I.O.S.

A MANUAL OF METHODS FOR THE CONTINUOUS FLOW DETERMINATION OF AMMONIA, NITRATE-NITRITE, PHOSPHATE AND SILICATE IN SEAWATER

BY

D. J. HYDES

REPORT NO. 177
1984

NATURAL ENVIRONMENT RESEARCH COUNCIL

INSTITUTE OF OCEANOGRAPHIC SCIENCES

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When citing this document in a bibliography the reference should be given as follows:-

HYDES, D.J. 1984 A manual of methods for the continuous flow determination of ammonia, nitrate-nitrite, phosphate and silicate in seawater. *Institute of Oceanographic Sciences, Report*, No. 177, 37pp.

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WORMLEY

A manual of methods for the continuous flow determination of ammonia, nitrate-nitrite, phosphate and silicate in seawater

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ABSTRACT

This report is aimed to provide the information required to successfully take to and run at sea the IOS - "Chemlab" continuous flow analysis system. Background information on the system is given, along with the details of the chemistries involved, and a full listing of the chemicals and subsidiary equipment needed at sea.

CONTENTS

INTRODUCTION

The purpose of this manual is to provide a basic source of information in addition to the manufacturers instrument manuals which should enable someone who has had two weeks training with the auto-analyser on dry land to successfully run the system at sea. The introduction summarises the most salient points required to understand the workings of the system. The setting up of the machinery for each of the individual chemical methods involved is described in the method section. This is followed by a section on trouble shooting the system, and a listing of the equipment which needs to accompany the instrument to sea. The hardware referred to in this report is that supplied by "Chemlab Ltd". A companion report (IOS Report 176) describes a system for automated data reduction using a CBM PET microcomputer.

THEORETICAL BASIS OF THE METHODS

All the the methods described here are "colorimetric", that is a chemical reaction is carried out with the micronutrient in the seawater sample to produce a coloured solution. The density of the colour is proportional to the concentration of the nutrient present (where the Bear-Lambert law holds) and can be measured accurately using a photometer containing filters corresponding to the colour developed in the solution.

The Beer-Lambert law

$$Ia = Io(1 - e^{-cx})$$

(Ia - light absorbed, I_o - incident light c - concentration of nutrient, x - constant for particular system) generally holds where the amount of light absorbed is less than about 85% of the incident light. Most spectrophotomers are calibrated in terms of <u>transmittence</u>,

$$\text{i.e.} \qquad \frac{Io - Ia}{Io}$$

and <u>absorbance</u> which is the log of the reciprocal of transmittence and is directly proportional to the concentration. However although on the "Chemlab" colorimeter the output from the photometer is fed through a logarithmic converter

to give a signal which is porportional to concentration (where the Beer Lambert law holds) it is not calibrated. This means that the linear working range has to determined empirically for each method. When developing a method the dilution of sample in the analysis stream is adjusted to keep to the maximum concentration likely to be encountered within the linear range of the Beer Lambert law. In operation the gain control on the colorimeter is adjusted to give full scale deflection on the chart recorder (being used to record the signal from the colorimeter) for the most concentrated sample.

The fundamental feature of continuous flow automated chemistry is segmentation of a flowing stream of sample and reagent with bubbles of air. The bubbles serve three purposes. Firstly they cause friction with the tubing creating turbulent flow which tends to mix sample and reagents. Secondly they prevent diffusional and turbulent mixing of one sample with the next, and thirdly they continuously scrub the walls of the tubing again limiting tailing of one sample into the next.

An important concept to grasp is that it is not necessary to have reactions go to completion to gain the increase in precision which is inherent in automated systems. This is because all operational conditions are maintained the same, so that each sample is subjected to exactly the same quantity of reagents, the same temperature, and the same mixing time as every other sample. Therefore each subsample between bubbles is repeatedly measured at some constant percentage of the completed chemical reaction. The precision of determinations is not therefore effected detrimentally although the sensitivity may be slightly lower than if the reactions had gone to completion.

We will now consider in greater detail two aspects of continuous flow analysis which have been noted above which are essential to understanding the operation of such a system and analysing the data output. They are firstly bubble segmentation and mixing, and secondly sampling rates and carryover.

BUBBLE SEGMENTATION AND MIXING

Bubble-size

When air and reagents are pumped into an injector fitting the air bubble increases in size until it fills the main tube and breaks away. The size of the bubble depends on the geometry of the injector and the surface tension of the reagent mixture. It is independent of the rate of air input. Increasing air input will increase the frequency of bubbles. The more frequent the bubbles the more effective will be their scrubbing action. The optimum size of bubble is one where the length should be about twice the tube diameter. Resolution is degraded when bubbles are larger or smaller than this.

Bubbles and coils

Bubbles in the flowing stream of reagents and sample, and, coiling of tubing induces mixing of solutions of different viscosities. As the two layers of different viscosity pass along straight tube the more viscous reagent moves to the rear of the segment. As the segments go round a coil because the outer circumference of the coil is greater than the inner, liquid on the outer edge moves relatively more quickly than that on the inner surface. This induces circulation within the individual segments producing mixing. For this effect to work best the segments should not be too long. About two and a half to three segments per turn of coil is optimal.

SAMPLING RATES AND CARRYOVER

Inside the colorimeter cell mixing of debubbled solutions can occur and this carryover of one sample into the next appears as overlapping peaks on the chart recording. The rate at which one sample is washed out of the cell by the next depends on the geometry of the colorimeter cells and the viscosities of the solutions. As a good approximation the degree of carryover of one peak to the next when expressed as a fraction of the previous peak is fairly constant for a particular set of conditions at a given time after the preceding peak. Hence the degree of carryover depends on the total time between peaks, i.e., sampling time plus wash time, and does not depend on the ratio of sample time to wash time. Measurement of peak heights will be most precise where sampling time is sufficiently long for the peak to reach a plateau. Practically speaking the liklihood of possible attainment of the plateau will be reduced by long wash relative to sample times. When deciding on sample and wash times a balance must be drawn between the following points.

