 ml	50	20
40	20 in 15 ml	50	40
60	30 in 15 ml	50	60
80	40 in 15 ml	50	80
100	50 in 15 ml	50	100
150	75 in 15 ml	250	30
200 *	100 in 14.9 ml †	250	40
250 *	125 in 14.9 ml †	250	50
300 *	150 in 14.9 ml †	250	60
400 *	200 in 14.8 ml †	250	80
500 *	250 in 14.8 ml †	250	100
1000 *	500 in 14.5 ml †	500	20
1500 *	750 in 14.2 ml †	500	30
2000 *	1000 in 14.0 ml †	500	40
2500 *	1250 in 13.7 ml †	500	50
3000 *	1500 in 13.5 ml †	500	60
3500 *	1750 in 13.2 ml †	500	70

* Required if dilution is necessary (see Appendix E).

† Alter volume of diluent syringe.

APPENDIX E

DILUTION OF SAMPLES

Sample dilution is necessary when silicate and nitrate concentrations are above 200 µg l^{-1} (i.e. samples collected at 100 m or greater depth). Other nutrient analyses are unlikely to require a dilution step. However, if different types of samples, such as estuarine or interstitial water samples, are used, dilution may be necessary before nutrient analyses can be performed on the autoanalyser.

The diluent must be a matrix of similar ionic strength to the sample and free from the nutrient being analysed. NFSW (see Section 2.2.4.) is suitable and enough should be prepared for all dilutions on a cruise.

Of the two methods for sample dilution -- in-line and pipette -- the former is usually used and is recommended.

E.1 In-line dilution

In-line dilution is most suitable when the samples in a batch have similar levels of concentration - for example, preserved samples from greater than 200 m can be run together. The method is less useful when samples within a batch require different dilutions.

A dilution circuit is inserted in the manifold of the nutrient for which high values are expected, so the sample is mixed with an in-coming diluent before reagents are added. This technique avoids the errors inherent in manual dilution.

The appropriate dilution circuits for nitrate and silicate are shown on the respective manifold diagrams (Figs 18 and 21). Other combinations are possible, but the maximum dilution with the in-line method is 1:260.

E.2 Pipette dilution

Pipette dilution is most suitable when samples within a batch require different dilutions, such as in a single cast where surface samples require no dilution, but samples from below 200 m require 1:10 dilution. It is vital that all standards be treated the same way to take into account the blank of the diluent.

The Hamilton Digital Diluter is preferred for performing dilutions at sea. The general procedure is :

1. insert the appropriate syringes into the Diluter (generally 10 ml for the diluent syringe and 1 ml or smaller for the sample syringe)

2. use the thumbwheels to select the percentage of full range required (e.g. 90% of a 10 ml diluent syringe and 99% of a 1 ml sample syringe would give a total volume of 9.99 ml and a dilution factor of 0.99:9.99 = approx. 0.1)

3. take up the diluent (NFSW) and sample, then discharge into a polyethylene centrifuge tube

4. add any extra diluent if required (for example, to make up a 15 ml volume)

5. cap the centrifuge tube and mix the contents thoroughly; check the dilution factor and enter it on the log sheet (Appendix F).

APPENDIX F

CUP DEFINITION FORM

F.1 Purpose of instructions

An autoanalyser cup definition form (Fig. 28) must be completed whenever the operator is recording data onto chart records.

These instructions will ensure operator uniformity in logging information. Although the explanations may seem lengthy and sometimes self-evident, these directions are necessary to avoid subsequent confusion in the interpretation of chart records and consequent loss of expensive data.

F.2 Key used in annotating log sheets

The following symbols and colour codes are used in the discussion of how to complete the log sheet.

