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Automatic analysis of sea water nutrients

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CONTENTS

	Page
Introduction	1
Instrumentation and technique	1
Methods	3
Automated determination of ammonia in sea water	4
Automated determination of nitrite in sea water	8
Automated determination of nitrate in sea water	12
Automated determination of phosphate in sea water	16
Automated determination of silicate in sea water	20

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AUTOMATIC ANALYSIS OF SEA WATER NUTRIENTS

by

A R Folkard

Introduction

Automated analysis techniques for the determination of nutrient salts in sea water have been introduced over the past 10-15 years and, where large numbers of samples are to be processed, have largely superceded the manual techniques. The most popular method employed in automated water analysis is the segmented continuous stream technique pioneered by Technicon Instruments and developed in their AutoAnalyser (R) apparatus. Earlier published work was concerned with methodology adapted to the AutoAnalyser I, a system which has now been replaced by the AutoAnalyser II. At the Fisheries Laboratory, Lowestoft we have over the past four years used the AutoAnalyser II system, both at sea and in the Laboratory, and in this paper we detail the analytical methods and general procedures adopted in the operation of the system.

Samples of sea water can be analysed for phosphate, silicate, nitrate, nitrite and ammonia and in addition to being used to analyse individual samples the system has the capability to be used on-line on board ship. On-line operation allows continuous recording of nutrient parameters from a vessel underway, either from a pumped source within the vessel itself (surface) or a towed depth pumping system.

Instrumentation and technique

Early sea trials of the AutoAnalyser II showed that ship's motion could seriously affect the maintenance of the bubble pattern within the analytical stream. The vertical acceleration produced by the ship's motion has an adverse effect upon the segmented exhaust stream and causes surging along the whole of the analytical stream and a consequent introduction of bubbles into the flowcell. To overcome this it has been necessary to fit a debubbler immediately prior to the flowcell. Manufacturers instructions advise that the sample volume should not exceed 0.60 ml min^{-1} as this will give rise to a larger air bubble between sample and wash than can be dealt with by the natural separation of air and liquid at the flowcell. Fitting of the debubbler to counteract the effects of ship's motion gives the added advantage that the sample stream can be increased to 1.00 ml min^{-1}, which in turn gives greater flexibility of operation when considering the automation of manual techniques.

The analytical apparatus was developed originally for clinical analysis with strongly coloured solutions where short path length flowcells could be used with low amplification factors in the colorimeter, and samples were dialysed or fed with diluent streams. Apart from some estuarine waters, most sea waters are relatively low in nutrient salts and often it is necessary to use higher amplification on the colorimeter, coupled with the use of longer flowcells to achieve the sensitivity required. Also the usual recommended procedure is to run with the reagent baseline adjusted to zero, but as we are working at low levels we consider it necessary to know the absorbance value of the reagent blank, therefore the system is set to zero when pumping distilled water only.

The sampler contains two wash receptacles, one of which is fed with distilled water and the other with sea water or synthetic sea water. The distilled water wash is used only at the beginning and the end of an analytical run, for initial zeroing and the determination of the reagent blank. To ensure its purity and to enable it to be used in the determination of the reagent blank it is pumped from its reservoir via a small cartridge of mixed bed resin (ZEROLIT DM-F) or a cation exchange cartridge for ammonia analysis (DOWEX 50W-X8) before it is fed to the wash receptacle. The sea water wash receptacle is fed from a reservoir of synthetic sea water when nitrate and nitrite analyses are being performed, but for phosphate, silicate and ammonia analyses a sea water low in these nutrients is used. This low nutrient sea water is best collected in the late spring after the main diatom outburst; it is then filtered and stored in polythene carboys. Under these conditions phosphate will be completely removed, silicate will remain at a very low level, but there may be some fluctuations in the levels of ammonia and nitrite. Many methods in the literature recommend the use of wetting agents either in the wash or one of the reagents, but we have never used these.

Atlas *et al* (1971) have reported, and our own work has confirmed, that there is a change in absorbance with respect to distilled water when sea water or synthetic sea water is passed through the system. They suggest that the change is due to flowcell geometry and differences in refractive index between distilled water and the more dense sea water. The consequence of this is to give a misleading low reagent blank (derived from distilled water) for sea water samples, because any bending of the light beams is recorded as absorbance; therefore all results from sea water samples exhibit a total absorbance, which is the sum of the absorbances derived from the reagent blank and the coloured solution produced plus an element of 'refractive' absorbance. The ideal method of assessing the 'refractive' absorbance is to determine the reagent blank in sea water free of the constituent that it is desired to measure. When this is impossible distilled water should be pumped through all lines and the base line set to zero; sea water is then pumped

through the sample line and the increase in 'absorbance' noted. This value is added to the distilled water derived reagent blank to give the true reagent blank for sea water samples. It must be borne in mind that this value of refraction 'absorbance' is dependent upon a particular flowcell, the wavelength used, the amplification of the colorimeter and the salinity. The range of salinity encountered in offshore samples is small and a constant correction may be applied. In our work we have found that the greatest effect of 'refractive absorbance' is shown in the phosphate analysis, but it should be determined and taken into account in all the analyses.

When the system is running steadily on sea water wash and reagents, analysis may begin. The sample tray is loaded first with 4 base sea water samples, followed by 4 samples of the same sea water which have been spiked to give a mid-range standard; samples are then loaded and the standardising procedure is repeated at the end of the run. If the run is a long one further sets of standards may be inserted at appropriate intervals. A work scheme to cover a long run of samples is set out below:-

1.	Distilled water all lines	Set zero
2.	Distilled water plus reagents	(2)-(1) Distilled water reagent blank I
3.	Sea water wash plus reagents	
4.	4 Samples of base sea water	
5.	4 Samples of base sea water plus spike	
6.	Sea water samples	
7.	4 Samples of base sea water	
8.	4 Samples of base sea water plus spike	
9.	Sea water samples	
10.	4 Samples of base sea water	
11.	4 Samples of base sea water plus spike	
12.	Sea water wash plus reagents	
13.	Distilled water plus reagents	
14.	Distilled water all lines	(13-(14) Distilled water reagent blank II
15.	Distilled water plus sea water in sample line	
16.	Distilled water all lines	(15)-(16) 'Refraction absorbance'

All peak heights are measured from a sea water reagent blank baseline which is drawn on the recorder chart by joining ((2)-(1)) + ((15)-(16)) to ((13)-(14)) + ((15)-(16)): this takes into account any drift of the baseline that has occurred. A factor for calibration is obtained by taking a mean from (5)-(4), (8)-(7) and (11)-(10). Some difficulty is encountered with the phosphate analysis when running such a work scheme. This will be discussed later under Phosphate method (7.1).