(1) The longer the sampling time, the more near will be the approach to steady state in the colorimeter cell and the more precise the measurement.
(2) The longer the sample and wash time the less will be the necessary correction.
(3) How much sample is available.
(4) The overall time for the analytical run.

SETTING UP AND CLOSING DOWN

Setting up

For the initial setting up.

(1) Place the pump tubing in the peristaltic pump manifolds in the order shown in in the method sheets.

(2) Connect these to the chemistry manifolds keeping the lengths of tubing to a minimum, trimming the pump tubes.

(3) Lengths of tubing into reagent bottles should be trimmed so they just reach the bottom of the bottles.

Daily

(1) One hour should be allowed for warmup of the colorimeter and heating baths.

(2) Start the reagents pumping through the lines after 20 minutes switch on the chart recorders and begin to observe the quality of the baselines. Check that the bubble pattern is steady, and that all the reagents are being pumped.

(3) If the nitrate column is to be used it should be fitted to the manifold after the buffer solution has been pumping for 10 minutes. If possible allow one hour after fitting the column before the start of an analytical run.

(4) Before starting the main run, determine 3 top of the range standards, and check that they are on scale and that the system is giving the expected response.

Closing down

(1) At end of run, remove the nitrate column.

(2) Place all reagent lines into distilled water containing the appropriate wetting agent, and leave pumping for at least 15 minutes.

(3) If nitrate/nitrite line has been used it is recommended to flush this out by pumping 10% "Decon" solution through the lines for five minutes before changing over to distilled water containing Brij-35. The Decon is more effective at removing any adsorbed colour from the flow cell than Brij-35.

(4) If the machine is not to be used for several days follow the wetting agent rinse by pumping distilled water through all the lines including the wash line for 15 minutes. Then pump all the lines dry.

(5) Open up the pump plattern and wipe the excess oil from the pump tubes.

(6) Relax the tubes to first peg at each side of plattern do not unhook them, because of the danger that if the pump is started before the blocks are hooked up one may jam in the rollers and damage the pump. Such damage is repairable but it takes time (see "Chemlab" manual).

METHODS

Note

For use with the automatic data reduction system developed with the Commodore PET Microcomputer, the colorimeter gains should be set for use with a chart recorder full scale of 100mV. A sampling rate of 90 seconds sample and 45 seconds wash has been decided on. This allows all four channels to be run simultaneously with a 2.5ml sample, "Anala R" grade chemical should be used throughout for the preparation of reagents.

AMMONIA

Ammonia in seawater is determined by a modification of the Bertholet reaction. Phenol and hypochlorite react with ammonia to form chloramine then aminophenol and finally indophenol blue which is measured at 625nm. Dichloroisocyanurate provides a convenient source of hypochlorite. A concentrated citrate buffer is used to prevent interference by magnesium.

Reagents

(1) Tri sodium citrate: Dissolve 34g in 500ml distilled water, containing 20ml of dilute sodium hydroxide and 1ml of Brij-35 (25% w/v).

(1a) Sodium hydroxide (dilute) Dissolve 10g in 500ml distilled water.

(2) Phenol (Stock solution): Dissolve 10g in 500ml distilled water.

(3) Sodium nitroprusside (stock solution): Dissolve 1g in 500ml distilled water.

(4) Sodium dichloroisocyanurate: Dissolve completely 2.5g Sodium hydroxide in 250ml distilled water. Dissolve 0.4g D.I.C. in this then make up to 500ml (stable 14 days).