Nutrient	*Symbol*	*Colour*
Ammonia	NH	red
Nitrate	N3	blue
Nitrite	N2	violet
Phosphate	PO	green
Silicate	SI	yellow

CSIRO MARINE LABORATORIES, HOBART, TASMANIA

AUTOANALYSER CUP DEFINITION FORM

CRUISE	DATE	RUN	START	END	OPERATOR	GAIN	S1	N3	PO	N2	NH
						DILUTION					

CUP	TUBE NUMBER	STATION	RPT.	DILUTION FACTOR	SILICATE		NITRATE		PHOSPHATE		NITRITE		AMMONIA	
					PEAK HEIGHT (MM)	CONC. (ug/L)	PEAK HEIGHT (MM)	CONC. (ug/L)	PEAK HEIGHT (MM)	CONC. (ug/L)	PEAK HEIGHT (MM)	CONC. (ug/L)	PEAK HEIGHT (MM)	CONC. (ug/L)

STANDARDS

CONC. (ug/L)	PEAK HEIGHT (MM)	SLOPE
SILICATE		
NITRATE		
PHOSPHATE		
NITRITE		
AMMONIA		

Figure 28. Autoanalyser cup definition form

F.3 Batch processing

All parts of the form should be completed (except the optional columns, i.e. cup re-labelling, ✓ , repeat, dilution factor, extra cups and remarks columns).

CRUISE:
Insert cruise name and number.

DATE:
Date of analysis.

RUN:
Run number of this set of samples (i.e. Run 1, 2 etc.) on this day.

START:
Time of starting sampler.

END:
Time of stopping sampler.

OPERATOR:
Operator's name or initials.

GAIN:
The gain settings on the colorimeters should be noted for each channel and inserted next to the symbol for the nutrient.

DIL:
If a dilution circuit is inserted in the pump to dilute a sample automatically, the dilution factor should be entered in the column under the appropriate nutrient label. (Note: MANUAL dilutions are entered in the DILUTION FACTOR column).

PROBE:
The label of the probe holder (A or B) should be inserted for the probes of each of the nutrients. Usually all probes are in the probe holder at the extremity of the arm (A). However, the silicate probe may occasionally be in the next holder if LNSW is contaminated with silicate and PW is used as wash. (See also 2.2.1.).

WASH:
The nature of the wash in each wash receptacle should be shown here. Usually, wash A holds LNSW and wash B holds PW.

PRE-RUN:
Any alterations to the systems should be recorded, for example - changes in tubing, manifolds, or new reagents.

BLANK COLUMN:

The blank column on the left-hand side of the sheet is used for two purposes. Firstly, the positions of starting and stopping of the A probe should be noted using a line and symbol A, as shown in Fig. 28. Secondly, cup-renumbering should be entered here. It is essential that every peak on the chart has a sequential and unique cup number. Cup re-numbering is necessary (i) when the tray is loaded and some samples are not analysed. In this case, the cup numbers on the log sheet should be adjusted so that for each peak on the chart the numbers on the log sheets are sequential, and (ii) when extra samples need to be included. These should be entered in the EXTRA CUPS, etc., column opposite the point where they were inserted and the subsequent cups relabelled to maintain the sequence.

CUP:

The cup number is pre-marked in this column and should not be altered unless re-numbering is necessary (as above).

√, RPT:

If it is necessary to repeat a sample by placing it in another position in the tray, this fact must be noted on the log sheet so that the peak is not included when the chart is read. When a sample is repeated the RPT column is slashed with the appropriate colour to indicate which nutrients require re-analysing. Once the sample has been satisfactorily repeated, a tick in the appropriate colour should be placed in the √ column.

Using this system, the following circumstances are covered:

If the sample needs to be repeated, this is indicated in the RPT column. When repeated, indicators in the √ column inform the reader that the original peak need not be entered in the data storage system.

If the sample needs to be repeated, indicators are shown in the RPT column, but if no *satisfactory* repeat analyses are made, there will be no indicators in the √ column, and the reader will enter the initial but unsatisfactory peak in the data system with a marker to indicate that the peak is questionable.

If the sample needs to be repeated for all or some nutrients, indicators are shown in the RPT column, but, if only some of the required number of repeat analyses are satisfactory, only the *satisfactory* analyses will be indicated in the √ column. For example, if it were necessary to repeat analyses of a sample for silicate, ammonia and nitrate, then a yellow, a red, and a blue slash would appear in the RPT column. If only the silicate peak was satisfactory in repeat analyses, in repeat analyses then only a yellow tick would appear in the √ column. The data reader would then store the original ammonia and nitrate peaks, with labels to indicate the questionable nature of the peak, and would record the repeated and satisfactory silicate peak, ignoring the original unsatisfactory silicate peak.