As mentioned previously the nutrient concentrations of sea water are generally low and flowcells of 50 mm have to be used at times in the colorimeters, but because of the high sensitivity of the nitrate and nitrite methods it is possible to use 15 mm flowcells. In many cases flowcells of 10 mm pathlength may be used for these two analyses and we have incorporated two Corning-Eel 254 single beam colorimeters into the system. These colorimeters use Hellma flowcells utilising the whole of the analytical stream, and the same debubbling method is employed as on the other colorimeters: both inlet and outlet tubes are pumped with the sample inlet pumping at a slightly higher rate than the sample outlet.

Multirange multispeed flat-bed recorders have been substituted for the original recorders thus giving a greater flexibility to the system. These recorders may be rack mounted, which results in space saving and a greater ease in handling when setting up the equipment on board ship.

If it is wished to run the system as a 2 or 3 channel analyser the rate of sampling has to be set to accommodate the slowest analysis and in so doing the amount of sample needed becomes greater than that contained in the sampling cup e.g. if nitrate, nitrite and phosphate were run together at a speed of 20 samples h^{-1} and a sample to wash ratio of 15:1, using the methods described later, 6.75 ml of sample would be needed, whereas the biggest sample cups (limited by the sampler itself) that we are able to use contain only 4 ml. In fact, this combination of three parameters is the only one possible as the silicate and ammonia samples are presented to the analyser separately. In practice we use two separate complete analysers with little loss of operating time.

So far we have discussed the AutoAnalyser as used to accept individual samples, but it may also be used as an on-line instrument. For inshore waters and offshore waters of high turbidity it is necessary that the stream presented for analysis be filtered and this can be achieved by demanding a small filtered volume (just in excess of sample line pump tube requirements) from a fast flowing pumped supply, which also serves to flush the surface of the filtering medium by virtue of its flow rate. The design of such a system requires that dead spaces be kept to a minimum and that short term (distance) salient features present in the sea are not smoothed out by sampling and analytical processes. The basic work scheme for individual samples may be followed when sampling on-line but with the individual base waters and standards replaced by sampling of the same for 5 min periods of time at suitable intervals during the

analysis. A reagent blank baseline can be determined and drawn on the recorder chart, and features of the record can be measured from this baseline, converted to concentration and then related to the ship's track. Great care should be taken to assess sample pumping lag and residence times of samples in the analyser so as to allow accurate position fixing of observed nutrient features.

Methods

The main advantage of automated analysis techniques over manual techniques is not so much their speed of operation as the ability of the system to handle large numbers of samples on a routine basis. Also each sample is treated in exactly the same manner within strictly prescribed and maintained operating conditions. Most manual techniques in use for nutrient salt analysis lend themselves to automation, and in the simplest cases it is only a matter of a straight conversion of volumes of samples and reagents to pumped volumes per minute on a 1:1 basis to automate a method. Where this is not possible it is necessary to manipulate the volumes of samples and reagents within the limits imposed by the analyser, but to end up with the reacting concentrations the same as in the manual method. Time may be added to the analytical stream e.g. to accommodate a longer colour development time, by addition of mixing coils and where necessary a heating coil may be added to hasten a reaction.

The manual phosphate method of Murphy and Riley (1962) with slight modification has been used for many years at the Fisheries Laboratory, Lowestoft and has proved to be a precise and trouble-free technique, and in practice this is the simplest of methods to automate. The manual method uses a sample volume of 50 ml and a mixed reagent volume of 10 ml. For automation this is easily converted into a pumped sample volume of 0.80 ml min^{-1} with the mixed reagent pumped at 0.16 ml min^{-1}. Colour development is complete after 5 min, while the residence time from reagent addition to the measurement of absorption in the colorimeter is about 6 min.

For nitrite, the method of Shinn (1941) later modified for use in sea water by Bendschneider and Robinson (1952) has satisfied the criteria of sensitivity and reproducibility with trouble-free application over many years in the field of manual analysis. The manual method requires the additions of 1 ml of each of the two reagents to a volume of 50 ml of sample, but because the automatic technique cannot accommodate this 50:1:1 volume ratio, the reagents have to be diluted by a factor of 6 and the final pumped volume ratio becomes 0.80 ml min^{-1}:0.10 ml min^{-1}:0.10 ml min^{-1}, but the reaction concentrations and conditions remain the same as before.

Manual analysis of sea water for nitrate is made after reduction to nitrite by cadmium-copper reductor column according to Wood, Armstrong and Richards (1967) with the addition of ammonium chloride as recommended by Strickland and Parsons (1968). The main disadvantage that ensues when automation is applied to this method stems from the fact that an unsegmented analytical stream has to be passed through the cadmium-copper reductor column and results in a certain amount of mixing between wash and samples and smoothing of the sample plateaux as displayed on the recorder. A recent modification as suggested by Stainton (1974) has been incorporated into our system and consists of a length of cadmium wire inserted into a length of the polythene tubing. The cadmium wire is copper coated *in situ* and then formed into a coil and the sea water sample plus ammonium chloride flows along the annulus between the cadmium wire and the inside of polythene tubing where it is reduced to nitrite. Thereafter the method is the same as that used for nitrite. Although an unsegmented stream is used through the reductor coil, there is no spoilage of the flow and sample plateaux are excellent.

A number of methods for the determination of ammonia in sea water that rely upon the formation of indophenol blue are to be found in the literature, but may prove troublesome because of high blanks and sometimes erratic production of indophenol blue. Liddicoat, Tibbitts and Butler (1975) by modification of the technique of Solorzano (1969) formulated a method which is much more reproducible in its application. Further work by Hampson (1977) produced more improvements and resulted in a method which lends itself to automation (Folkard and Hampson, 1977). Optimal colour development in this method involves heating to 30°C and irradiation by low power, long wave UV light and both these facilities are easily accommodated in the analytical module.

For the determination of reactive silicate in sea water a modification of Technicon method No. 186-72 W is used. With increased acidity and changes in reagent concentrations that allow a greater sample/reagent volume ratio, an overall sensitivity increase of approximately x 3.5 is possible. Theoretically this could be increased twofold again by substituting the 660 nm filters with 810 nm filters. A similar method for manual use has been described by Koroleff (1976).

For the various methods used estimates of standard deviation have been obtained at a number of concentration levels and at differing absorbance ranges by replicate sampling from bulk homogeneous samples (Tables 1–5). It may be argued that this gives an enhanced estimate of precision over that obtainable in practice as far as individual samples are concerned, but it was also necessary to assess the precision of values obtained on-line (where there is no wash interruption between samples and one might expect a better precision), and therefore the method is something of a compromise between the two systems. Reference to Phosphate method (7.1.) will show that mixing of samples of high and low concentrations could lead to higher values for standard deviation and therefore less precise results. The number of replicates from a bulk sample varied between 10 and 20 and where runs were repeated the worst estimate has been taken. Precision is given at the 95%

confidence limit and is taken as ±2 standard deviations and the limit of detection is taken as 3 standard deviations at the lowest level measured.