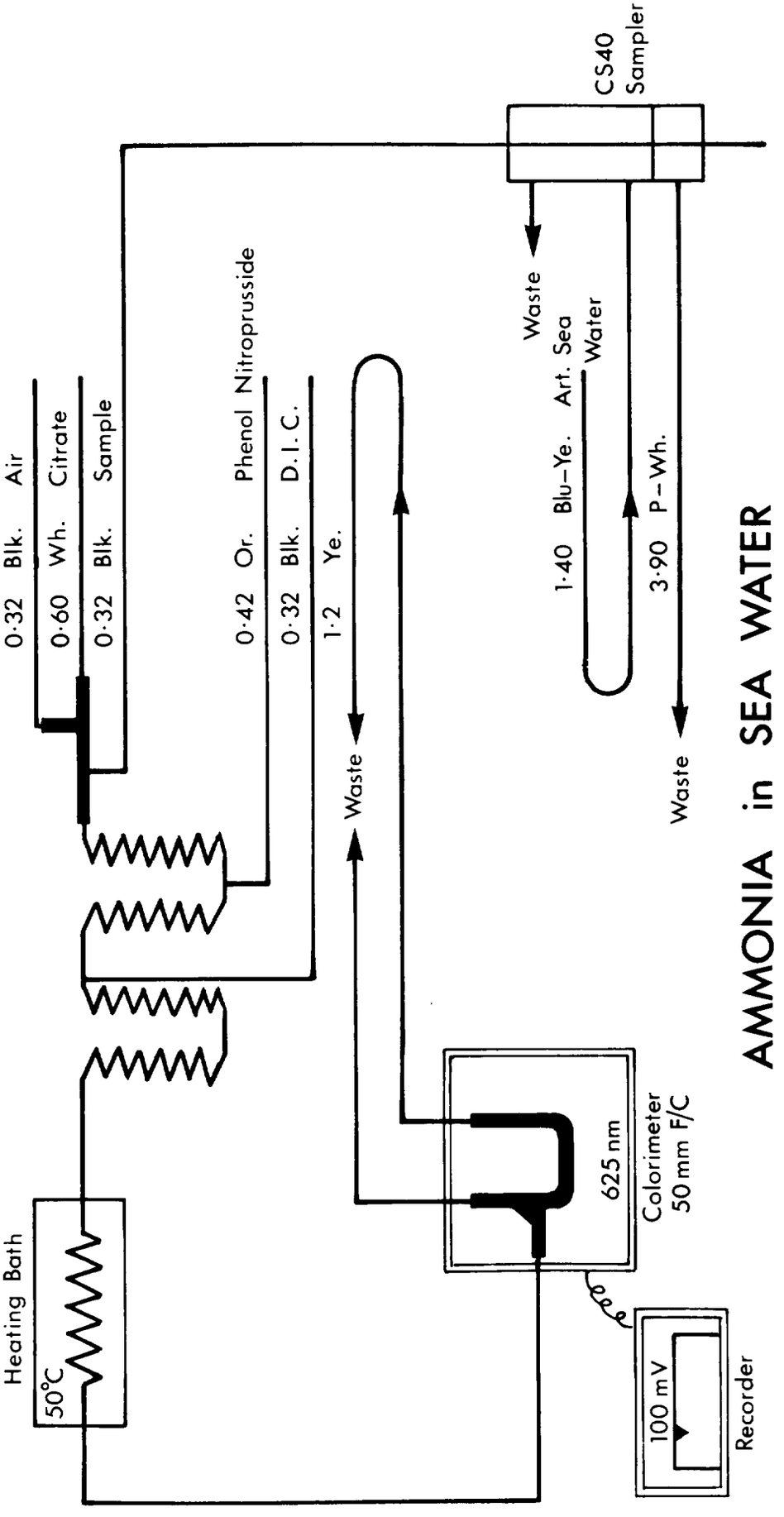
(6) Mixed phenol/nitroprusside 10 volumes of phenol plus 1 volume of nitroprusside (prepare fresh daily).

Standard Solutions

Dissolve 0.134g ammonium chloride in 500ml distilled water (5mM). Prepare the following working standards by dilution in 100ml of freshly collected surface seawater.

Standards

500µl = 25µM
375µl = 18.75µM
250µl = 12.5µM
125µl = 6.25µM


AMMONIA in SEA WATER
CS40 Sampler
Waste
Art. Sea Water
1·40 Blu–Ye.
3·90 P–Wh.
Waste
Waste
0·32 Blk. Air
0·60 Wh. Citrate
0·32 Blk. Sample
0·42 Or. Phenol Nitroprusside
0·32 Blk. D.I.C.
1·2 Ye.
Heating Bath
50°C
625 nm
Colorimeter
50 mm F/C
100 mV
Recorder

Lines

(1) Citrate Buffer (White 0.6ml/min)

(2) Air (Black 0.32ml/min)

(3) Sample (Black 0.32ml/min)

(4) Phenol. Nitroprusside (Orange 0.42ml/min)

(5) D.I.C. (Black 0.32ml/min)

(6) Return (Yellow 1.2ml/min)

NITRATE-NITRITE

The analysis of nitrate requires the reduction of nitrate to nitrite. Nitrite is determined by forming a diazo compound and then an azo dye, which is measured at 540nm. For seawater the most practical way of reducing nitrate to nitrite is heterogeneously using cadmium metal. We have developed an on-line column filled with a copper-cadmium alloy for this purpose.

Reagents

(1) Ammonium chloride: Dissolve 50g in 2L distilled water.

(2) 4-Amino-acetophenone Dissolve 0.15g in 100ml of 50% (v/v) acetic acid. Add 1m Brij-35 (25%w/v) when dissolved.

(3) 1-Analinoanaphthalene-8-sulphonic acid Dissolve 0.06g in 100ml distilled water. Add 1ml Brij-35 (25%w/v) when dissolved.