Note: Only samples to be repeated because of unsatisfactory result should have entries in the √ and RPT columns. The repeat analysis itself does not, if it is satisfactory, does not require any entry in the √ and RPT columns. In fact, if entries do appear against the repeat, it will be assumed that

this sample has later been repeated for a second time, and peaks will not be read and stored.

SAMPLE No. NUTRIENT:

When samples are being processed, the sample number should appear in this column. The sample number will be obtained either from a label on the sample bottle or from a log sheet specifying the sample number. When standards are being processed, the nutrient label (NH, N3, N2, PO or SI) should appear in this column.

SAMPLE CODE/CONC. STD:

When samples are being processed, the sample code (that is, a label to describe the history or container type) is recorded in this column; when standards are being processed, the concentration of the standard is recorded in this column. Usually, concentration units are $\mu g\ l^{-1}$; however, if other units are required, they should be clearly indicated.

DIL FACT:

If manual dilution of a sample is made, the dilution factor should be entered in this column. Dilution factor (DF) is calculated as :

$$DF = \frac{\text{Volume of sample}}{\text{Final volume of diluted sample}}$$

NH, N3, N2, PO, SI

These columns are used to indicate which cup numbers are to be rejected during manual chart reading or digitisation (see - Airey *et al.*, in prep.).

EXTRA CUPS, ETC:

Since the log sheet is filled out at the beginning of a run, the CUP and SAMPLE No. columns will be fully filled. If it is necessary to insert any samples during a run, the cup numbers and sample numbers should be entered in this column, close to the cup after which the interruption is made. Note that it will be necessary to re-label the cup numbers in the main body of the sheet to keep the sequence.

REMARKS:

Any comments about the sample as it is fed into the autoanalyser, or as it is being processed, should be entered here. Such comments could include:

- sample quantity or quality (for example, sample has green tinge)
- handling (including possible contamination) of the sample (e.g., finger in sample, smoker entered lab, or laboratory fire extinguished with mono-ammonium phosphate extinguisher, or carbon dioxide extinguishers used near nutrient autoanalyser)
- labelling of the sample (for example, label illegible)
- events during processing that could affect the recorder trace obtained, (e.g. pauses, light source fluctuations, power fluctuations, chart jamming, manifold connectors breaking, or other instrument malfunctions).

STANDARDS:

As standards are analysed, the peaks on the chart recorder should be recorded directly on this log sheet for immediate comparison with previous standard curves to determine whether the standards are acceptable. The columns are used as follows:

- the concentrations of the standards for each nutrient are entered in the column CONC. STD
- the peak height is read directly from the chart for each of the standards and entered in the column RAW PEAK HEIGHT
- the value of the peak height for the blank standard is subtracted from the peak height values and the results are entered in the column CORR. PEAK HEIGHT
- a calibration plot of concentration vs absorbance is drawn using CORR. PEAK HEIGHT values; a line of best fit is drawn through the data points and the accurate peak height is read from the graph for each of the standards; these values are entered in the ACC. PEAK HEIGHT column.

This procedure allows (a) the operator to compare standards from any run with previous standards, to detect any errors in their preparation, and to note deterioration of tubing or any malfunction in the manifold; and (b) the data reader to produce concentration value files using a least squares fit of a line to the corrected peak height values. (See Section 3.2.9).

F.4 Continuous Mode Operation

The log sheet used for batch mode operation is also used for continuous mode operation, with some modifications to the entries. Only entries that from batch mode entries are listed below.

PROBE:

Left blank in continuous mode operation.

BLANK COLUMN:

Left blank in continuous mode.

CUP:

Cup numbers are used only when standards are being run (i.e. discrete samples).

SAMPLE No./NUTRIENT:

This column will list the standard designations - NH, N3, N2, PO, SI - when standards are being run. Otherwise, it should record the time (in 24 h clock notation) of the start and finish of a continuous sampling run. If the intake line is held at a particular depth for sampling, the depth or length of cable and hydrology-wire angle and the times of commencement and conclusion of the pause should be noted here (also mark the recorder chart).

REMARKS:

The chart speed should be noted here in order to match the chart record with the log sheet. If continuous profiling mode is being used, and if the rate of descent of the intake line is not regular, the rates of descent in each portion of the profile should be noted here.