Automated determination of ammonia in sea water

1. Performance characteristics of the method

1.1	Substance determined	Ammonia
1.2.	Type of sample	Sea water
1.3.	Basis of method	Ammonia is converted to monochloramine at a suitable pH. This monochloramine then reacts with phenol to form indophenol blue whose absorbance is measured at 630 nm.
1.4.	Range of application	0-75 μg at NH_4-Nl^{-1} (0-1050 μg NH_4-N l^{-1})
1.5.	Calibration	Linear to at least 75 μg at NH_4-N l^{-1}
1.6.	Statistical data	See Table 1
1.7.	Limit of detection	See Table 1
1.8.	Sensitivity	See Table 1
1.9.	Bias	No bias detected
1.10.	Interferences	Amines and nitrite interfere at high concentrations in sea water, but at their natural concentration in sea water their effect is negligible.
1.11.	Time required for analysis	Samples are run at 40 h^{-1} with a sample to wash ratio of 6:1. Set-up and run down times (approximately 45 min each) must be added to give an overall estimate.

2. Principle

2.1. Ammonia is converted to monochloramine at a suitable pH. This monochloramine then reacts with phenol to form indophenol blue whose absorption is measured at 630 nm. The reaction stream is heated to 30°C after the addition of reagents and then irradiated with long wave low power UV light for 16 min before the measurement of absorbance. Standardisation is carried out at the beginning and the end of a run of samples.

3. Safety note

3.1. Phenol is destructive of human tissues and should be regarded as a hazard. Skin contact with it and reagents incorporating it should be avoided.

4. Reagents

Use analytical grade reagents and water that has been freshly deionised by passing through a cation exchanger column.

4.1. Ethanol 37% v/v

37± 1 ml of absolute ethanol made up to 100± 2 ml with water. Prepare fresh daily.

4.2. Ethanol - Phenol

Dissolve 3.9± 0.05 g of Phenol C_6H_5OH in 100± 2 ml of 37 v/v ethanol (4.1.). Prepare fresh daily.

4.3. Sodium Hydroxide 1.5 M

Dissolve 6 ± 0.1 g of sodium hydroxide NaOH in water and make up to 100± 2 ml. Prepare fresh daily.

4.4. Trisodium Citrate

Dissolve 390± 2 g of trisodium citrate dihydrate $Na_3C_6H_5O_7.2H_2O$ in water and make up to 1000 ml.

4.5. Chlorinating agent

Measure 200± 2 ml of trisodium citrate solution (4.4.) and 67± 1 ml of 1.5 M sodium hydroxide solution (4.3.) into a 500 ml flat bottomed round flask and boil for 15 min. Cool and add deionised water and make up to original volume. Dissolve 0.39± 0.02 g of sodium dichloroisocyanurate in 33± 1 ml of 1.5 M sodium hydroxide solution (4.3.) and add to the 267 ml of sodium hydroxide - trisodium citrate. Prepare fresh daily.

4.6. Potassium ferrocyanide

Dissolve 0.194± 0.002 g of potassium ferrocyanide trihydrate $K_4Fe(CN)_6.3H_20$ in water and make up to 100 ml with water. Prepare fresh daily.

4.7. Standard ammonia solution (stock) 100μg at NH_4-N ml^{-1}.

Dissolve 3.3030 g of ammonium sulphate $(NH_4)_2SO_4$ in 500 ml of water in a graduated flask and mix well. Add 1 ml of chloroform as a preservative and

store in a dark glass bottle. The solution is stable for many months in the absence of evaporation.

4.8. Standard ammonia solution (dilute) 1 μg at NH_4-N ml^{-1}.

Dilute 1.00 ml of stock standard ammonia solution (4.7.) to 100 ml with water in a graduated flask and mix well. Prepare fresh daily.

4.9. Standard ammonia solution (working)

See Calibration (7.2.)

5. Apparatus

5.1. Analytical apparatus

A segmented stream analyser (Technicon AA II) is used for this method. The colorimeter uses 630 nm interference filters and 50 mm flowcells. A single cell colorimeter (Corning-Eel 254) with a 10 mm Hellma flowcell may also be used, particularly at the higher concentrations. The cover of the analytical cartridge is modified to take an array of two 8 inch, 15 W, 365 nm UV lamps which is positioned about 2 cm above the last 4 double mixing coils in the analytical stream. The air for segmenting the stream is acid scrubbed by bubbling through 0.5 M sulphuric acid before entering the sample stream.

6. Sampling and samples

6.1. Samples from coastal waters (this includes the North Sea, Irish Sea and English Channel) are filtered through a glass fibre filter (Whatman GF/C or equivalent) and analysed with the minimum delay. Use 4 ml polystyrene sample cups that have been acid washed and rinsed immediately prior to filling for analysis.

7. Procedure

7.1. Pump deionised water through all lines and set colorimeter control to suit concentration range of samples.

Absorbance range	Concentration range	
0 - 0.5	0 - 10 μg at NH_4-N l^{-1})	50 mm flowcell
0 - 1.0	0 - 15 μg at NH_4-N l^{-1})	
0 - 1.0	0 - 75 μg at NH_4-N l^{-1})	10 mm flowcell

Allow system to equilibrate for 30 min and set baseline as required on chart recorder. Introduce reagents into pump lines and 10 min later change sample line to low ammonia sea water wash, and run for a further 10 min before introducing samples. Load samples into tray with 4 mid-range standards at the beginning and end of each run. At the end of each run reverse the order of operations until the system is pumping deionised water in all lines.

7.2. Calibration

Calibration is always carried out by addition of known amounts of ammonia to low ammonia sea water, 4 samples of this base sea water are placed in the sample tray followed by 4 samples of the spiked base water. As the concentration/absorbance relationship is linear over the range of concentration used, single mid-range standards are used as detailed below.

Absorbance range	Standard
0 - 0.5	Dilute 0.05 ml of dilute standard ammonia solution (4.8.) to 100 ml with low ammonia sea water. Added ammonia = 5 μg at NH_4-N l^{-1}
0 - 1.0	Dilute 1.00 ml of dilute standard ammonia solution (4.8.) to 100 ml with low ammonia sea water. Added ammonia = 10 μg at NH_4-N l^{-1}
0 - 1.0	Dilute 2.50 ml of dilute standard ammonia solution (4.8.) to 100 ml with low ammonia sea water. Added ammonia = 25 μg at NH_4-N l^{-1}

7.3. Calculation

See general notes on automatic analysis.