Preparation of the copper-cadmium column

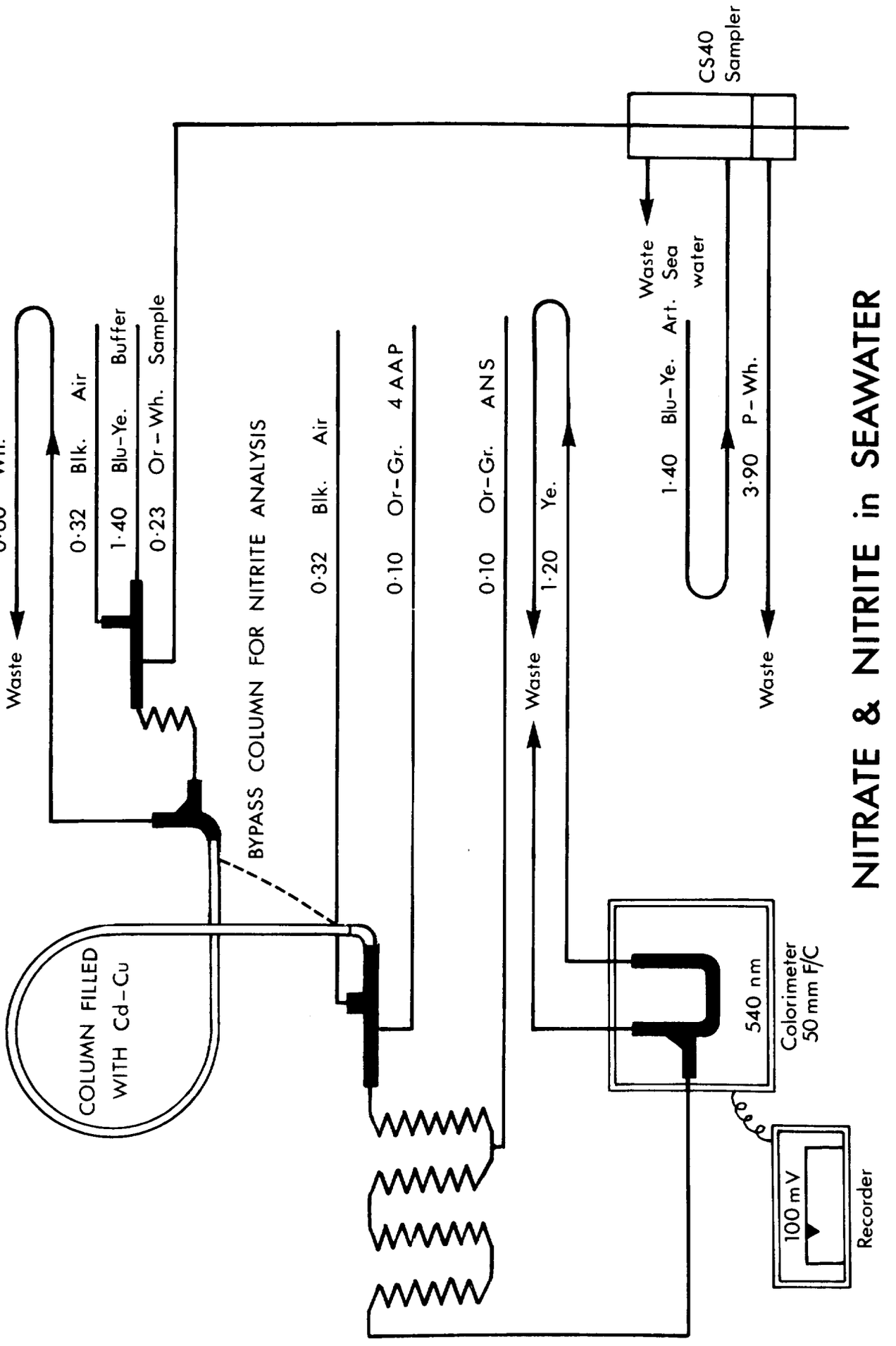
Crush copper-cadmium alloy (50/50w/w) in a hammer mill, and sieve to retain the 500-350 micron size fraction. Plug the end of a 25cm length of transmission tubing with fibre glass. Fill with ammonium chloride buffer solution and then add the Cd/Cd graduals until they fill 20cm of tube. Plug the top with glass wool and trim to length. Activate the column by pumping ammonium chloride solution through it at 1ml/min for four hours.

Standard Solutions

NO_3 Dissolve 0.510g potassium nitrate in 500ml distilled water. Transfer to screw top bottle for storage. Add 1ml of chloroform as preservative. This gives a 10mM solution.

NO_2 Dissolve 0.345g sodium nitrite in 500ml distilled water. Transfer to screwtop bottle for storage. Add 1ml chloroform as preservative. This gives a 10mM solution.

Prepare working standards by further dilution in 100ml of artificial sea water or surface sea water. The calibration curve is linear up to 50µM.


CS40 Sampler
Waste
Sea water
Art.
Blu-Ye.
1·40
P-Wh.
3·90
Waste
0·60 Wh.
Waste
0·32 Blk. Air
1·40 Blu-Ye. Buffer
0·23 Or-Wh. Sample
BYPASS COLUMN FOR NITRITE ANALYSIS
0·32 Blk. Air
0·10 Or-Gr. 4 AAP
0·10 Or-Gr. ANS
Waste
1·20 Ye.
COLUMN FILLED WITH Cd-Cu
540 nm
Colorimeter 50 mm F/C
100 mV
Recorder
NITRATE & NITRITE in SEAWATER

Standards

500µl = 50µM
375µl = 37.5µM
250µl = 25µM
125µl = 12.5µM

Lines

(1) Buffer (Yellow-Blue 1.40ml/min)

(2) Air (Black 0.32ml/min)

(3) Sample (Orange-White 0.23ml/min)

(4) Return (White 0.60ml/min)

(5) Air (Black 0.32ml/min)

(6) 4-Amino-acetophenone (Green-Orange 0.1ml/min)

(7) 1-Analinonapthalene sulphonic acid (Green-Orange 0.1ml/min)

(8) Return (Yellow 1.2ml/min)

PHOSPHATE

Phosphate is reacted with acidified molybdate reagent to give a phosphomolybdate complex which is then reduced to a highly coloured blue compound. Ascorbic acid is used as the reducing agent with potassium antimonyl tartrate in a single solution reagent. The mixed reagent reacts rapidly with phosphate ions to give a blue-purple complex containing antimony and phosphorus in a 1:1 atomic ratio. Measurement is made at 880nm.

Reagents

(1) Ammonium molybdate: Dissolve 15g in 500ml distilled water.

(2) Ascorbic acid: Dissolve 25g in 500ml distilled water.

(3) Potassium antimonyl tartrate: Dissolve 0.68g in 500ml distilled water.

(4) Sulphuric acid: Dilute 140ml concentrated H_2SO_4 in 900ml distilled water.

(5) Sodium dodecyl sulphate - SDS (Sodium lauryl sulphate) dissolve 25g in 500ml distilled water.

(5a) Diluent Add 12.5ml SDS to 500ml distilled water.