(Note: The period for which a water mass of one concentration must be sampled in order to reach the maximum colour development for samples to be related to the standards must be established for each profiling sampler hose. This can be done on deck by inserting the profiling pump in a large container containing a high standard. Ensure that the rate of descent of the pump is not too rapid to differentiate interesting water pockets. It may, for example, be necessary to pass through the thermocline at a much slower rate).

APPENDIX G

TYPICAL RECORDER TRACES

Nutrient peaks on recorder charts are shown in Fig. 29a-e. The peaks obtained from nitrate and silicate manifolds with dilution circuits are shown in Fig. 29f,g.

For batch processing, a compromise had to be made between obtaining a maximum value for each sample peak (indicative that pure sample rather than sample plus the last traces of LNSW wash had reached the flow cell) and limiting the time of sampling so that all five nutrient samples could be determined from a 15 ml seawater sample. Hence, not all recorder peaks have plateaux. However, the time of transit in the manifold line is constant for each manifold, i.e. standards and samples are treated identically. It is therefore permissible to operate with sub-maximal colour development.

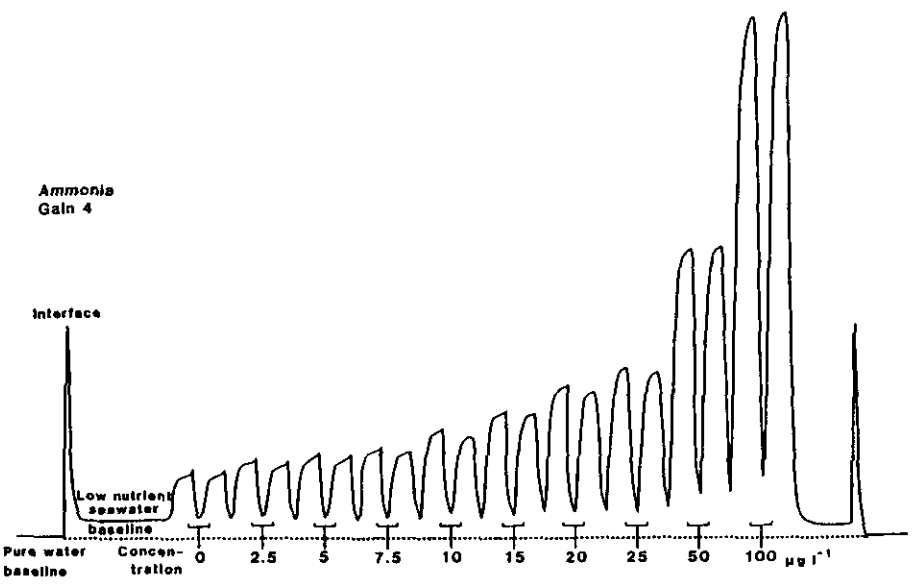

Figure 29a. Typical recorder traces - ammonia

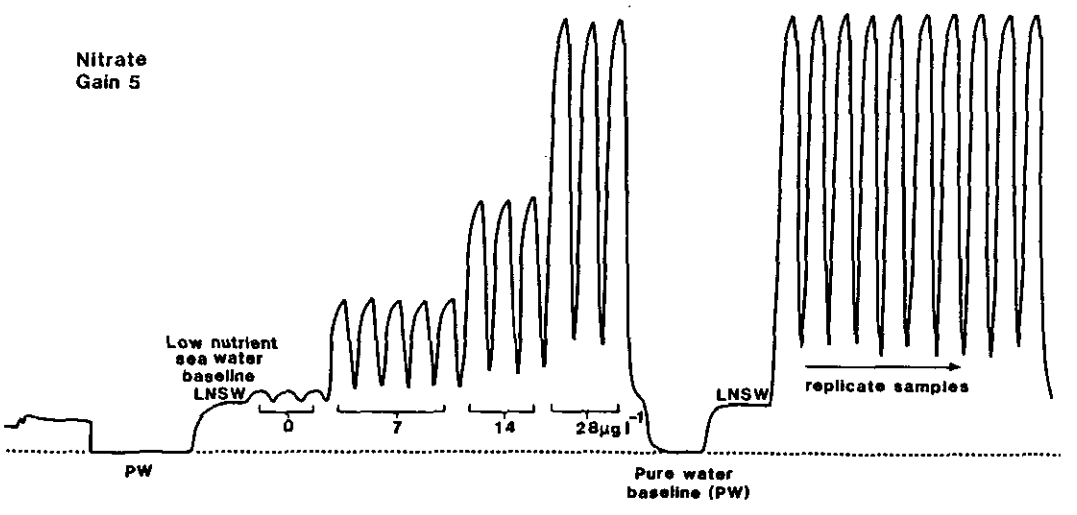

Figure 29b. Typical recorder traces - nitrate

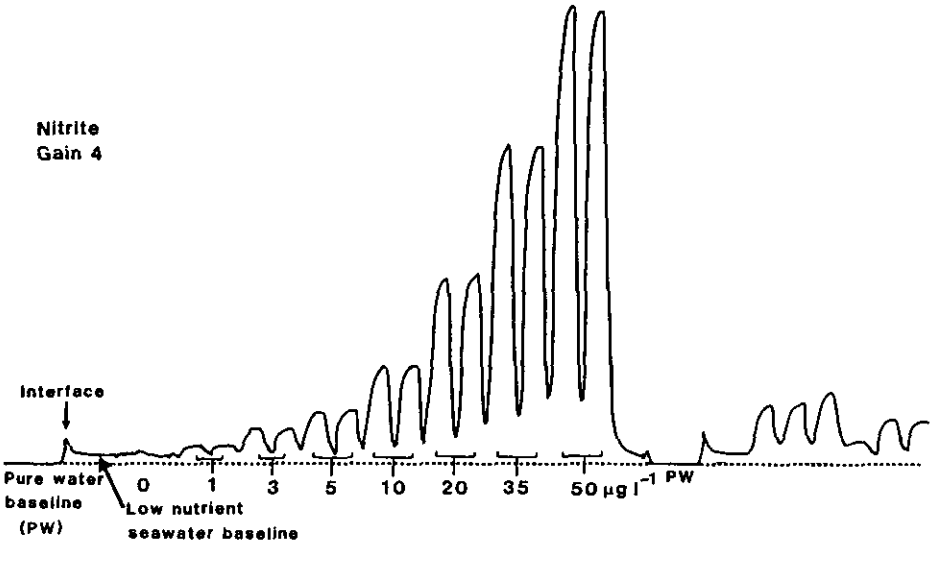

Figure 29c. Typical recorder traces - nitrite

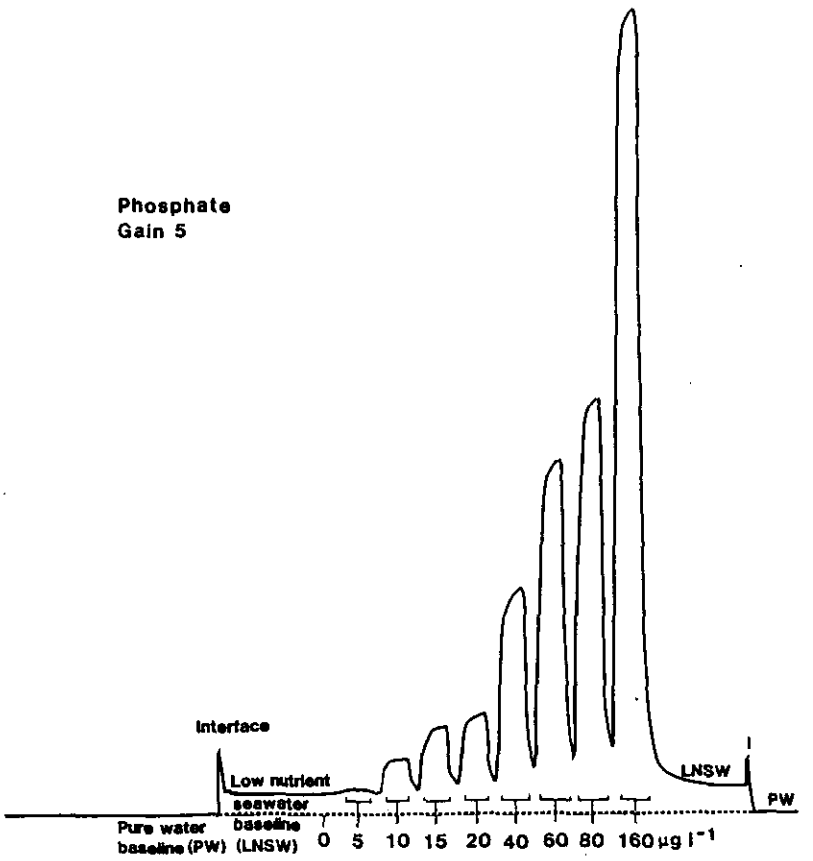

Figure 29d. Typical recorder traces - phosphate

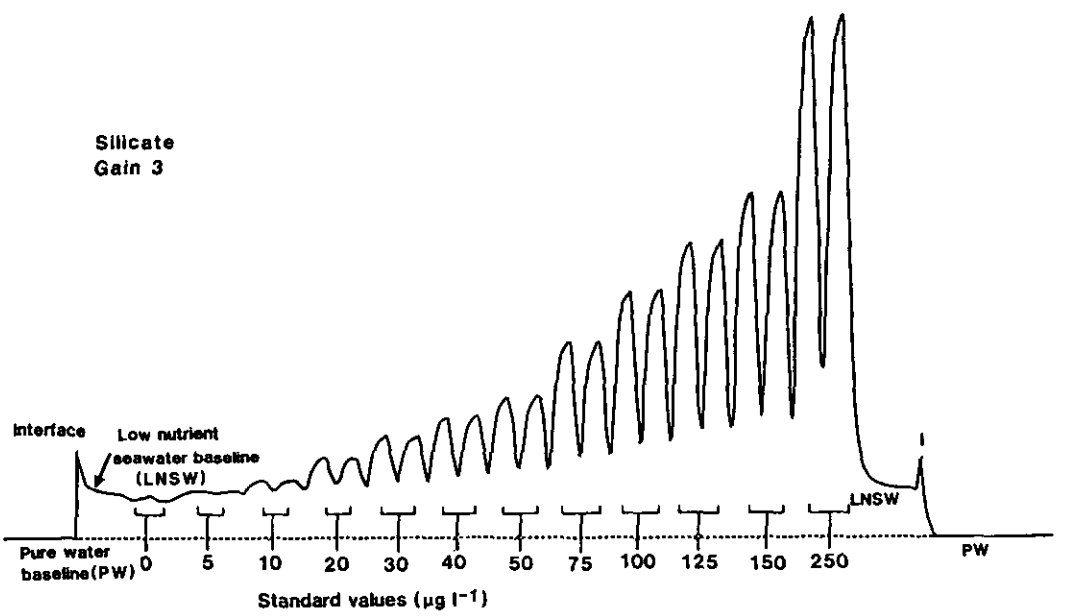

Figure 29e. Typical recorder traces - silicate

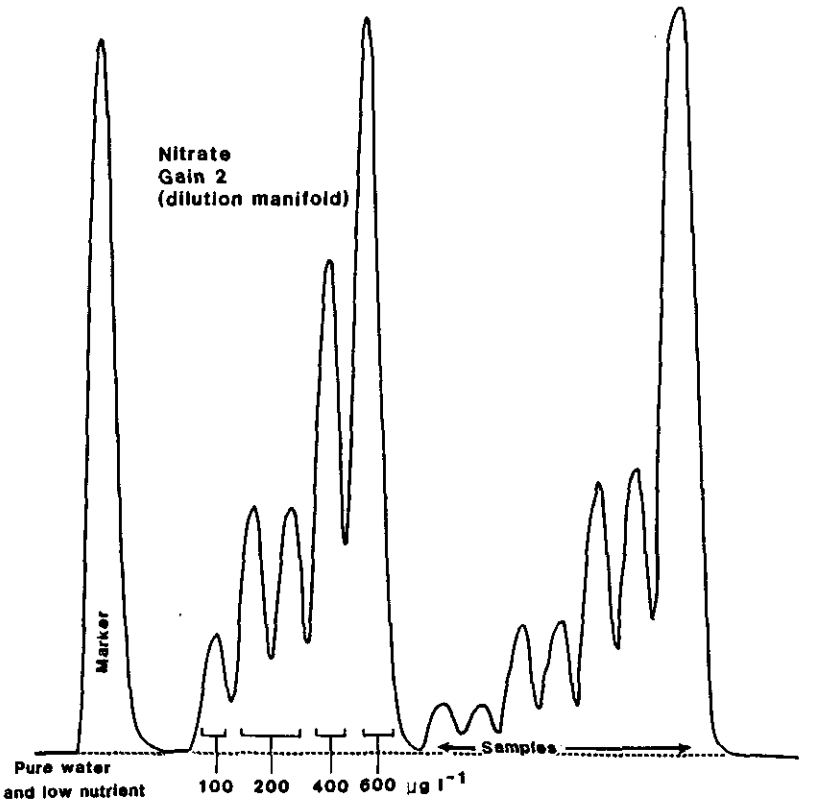

Figure 29f. Typical recorder traces - nitrate line with dilution circuit

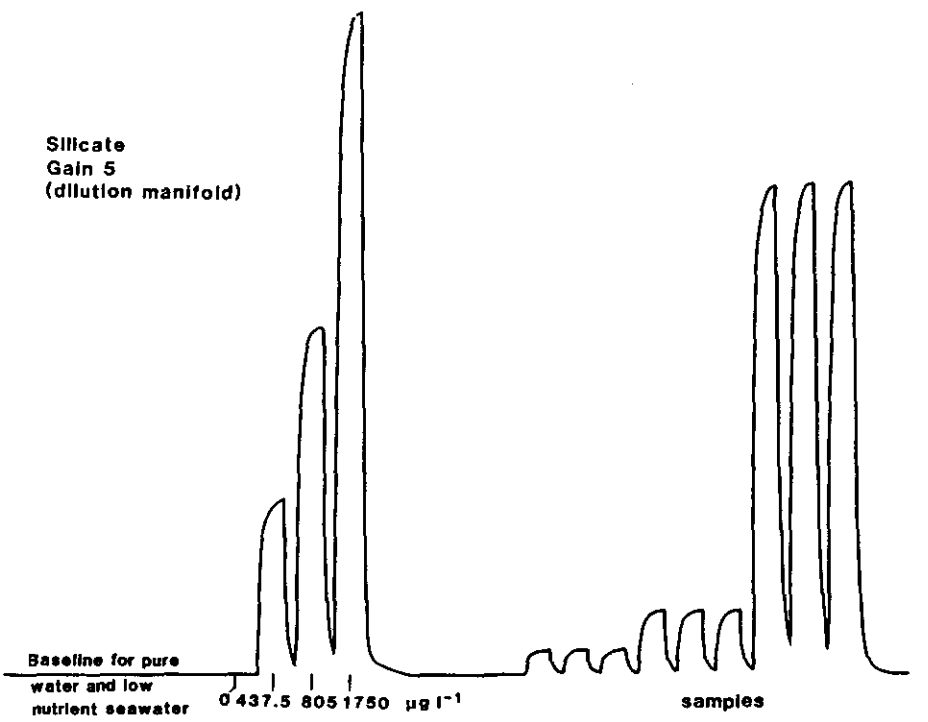

Figure 29g. Typical recorder traces - silicate line with dilution circuit

APPENDIX H

COMPONENTS AND SUPPLIERS

ITEM	SUPPLIER
Kayline polypropylene centrifuge tubes: 17 ml	H.L.S. Scientific 3 Schofield St Riverwood
Nasco Whirlpak® bags 6 oz, 18 oz	A.H.S. Australia Pty Ltd. 25 Paul St North Ryde
Mono pump	Mono House Ethel Rd Kirrawee
Nylon hose, 1/2 inch diameter	Nylex Corporation 361 Horsley Rd Milperra
Filter and filter holder Whatman Gamma 12/80	F.S.E. Scientific 40 Hilly St Mortlake
Flow regulator Fischer and Porter series 3135	Fischer and Porter 156 Pacific Highway St. Leonards
Peristaltic Pump Watson and Marlow	Chemical Construction Equipment Co. Pty Ltd 36 John St Mascot