Table 1 Automated determination of ammonia in sea water. Statistical data

Level µg at NH_4-N l^{-1}	Colorimeter setting	Absorbance range	Flowcell length mm	Standard deviation S.D.	Precision ±2. S.D. µg at NH_4-N l^{-1}	Detection limit: 3. S.D. µg at NH_4-N l^{-1}	Sensitivity
1.25	Std. cal. 300	0-0.5A	50	0.02	±0.04	0.06	
2.50				0.02	±0.04		5 µg at NH_4-N l^{-1}
5.00				0.02	±0.04		
7.50				0.06	±0.12		≡ 0.2A
10.00				0.05	±0.10		
2.50	Std. cal. 100	0-1.0A	50	0.02	±0.04	0.06	
5.00				0.05	±0.10		10 µg at NH_4-N l^{-1}
7.50				0.04	±0.08		
10.00				0.07	±0.14		≡ 0.4A
12.50		0-1.0A	10	0.04	±0.08	0.12	
25.00				0.08	±0.16		25 µg at NH_4-N l^{-1}
50.00				0.08	±0.16		
75.00				0.15	±0.30		≡ 0.29A

o	Red/Red	0.80 ml min^{-1}	Sample
o	Black/Black	0.32 ml min^{-1}	Air (acid scrubbed)
o	Orange/Green (Solvaflex)	0.10 ml min^{-1}	Ethanol-phenol
o	Orange/Yellow	0.16 ml min^{-1}	Chlorinating agent
o	Orange/Green	0.10 ml min^{-1}	Potassium ferrocyanide
o	Orange/Orange	0.42 ml min^{-1}	Debubbler
o	Red/Red	0.80 ml min^{-1}	Flowcell pull through

Figure 1a Arrangement of pump tubes for the automated determination of ammonia in sea water

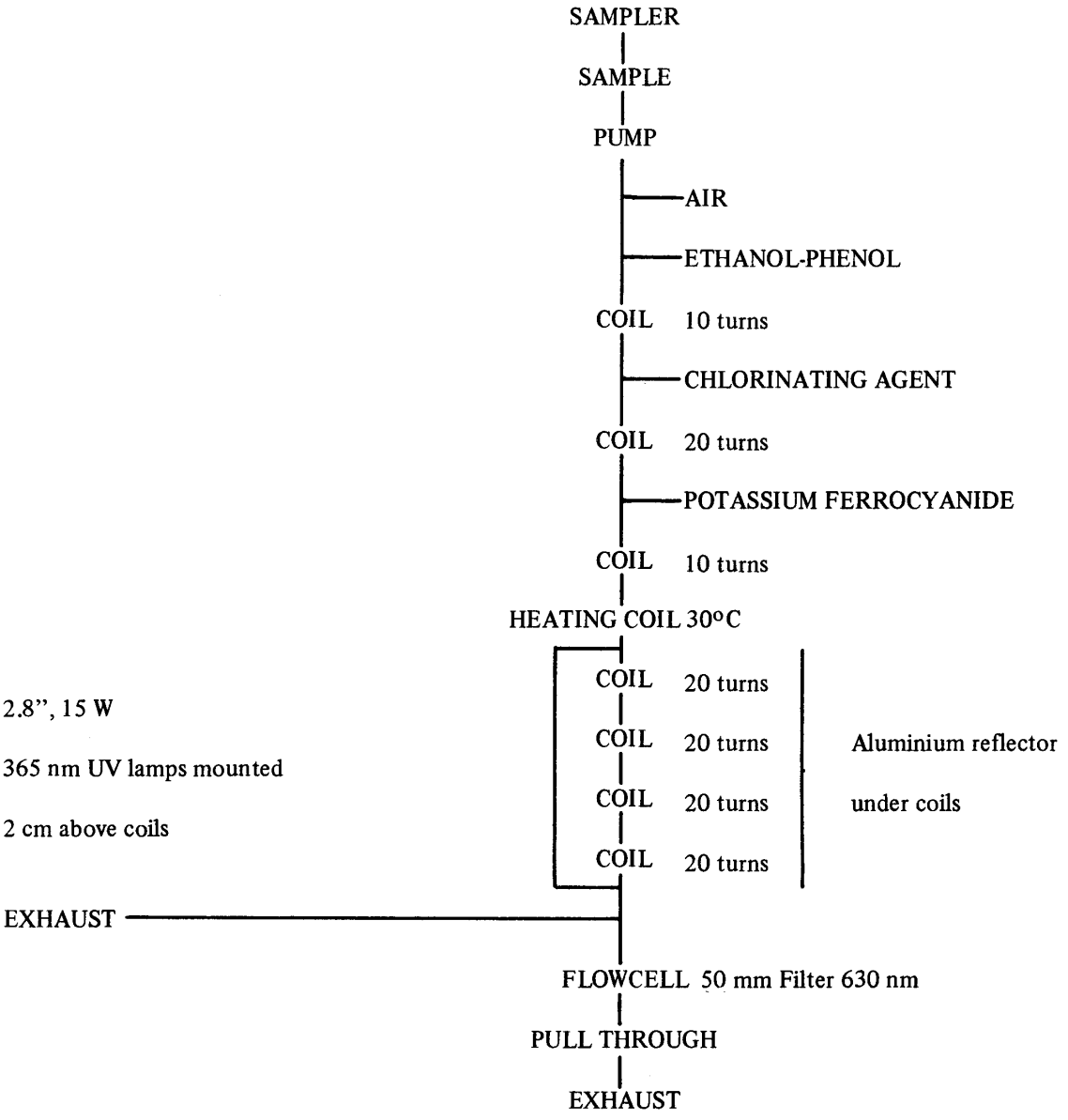

Figure 1b Flow diagram for the automated determination of ammonia in sea water

Automated determination of nitrite in sea water

1. Performance characteristics of the method

1.1.	Substance determined	Nitrite
1.2.	Type of sample	Sea water
1.3.	Basis of method	Nitrite ions are diazotised with sulphanilamide and then coupled with N-(1-naphthyl) ethylenediamine to form a magenta coloured azo-dye whose absorbance is measured at 550 nm.
1.4.	Range of application	0-5 μg at NO_2-N l^{-1} (0-70 μg, NO_2-N l^{-1})
1.5.	Calibration	Linear to 10 μg at NO_2-N l^{-1}
1.6.	Statistical data	See Table 2
1.7.	Limit of detection	See Table 2
1.8.	Sensitivity	See Table 2
1.9.	Bias	No bias detected
1.10.	Interferences	A number of substances are known to interfere, but none are expected to be present in significant amounts in oceanic and most inshore waters.
1.11.	Time required for analysis	Samples are run at 40 h^{-1} with a sample to wash ratio of 6:1. Set up and run down time (approx. 30 min each) must be added to give an overall estimate.