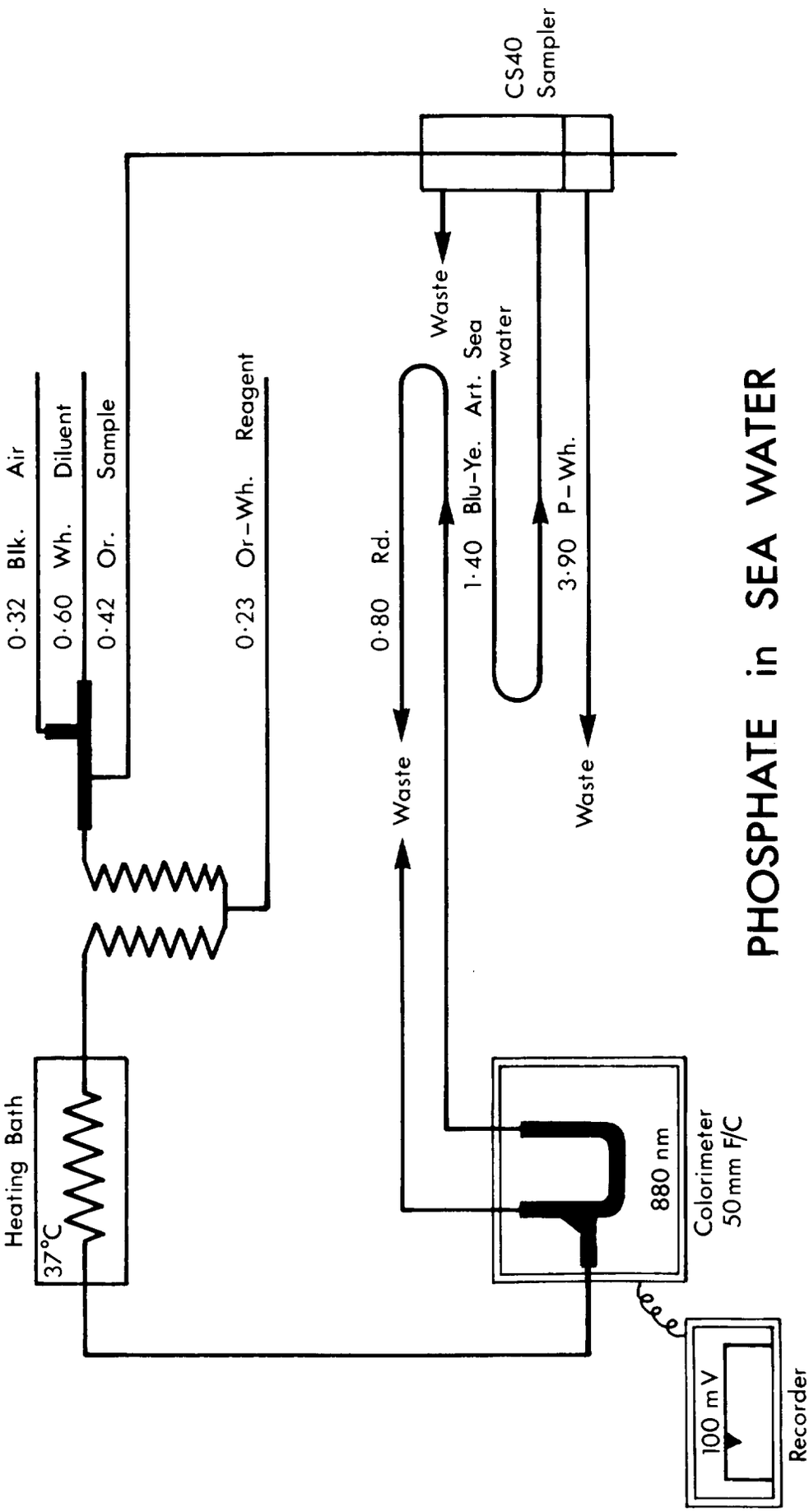
(6) Mixed Reagent
- 20ml Ammonium molybdate
- 50ml Sulphuric Acid diluted
- 20ml Ascorbic Acid
- 10ml Potassium Antimoyl Tartrate

Prepare and used within 6 hours.

Standard Solutions

(A) Dissolve 0.681g potassium dihydrogen phosphate in 500ml distilled water. Transfer to a screw top bottle for storage: add 1ml chloroform as a preservative.

(B) To prepare working standards dilute the primary standard 5 parts + 95 parts distilled water. Then the following sets of standards can be prepared by dilution in 100mls of artificial sea water or phosphate free surface sea water.



PHOSPHATE in SEA WATER

Standards

Water Column		Pore Waters	
500µl	= 2.50µM	1000µl	= 5.0µM
375µl	= 1.875µM	750µl	= 3.75µM
250µl	= 1.25µM	500µl	= 2.5µM
125µl	= 0.625µM	250µl	= 1.25µM

Lines

(1) Diluent (White 0.6ml/min)

(2) Air (Black 0.32ml/min)

(3) Sample (Orange 0.42ml/min)

(4) Mixed Reagent (Orange-White 0.23ml/min)

(5) Return (Red 0.80ml/min)

Notes

(1) Polythene volumetric flasks appear to absorb phosphate. Use of glass flasks to avoid memory effects distorting standard concentrations is recommended.

(2) Where possible phosphate free surface sea water should be used as the blank. Because of the high gain on which the colorimeter is run for this method spurious signals due to density differences between even artificial sea water and sea water are seen.

SILICATE

Dissolved silicate in seawater reacts rapidly in acidic molybdate solutions to form yellow silicomolybdic acid. This may be reduced using a number of reducing agents to give an intense blue coloured compound. Oxalic acid is added prior to the reduction step to prevent interference from phosphate. Ascorbic acid is recommended here as the reducing agent because it is the simplest and most stable one to use.