ITEM	SUPPLIER
Polyethylene tube I.D. 1/8 inch; O.D. 1/4 inch Cole Parmer Cat. No. 6407-02	Edwards Laboratories Pty Ltd 29 Bellingham St Narellan
Polypropylene tube I.D. 1/20 inch; O.D. 1/8 inch Technicon Cat. No. 562-5001-01	Technicon Equipment Pty Ltd 80 Talavera Rd North Ryde
Filter 0.45 µm Whatman GFF	F.S.E. Scientific (see above)
Filter holder Millipore Cat. No. SX002500 Swinnex 25 mm	Millipore & Water Associates Lock Bag 18 Lane Cove NSW 2066
Disposable syringe 20 ml Millipore Cat No. XXII 02012	Millipore & Water Ass. (see above)
Batch filtration unit Millipore Cat. No. XX27 05040 3025 sampling manifold Replaced by 1225 sampling manifold holder	Millipore & Water Associates (see above)
Sampler IV and cams Technicon Cat. No.171-B100-04	Technicon Equipment Pty Ltd (see above)
Sample Holder Technicon Cat. No. 171-B100-04	Technicon Equipment Pty Ltd (see above)
Sample-baseline switching valves (3 way) single key Omnifit Cat. No. 1109	Amersham Australia P.O. Box M321 Strawberry Hills NSW
Nalgene hedpaks; 4-1 Cole Parmer Cat. No. 6061	H.L.S. Scientific (see above)
Solution Administration Set Cat. No. 30-500	Tuta Laboratories 332 Burns Bay Rd Lane Cove
Reagent wash changeover unit Chemlab	Nalco Industries 73 Kissing Point Rd Dundas
Peristaltic pump CPP-30 Chemlab	Nalco Industries (see above)

ITEM	SUPPLIER
Peristaltic pump III Technicon Cat. No. 133 A014 01	Technicon Equipment Pty Ltd (see above)
Elkay flow-rated pump tubing Series 116-0549	Nalco Industries (see above)
Elkay standard autoanalyser tubing I.D. 0.025 inch Cat. No. 116-0536 - 060	Nalco Industries (see above)
Manifold housings Type B Technicon Cat. No. 170-0108-G	Technicon Equipment Pty Ltd (see above)
Manifold trays Technicon Cat. No. 170-010-H	Technicon Equipment Pty Ltd (see above)
Manifold tray covers Technicon Cat. No. 170-0233-O	Technicon Equipment Pty Ltd (see above)
Five-channel colorimeter Mark III Chemlab Cat. No. MC1002	Nalco Industries (see above)
50-mm flow cells Chemlab. Cat. No. MC1002/2	Nalco Industries (see above)
Filters nm Chem Cat. No. Ammonia 630 MC1000/5+ Nitrate 540 MC1000/5+ Nitrite 540 " Phosphate 880 MC1000/6+ Silicate 820 "	Nalco Industries (see above)
Single channel colorimeter Technicon Cat. No. 199-0001-01	Technicon Equipment Pty Ltd (see above)
50-mm flowcells Technicon Cat. No. 199-B007-03	Technicon Equipment Pty Ltd (see above)
Filters nm Technicon Cat No. Ammonia 630 170.B070-27 Nitrate 550 170-B070-40 Nitrite 550 170-B070-40 Phosphate 880 170-B070-48 Silicate 815 170-B070-XX	Technicon Equipment Pty Ltd (see above)
Wastelines Polypropylene tube, I.D. > 2.00 mm	
Recorders - 1 pen Hewlett Packard Cat. No. 7123-A	Hewlett-Packard Pty Ltd 31 Bridge St Pymble

ITEM	SUPPLIER
Recorders - vertical pen YEW Cat No. 3056 2 or 3 pen	Parameters Pty Ltd 68 Alexander Rd Crows Nest
Chart paper YEW Cat. No. XT127	Parameters (see above)
Digital Diluter Hamilton Cat. No. 100200	F.S.E. Scientific (see above)
Sieves: Mesh 300 µm, 700 µm BS410	H.L.S. Scientific (see above)
Vials, Pyrex 50-ml Teflon-lined cap	Ramsay Surgical Ltd 7-9 Khartoum Rd North Ryde
20 cm drying tube plastic I.D. 2 cm (for zirconiumhydroxide) Bel-Art Cat. No. F19962	John Morris Scientific PO Box 80 Chatswood
Pipette: 0.2-ml; 2-ml Gilmont	H.L.S. Scientific (see above)

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