2. Principle

2.1. Nitrite ions are diazotised with sulphanilamide in acid solution and then coupled with N-(1-naphthyl) ethylenediamine to form an azo-dye. The absorbance of this magenta dye is measured at 550 nm and related to the nitrite concentration by means of a factor obtained by standardization at the beginning and the end of a run of samples.

3. Safety note

3.1. The N-(1-naphthyl) ethylenediamine should be regarded as a hazard and skin contact with it and reagents containing it must be avoided.

4. Reagents

Use analytical grade reagents and distilled water or equivalent throughout.

4.1. Sulphanilamide solution (stock)

5 ± 0.1 g of sulphanilamide dissolved in a mixture of 50± 1 ml of concentrated hydrochloric acid HCl and 300 ml of water. Dilute to 500± 5 ml with water. Store in glass bottle.

4.2. Sulphanilamide solution (working)

Dilute 30± 1 ml of stock sulphanilamide solution (4.1.) to 180± 5 ml with water. Prepare fresh daily.

4.3. N-(1-naphthyl) ethlylenediamine solution (stock)

0.5 ± 0.02 g of N-(1-naphthyl) ethylenediamine dihydrochloride dissolved in 500± 5 ml water. Store in a dark glass bottle and renew after a month or when a strong brown colouration develops.

4.4. N-(1-naphthyl) ethylenediamine solution (working)

Dilute 30± 1 ml of stock N-(1-naphthyl) ethylene-diamine solution (4.3.) to 180± 5 ml with water. Prepare fresh daily.

4.5. Standard nitrite solution (stock) 5 μg at NO_2-N ml^{-1}

Dissolve 0.3450 gm of sodium nitrite $NaNO_2$ which has previously been dried at 110°C for 1 h in 1000 ml of water in a graduated flask and mix well. Store in a dark glass bottle with 1 ml of chloroform as preservative. The solution should be stable for at least 1-2 months.

4.6. Standard nitrite solution (dilute) 0.05 μg at NO_2-N ml^{-1}

Dilute 1.00 ml of stock standard nitrite solution (4.5.) to 100 ml in a graduated flask and mix well. Prepare fresh daily.

4.7. Standard nitrite solution (working)

See calibration (7.2.)

4.8. Synthetic sea water

Dissolve 310± 2 g of sodium chloride NaCl, 100± 1 g of magnesium sulphate heptahydrate $MgSO_4.7H_20$ and 0.50± 0.01 g of sodium bicarbonate $NaHCO_3.H_20$ in 10 l of distilled water.

5. Apparatus

5.1. A segmented stream analyser (Technicon AA II) is used. The colorimeter employs 550 nm interference filters and a 50 mm flowcell. A single cell colorimeter (Corning-Eel 254) with a 10 mm Hellma flowcell has also been used and works satisfactorily particularly at high concentrations.

6. Sampling and samples

6.1. Samples from coastal waters (this includes the North Sea, Irish Sea and English Channel) are filtered through a glass fibre filter (Whatman GF/C or equivalent but avoid using cellulose nitrate membrane filters) and stored in glass bottles with 1 ml of chloroform added as a preservative. The samples are kept cool and dark and analysis is carried out as soon as possible. Use 4 ml polystrene sample cups that have previously been acid washed.

7. Procedure

7.1. Pump distilled water through all lines and set colorimeter control to suit concentration range of samples.

Absorbance range	Concentration range	
0 - 0.2	0 - 0.5 µg at NO_2-N l^{-1})	50 mm flowcell
0 - 0.3	0 - 1.0 µg at NO_2-N l^{-1})	
0 - 0.5	0 - 2.0 µg at NO_2-N l^{-1})	
0 - 1.0	0 - 5.0 µg at NO_2-N l^{-1})	

Allow system to equilibrate for 30 min and set baseline as required on chart recorder. Introduce sulphanilamide and N-(1-naphthyl) ethylenediamine reagents into sample stream and 10 min later change sample line from distilled water to synthetic sea water wash. Load samples into tray with 4 midrange standards at the beginning and end of each run. At the end of each run reverse the order of operations until the system is pumping distilled water in all lines.

7.2. Calibration

Calibration is always carried out by addition of known amounts of nitrite to synthetic sea water or low nitrite sea water, 4 samples of this base water are placed in the sample tray followed by 4 samples of the spiked base water. As the concentration/absorbance relationship is linear over the range of concentration used, single mid-range standards are used as detailed below.

Absorbance range	Standard
0 - 0.2	Dilute 1.00 ml of dilute standard nitrite solution (4.6.) to 100 ml with synthetic sea water (4.8.). Added nitrite = 0.5 µg at NO_2-N l^{-1}
0 - 0.3	As above
0 - 0.5	Dilute 1.00 ml of dilute standard nitrite solution (4.6.) to 50 ml with synthetic sea water (4.8.). Added nitrite = 1.0 µg at NO_2-N l^{-1}.
0 - 1.0	Dilute 5.00 ml of dilute standard nitrite solution (4.6.) to 100 ml with synthetic sea water (4.8.). Added nitrite = 2.5 µg at NO_2-N l^{-1}.

7.3. Calculation

See general notes on automatic analysis.

Table 2 Automated determination of nitrite in sea water. Statistical data

Level µg at NO_2-N l^{-1}	Colorimeter setting	Absorbance range	Flowcell length mm	Standard deviation S.D.	Precision ±2. S.D. µg at NO_2-N l^{-1}	Detection limit: 3. S.D. µg at NO_2-N l^{-1}	Sensitivity
0.25	Std. cal. 750	0-0.2A	50	0.004	±0.008	0.012	0.5 µg at NO_2-N l^{-1}
0.50				0.005	±0.010		≡ 0.08A
0.25	Std. cal. 500	0-0.3A	50	0.008	±0.016	0.024	1 µg at NO_2-N l^{-1}
0.50				0.005	±0.010		
1.00				0.006	±0.012		≡ 0.15A
0.25	Std. cal. 300	0-0.5A	50	0.005	±0.010	0.015	1 µg at NO_2-N l^{-1}
0.50				0.005	±0.010		
1.00				0.007	±0.014		≡ 0.35A
2.00				0.003	±0.006		
2.50	Std. cal. 100	0-1.0A	50	0.018	±0.036	0.054	2.5 µg at NO_2-N l^{-1}
5.00				0.003	±0.006		≡ 0.37A

o	Red/Red	0.80 ml min^{-1}	Sample
o	Black/Black	0.32 ml min^{-1}	Air
o	Orange/Green	0.10 ml min^{-1}	Sulphanilamide
o	Orange/Green	0.10 ml min^{-1}	N-1-Naphthyl ethylenediamine
o	Orange/Orange	0.42 ml min^{-1}	Debubbler
o	Red/Red	0.80 ml min^{-1}	Flowcell pull through