Reagents

(1) Ammonium molybdate Stock solution - dissolve 15g in 500ml distilled water.

(1a) Working solution To 80ml stock solution add 10ml dilute sulphuric acid and 25ml stock SDS then dilute to 250ml. Dilute 2+1 distilled water for pore water determinations.

(2) Sulphuric acid Dilute 140ml concentrated H_2SO_4 in 900ml distilled water.

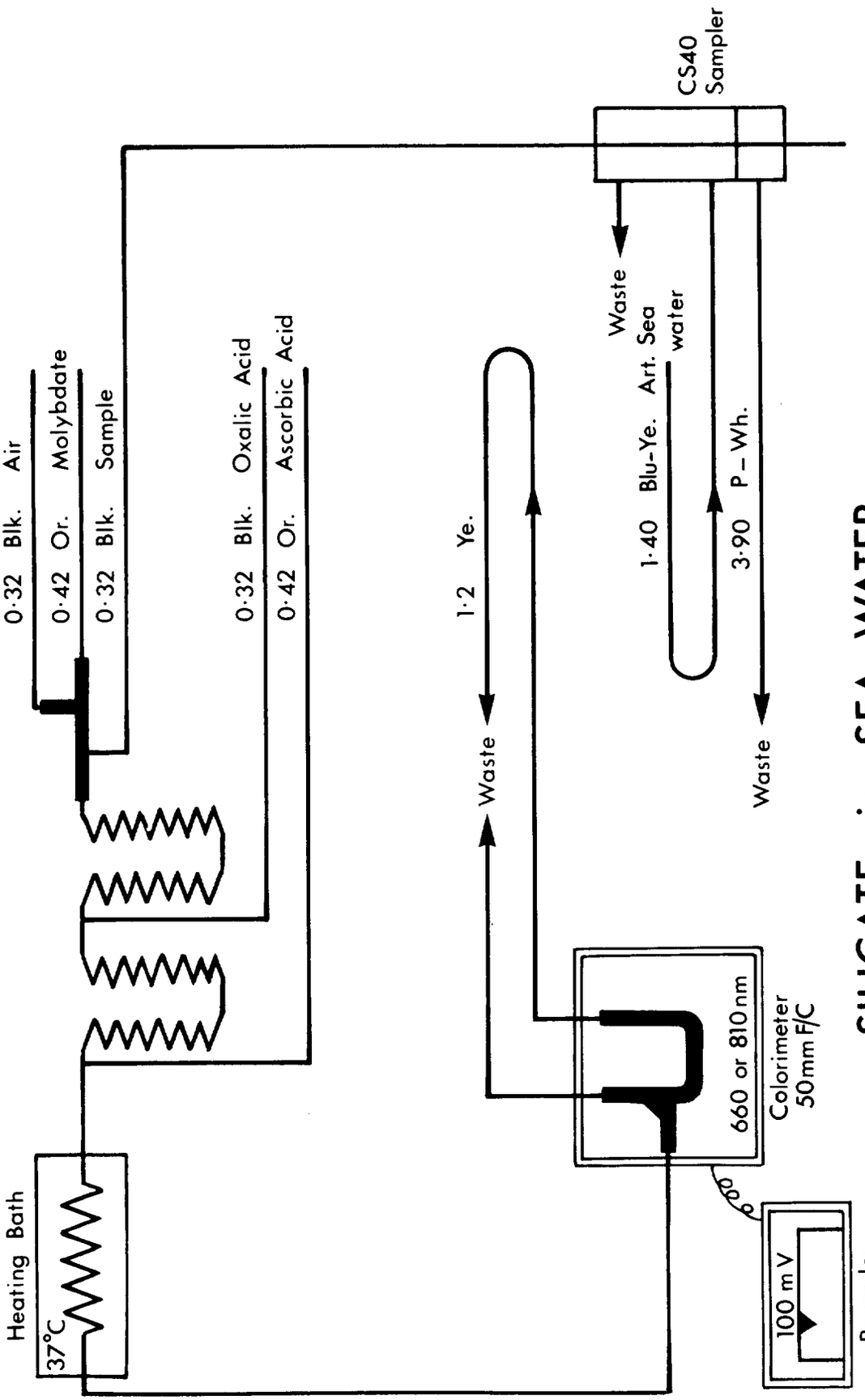
(3) Sodium dodecyl sulphate - SDS (sodium lauryl sulphate) dissolve 25g in 500ml distilled water.

(4) Oxalic acid: Dissolve 25g in 500ml distilled water.