Figure 2a Arrangement of pump tubes

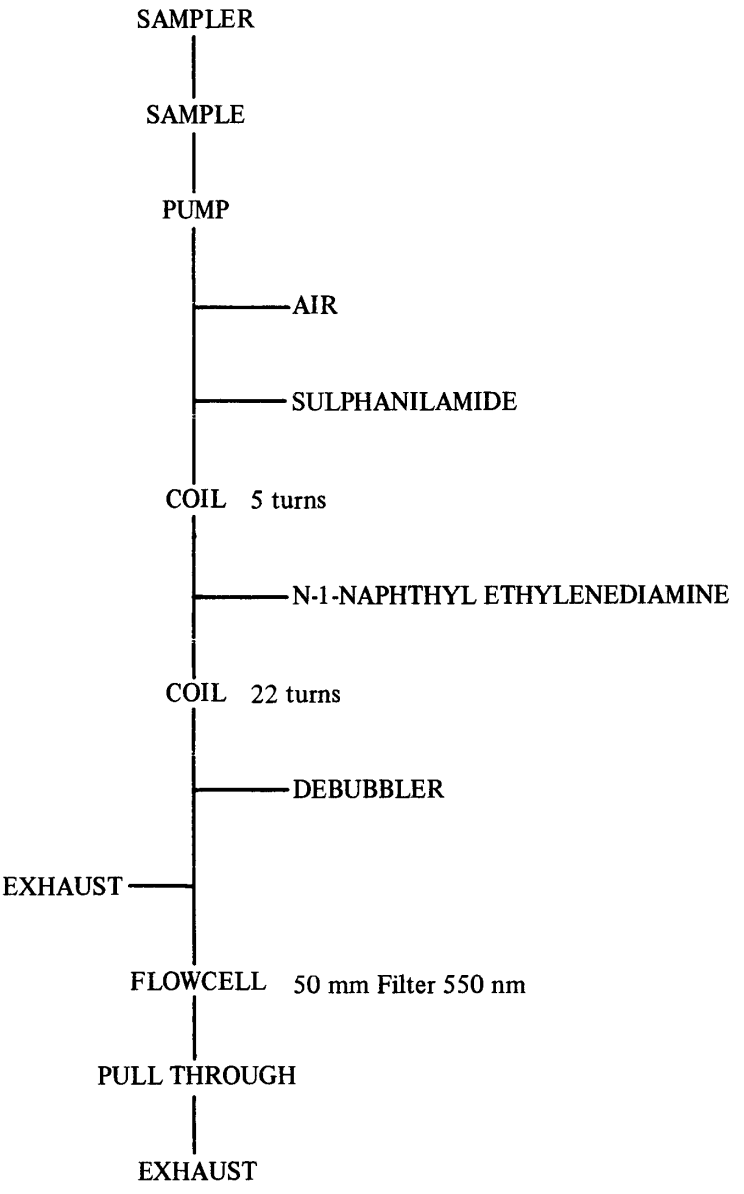

Figure 2b Flow diagram of the automated determination of nitrite in sea water

Automated determination of nitrate in sea water

1.	Performance characteristics of the method	
1.1	Substance determined	Nitrate
1.2.	Type of sample	Sea water
1.3.	Basis of method	Nitrate is reduced to nitrite by passing the sample through a coil containing copper coated cadmium wire. The total nitrite is diazotized with sulphanilamide and coupled with N-(1-naphthyl) ethylenediamine to form a magenta coloured azo-dye whose absorbance is measured at 550 nm.
1.4.	Range of application	0-50 μg at NO_3-N l^{-1} (0-700 μg NO_3-N l^{-1})
1.5.	Calibration	Linear to 50 μg at NO_3-N l^{-1}
1.6.	Statistical data	See Table 3
1.7.	Limit of detection	See Table 3
1.8.	Sensitivity	See Table 3
1.9.	Bias	No bias detected
1.10.	Interferences	It has been reported that the method can tolerate up to 2 mg sulphide l^{-1} although the cadmium may become deactivated after repeated analyses. Amines, oxidising or reducing agents, acid, alkalis, heavy metals, colour, organic matter, iodate and selenium all interfere, but sea water samples will normally be free of such agents. Nitrite will be quantitatively determined but can be corrected for by carrying out a separate nitrite determination and subtracting from the total.
1.11.	Time required for analysis	Samples are run at 20 h^{-1} with a sample to wash ratio of 15:1. At a speed of 30 h^{-1} and a sample to wash ratio of 7:1 there is no appreciable loss of precision. To the time taken for the actual analysis must be added the set-up time and stabilisation at the beginning of the day (approximetly 1 h) and the run down time at the end of the day (approximately half an hour).

2. Principle

2.1. Nitrate ions are reduced to nitrite ions by passage through a polyethylene coil containing a 1 m length of cadmium wire coated with copper. The total nitrite content of the sample is then diazotised with sulphanilamide in acid solution and coupled with N-(1-naphthyl) ethylenediamine to form an azo-dye the absorbance of which is measured at 550 nm and related to concentration by means of a factor obtained by standardisation at the beginning and end of a run of samples.

3. Safety note

Cadmium metal and its compounds are harmful if taken orally or by inhalation. Gloves should be worn when cadmium wire is being handled. The N-(1-naphthyl) ethylenediamine should be regarded as a hazard and skin contact with it and reagents incorporating it must be avoided.

4. Reagents

Use analytical grade reagents and distilled water or equivalent throughout.

4.1. Ammonium chloride solution (35% w/v)

Dissolve 175$\pm$ 5 g of ammonium chloride NH_4Cl in 500$\pm$ 5 ml of water and store in a glass or plastic bottle.

4.2. Ammonium chloride solution (approximately 11% w/v)

Dilute 60$\pm$ 2 ml of 35% w/v ammonium chloride (4.1.) to 180$\pm$ 5 ml with water. Store the solution in a dark glass bottle.

4.3. Ammonium chloride solution (approximately 0.7% w/v)

Dilute 2$\pm$ 0.1 ml of 35% w/v ammonium chloride (4.1.) to 100$\pm$ 2 ml with water. Store in a dark glass bottle.

4.4. Copper sulphate solution (2% w/v)

Dissolve 2± 0.1 g of copper sulphate pentahydrate $CuSO_4.5H_2O$ in 100± 2 ml of water.

4.5. Hydrochloric acid 1M.

4.6. Sulphanilamide solution (stock)

Dissolve 5± 0.1 g of sulphanilamide in a mixture of 50± 1 ml of concentrated hydrochloric acid HCl and 300 ml of water. Make up to 500± 5 ml with water.

4.7. Sulphanilamide solution (working)

Dilute 30± 1 ml of stock sulphanilamide solution (4.6.) to 180± 5 ml with water. Prepare fresh daily.

4.8. N-(1-naphthyl) ethylenediamine solution (stock)

Dissolve 0.5± 0.02 g of N-(1-naphthyl) ethylenediamine dihydrochloride in 500± 5 ml of water. Store in a dark glass bottle and renew after a month or when a strong brown colouration develops.

4.9. N-(1-naphthyl) ethylenediamine solution (working)

Dilute 30± 1 ml of stock N-(1-naphthyl) ethylenediamine (4.8.) to 180± 5 ml with water. Prepare fresh daily.

4.10. Standard nitrate solution (stock) 12.5 µg at NO_3-N ml^{-1}

Dissolve 1.275 g of anhydrous potassium nitrate KNO_3, which has previously been dried at 105°C, in water and dilute to 1 l in a graduated flask and mix well. The solution is stable indefinitely in the absence of evaporation.

4.11. Standard nitrate solution (dilute) 0.5 µg at NO_3-N ml^{-1}

Dilute 2.00 ml of stock standard nitrate solution to 50 ml with water in a graduated flask and mix well. Prepare fresh daily.

4.12. Standard nitrate solution (working)

See calibration 7.2.

4.13. Synthetic sea water

Dissolve 310± 2 g of sodium chloride NaCl, 100± 1 g of magnesium sulphate heptahydrate $MgSO_4.7H_2O$ and 0.50± 0.01 g of sodium bicarbonate $NaHCO_3.H_2O$ in 10 l of distilled water.

5. Apparatus

5.1. Cadmium reductor coil

Measure off 1 m of 1 mm diameter cadmium wire. Measure off 1 m 10 cm of 1.5 mm internal diameter polyethylene tubing. Insert cadmium wire into the polyethylene tubing leaving a space of 5 cm at each end. By means of a 20 ml plastic syringe pass 10 ml of 1M hydrochloric acid (4.5.) through the tubing followed by 10 ml of distilled water to flush out any remaining acid. Inject 10 ml of 2% w/v copper sulphate (4.4.) solution followed by two or three washes with distilled water to remove any sediment of deposited copper. Keep the tube full of distilled water and wind into a coil around a former of approximately 25 mm diameter. Connect the coil ends to adjacent ports of a 4-way chromatography valve and with the plastic syringe flush through with about 30 ml of 0.7% w/v ammonium chloride solution (4.3.). Then turn the valve taps to isolate the coil now fitted with 0.7% w/v ammonium chloride solution.

5.2. Regeneration of the cadmium reductor coil

When the efficiency of the cadmium reductor coil falls it can be regenerated either by stripping down to component parts and repeating the operation of setting up the coil, or it can be regenerated *in situ* by connecting the syringe adaptor to one of the remaining ports of the chromatography valve and pumping through the reagents in order.

5.3. Analytical apparatus

A segmented stream analyser (Technicon AA II) is used. The colorimeter uses 550 nm interference filters and a 15 mm flowcell. A single cell colorimeter (Corning-Eel 254) with a 10 mm Hellma flowcell may also be used over the range of nitrate concentrations to be found in the sea.

6. Sampling and samples

6.1. Samples from coastal waters (this includes the North Sea, Irish Sea and English Channel) are filtered through a glass fibre filter (Whatman GF/C or equivalent but avoid using cellulose nitrate membrane filters) and stored in glass bottles. 1 ml of chloroform is added as a preservative. The samples are kept cool and dark and analysis is carried out as soon as possible. Use 4 ml polystyrene sample cups that have been previously acid washed.

7. Procedure

7.1. Pump distilled water through all lines except the 11% w/v ammonium chloride (4.2.) line and set colorimeter control to suit concentration range of samples.

Absorbance range	Concentration range	
0 - 0.5	0 - 10 µg at NO_3-N l^{-1})	) 15 mm
0 - 1.0	0 - 20 µg at NO_3-N l^{-1})	flowcell)
0 - 2.0	0 - 30 µg at NO_3-N l^{-1})	
0 - 2.0	0 - 40 µg at NO_3-N l^{-1}	10 mm flowcell

Allow system to equilibrate for 45 min and set baseline as required on the chart recorder. Introduce sulphanilamide and N-(1-naphthyl) ethylenediamine reagents into pump lines and 10 min later change sample line to synthetic sea water wash. Load samples into tray with 4 mid-range standards at the beginning and end of each run. At the end of each run reverse the sequence of operations until all lines, except the ammonium chloride line, are pumping distilled water. Before close down isolate the cadmium coil reductor.

7.2. Calibration

Calibration is always carried out by addition of known amounts of nitrate to synthetic sea water or low nitrate sea water. 4 samples of this base sea water are placed in the sample tray followed by 4 samples of the spiked base water. As the concentration/absorbance relationship is linear over the range of concentration used, single mid-range standards are used as detailed below. If higher values of nitrate are encountered (see 1.4. and 1.5.) then a calibration curve has to be constructed or the samples re-analysed after dilution with synthetic sea water.

Absorbance range	Standard
0 - 0.5	Dilute 1.00 ml dilute nitrate standard (4.11.) to 100 ml with synthetic sea water (4.13.) Added nitrate = 5 µg at NO_3-N l^{-1}
0 - 1.0	Dilute 1.00 ml dilute nitrate standard (4.11.) to 50 ml with synthetic sea water (4.13.) Added nitrate = 10 µg at NO_3-N l^{-1}
0 - 2.0	Dilute 2.00 ml dilute nitrate standard (4.11.) to 50 ml with synthetic sea water (4.13.) Added nitrate = 20 µg at NO_3-N l^{-1}

7.3. Calculations — See general notes on automatic analysis.

Table 3 Automated determination of nitrate in sea water. Statistical data.