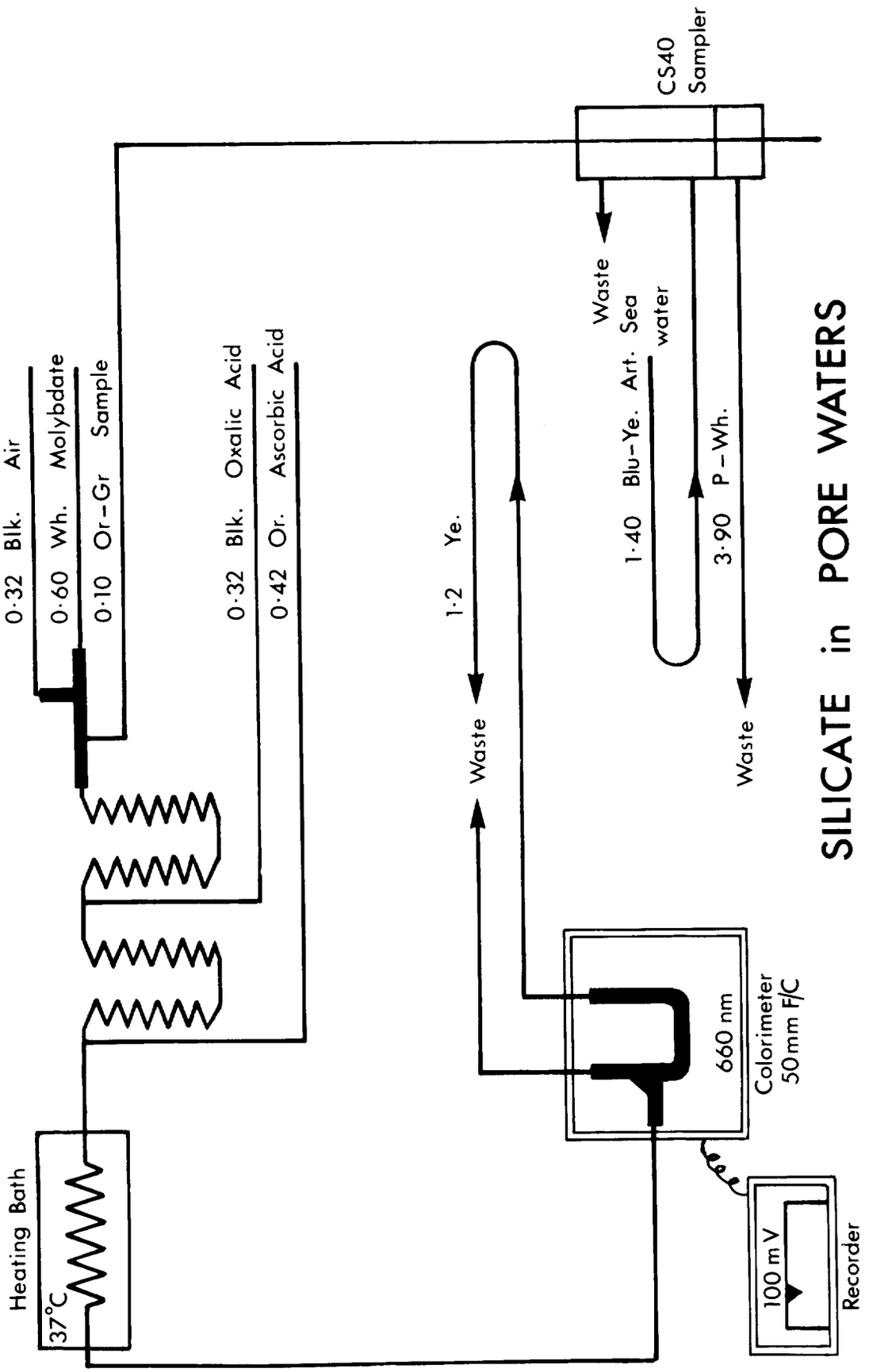
(5) Ascorbic acid: Dissolve 8.8g in 500ml distilled water.

Standard Solutions

Dissolve 0.960g sodium silica fluoride in 500ml distilled water. Start dissolution in 100ml plastic beaker by grinding the fluoride powder using a plastic rod to a paste with a few drops of water. Transfer to a screw top bottle for storage. Prepare the following standards by dilution of the primary standard in 100ml of artificial sea water or silica free surface sea water.



SILICATE in SEA WATER


SILICATE in PORE WATERS
CS40 Sampler
Waste
1·40 Blu–Ye. Art. Sea water
3·90 P–Wh.
Waste
1·2 Ye.
Waste
0·32 Blk. Air
0·60 Wh. Molybdate
0·10 Or–Gr Sample
0·32 Blk. Oxalic Acid
0·42 Or. Ascorbic Acid
Heating Bath
37°C
660 nm
Colorimeter
50 mm F/C
100 m V
Recorder

Standards

Water Column

500µl = 50µM

375µl = 37.5µM

250µl = 25µM

125µl = 12.5µM

Lines

(1) Molybdate (Orange 0.42ml/min)

(2) Air (Black 0.32ml/min)

(3) Sample (Black 0.32ml/min)

(4) Oxalic acid (Black 0.32ml/min)

(5) Ascorbic acid (Orange 0.42ml/min)

(6) Return (Yellow 1.2ml/min)

Standards

Pore Waters

3.0ml = 399µM

2.0ml = 200µM

1.0ml = 100µM

0.5ml = 50µM

Lines

(1) Molybdate (White 0.6ml/min)

(2) Air (Black 0.32ml/min)

(3) Sample (Orange-Green 0.10ml/min)

(4) Oxalic acid (Black 0.32ml/min)

(5) Ascorbic acid (Orange 0.42ml/min)

(6) Return (Yellow 1.2ml/min)

Notes

(1) Using 660nm filter in the colorimeter gives linear responses with the above conditions up to 50μM and 300μM for water column and pore waters respectively.

(2) For detailed studies of silicate distributions in coastal and near surface ocean waters. The sensitivity of the method can be increased by using the 810nm filter in the colorimeter. The linear working range is up to 12.5μM.

(3) Mixed standard solutions can be prepared in well rinsed glass volumetric flasks (these should be prepared fresh daily) without contaminating the samples.

TROUBLE SHOOTING

If you prepared all the reagents carefully and cleanly and checked tube tensions in the pump then the only problem you should have and that hopefully not at all is a mechanical breakdown.

Likely Problems

(1) Undulating baseline, with period similar to the movement of the pump rollers is probably due to the tubes in peristaltic pump requiring tensioning.

(2) Erratic spiky noise-low applitude. Particularly on the nitrate line is due to lack of wetting agent in either or both reagents. This can occur without apparent surging in the flow line.

(3) Erratic spiky noise-high applitude. Is caused by bubbles entering the flow cell. The most common cause is running out of one or more reagents or sample, particularly where the operator has forgotten to set the auto-stop on the sample tray. Lack of wetting agent in the phosphate diluent or silicate molybdate reagent, can lead to irregular bubble patterns and considerable surging that sends bubbles into the flow cell.

(4) An off scale signal which does not return to the baseline. Look at the reagents! The phosphate mixed reagent or the silicate molybdate reagents may become contaminated and spontaneously go blue with the above mentioned consequences. Scrupulously wash out the lines and containers and prepare a fresh batch of reagent.

(5) Erratic spiky noise-low applitude and variable drift on the baseline. Can occur on the phosphate channel because due to the high gain used on this channel it can be sensitive to changes in the ambient light levels outside the colorimeter. Keep colorimeter out of direct sunlight and seal the lid on the phosphate channel with black tape.

Equipment List - Chemlab Autoanalyser

Running

Cups 2.5ml
Cups 8ml
Transmission tubing 0.062 ins ID
" " 0.02 ins ID
" " 0.125 ins ID
Pump tubing - see separate method sheets
JJL chart recorder paper
" " " pens - black
" " " pens - red

Spares

Colorimeter lamp
Colorimeter power supply
Shear pin kit
Heating bath repair kit
Heating bath coil G(PO_4 and NH_4)
" " " H(SiO_2)
Colorimeter Flow Cell (50mm)

Preparation of Reagents and Standards, etc.

	Quantity for 4-week cruise running 4 channels.
Micro master pipette 25-100µl	1
" " " 125-500µl	1
Pipette Tips - Blue	200
- Yellow	200
Finnpipette - 1 - 5ml	1
Finnpipette tips	50
Syringes 5ml	10
Scalple	1
Scalple blades	5

Mini stirrer	1
Stirrer bars	2
Plastic funnel small	2
Plastic beaker 100ml	2
Plastic rods	2
Plastic beaker 1000ml	2
Squeeze bottle 500ml	2
Disposable beakers 30ml	50
Volumetric Flasks Plastic 500ml	2
" " Plastic 100ml	4
" " Glass 100ml	8
Polypropylene bottles - 100ml	2
Polythene bottles - 1 pint	16
- 250ml square	12
- 2000ml square	8
Measuring cylinder - 50ml	2
Aspirator - 10L	1
- 30L	3
Double distilled water	90L
Sample containers (Elkay-Dilu-Vial 30ml)	*

(* May be recycled if samples not being stored for further analyses).

Chemicals Chemlab Auto Analyser

Silicate, Phosphate, Nitrate/ite, Ammonia	Tube Size	Volume	Run. Time h.
Silicate			
(A) 15g Ammonium molybdate		500ml	
diluted 1 + 2	0.42	1500ml	59
(B) 25g Oxalic aicd	0.32	500ml	26
(C) 8.8g Ascorbic acid	0.42	500ml	20
(D) Sulphuric acid 140ml in 900ml DW		500ml	491
6ml/150ml molybdate mixed reagent			
(E) 25g Sodium dodecyl sulphate		500ml	196
15ml SDS/150 molybdate mixed reagent			
Phosphate			
(A) 15g Ammonium molybdate		500ml	
diluted 1 + 4	0.23	2500ml	181
(B) 27g Ascorbic acid		500ml	
diluted 1 + 4	0.23	2500ml	181
(C) 0.68g Potassium antimonyl tartrate		500ml	
diluted 1 + 9	0.23	5000ml	362
(D) Sulphuric acid 140ml in 900ml DW		500ml	
diluted 1 + 1	0.23	1L	72
(E) 25g Sodium dodecyl sulphate		500ml	
12.5ml SDS/500ml dilutent		2000ml	555

Nitrate

(A) 50g Ammonium chloride	1.40	2L	23
(B) 0.15g 4-Amino-acetophenone	0.1	100ml	16
(C) 0.06g 1-Anilinonaphthalene 8-sulphonic	0.1	100ml	16
(D) 50% v/v Glacial acetic acid	0.1	500ml	80
(E) 25% w/v Brij-35 1ml/100 (B) and (C)		150ml	1250

Ammonia

(A) 10g Sodium hydroxide diluted 1 + 24 in Citrate		500ml	
(B) 34g Tri-sodium citrate	0.6	500ml	13
(C) 10g Phenol		500ml	
diluted 10 + 1	0.42	500ml	20
(D) 0.4g Sodium dichloroisocyanurate 2.5g Sodium hydroxide	0.32	500ml	26
(E) 1g Sodium nitroprusside		500ml	
dilute 1 + 10 (Phenol)	0.42	5500ml	210

	Mol wt	Volume	Molority mM
Standards			
Si			
0.960g Sodium Silica-Fluoride $Na_2 Si F_6$ (Assay 98%) Dry at 105°C.	188.8	500	10.0
No_3			
0.510g Potassium Nitrate KNO_3	101.11	500	10.0
NO_2			
0.345g Sodium Nitrite Na NO_2 Dry at 110°C, 1 hour	69.00	500	10.0
PO_4			
Potassium dihydrogen Phosphate 0.6805g$KH_2 PO_4$ Dry at 110°C, then place in desiccator	136.09	500	10.0
NH_3			
0.134g Ammonium Chloride NH_4Cl Dry at 100°C	53.49	500	5.0

ACKNOWLEDGEMENTS

The generous and encouraging help of Christopher Cook of ChemLab Instruments Limited, Hornchurch, Essex, in getting the I.O.S. analyser running is gratefully acknowledged.