Level µg at NO_3-N l^{-1}	Colorimeter setting	Absorbance range	Flowcell length mm	Standard deviation S.D.	Precision ±2. S.D. µg at NO_3-N l^{-1}	Detection limit:3. S.D. µg at NO_3-N l^{-1}	Sensitivity
1.25	Std. cal. 300	0-0.5A	15	0.02	±0.04	0.06	5µg at NO_3-N l^{-1}
2.50				0.02	±0.04		
5.00				0.06	±0.12		≡ 0.2A
10.00				0.11	±0.22		
2.50	Std. cal. 100	0-1.0A	15	0.07	±0.14	0.21	10µg at NO_3-N l^{-1}
5.00				0.03	±0.06		
10.00				0.04	±0.08		≡ 0.4A
20.00				0.08	±0.16		
5.00	Std. cal. 000	0-2.0A	15	0.05	±0.10	0.15	20µg at NO_3-N l^{-1}
10.00				0.06	±0.12		
20.00				0.17	±0.34		≡ 0.8A
40.00				0.07	±0.14		

o	Red/Red	0.80 ml min^{-1}	Sample
o	Blue/Orange	0.05 ml min^{-1}	Ammonium chloride
o	Black/Black	0.32 ml min^{-1}	Air
o	Orange/Green	0.10 ml min^{-1}	Sulphanilamide
o	Orange/Green	0.10 ml min^{-1}	N-1-Naphthyl ethylenediamine
o	Orange/Orange	0.42 ml min^{-1}	Debubbler
o	Red/Red	0.80 ml min^{-1}	Flowcell pull through

Figure 3a Arrangement of pump tubes

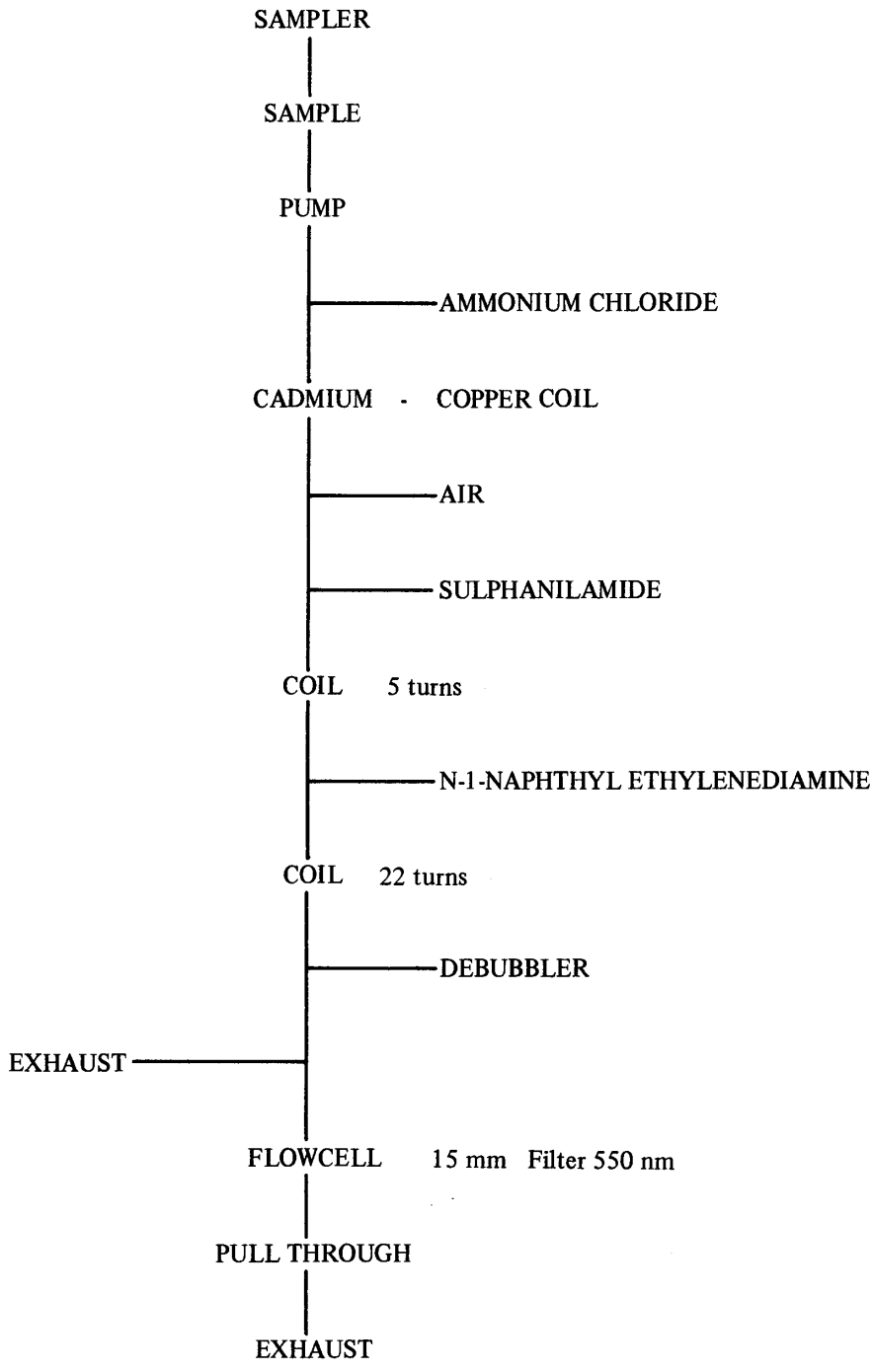

Figure 3b Flow diagram of the automated determination of nitrate in sea water

Automated determination of phosphate in sea water

1. Performance characteristics of the method

1.1.	Substance determined	Dissolved inorganic reactive phosphate
1.2.	Type of sample	Sea water
1.3.	Basis of method	Orthophosphate ions react with a mixed reagent containing molybdic acid, trivalent antimony and ascorbic acid. The resulting hetero-poly acid is reduced *in situ* to give a blue solution whose absorb-ance is measured at 880 nm.
1.4.	Range of application	0-10 µg at PO_4-P l^{-1} (0-310 µg PO_4-P l^{-1})
1.5.	Calibration	Linear to 28 µg at PO_4-Pl^{-1}
1.6.	Statistical data	See Table 4
1.7.	Limit of detection	See Table 4
1.8.	Sensitivity	See Table 4
1.9.	Bias	No bias detected
1.10.	Interferences	Interference from copper and iron is insignificant. Silicon at a level of 100 µg at Si l^{-1} is reported to interfere equivalent to 0.04 µg at PO_4-P l^{-1}. Arsenate produces a colour similar to that of phosphate, but concen-trations of arsenate encountered in sea water are unlikely to produce any significant inter-ference. Salt error is less than 1%.
1.11.	Time required for analysis	Samples are run at 20 h^{-1} with a sample to wash ratio of 15:1. To the time taken for analysis must be added the set-up and run-down times and stabilisa-tion time (see 7.1.).

2. Principle

2.1. Orthophosphate ions react with a mixed reagent containing acid ammonium molybdate with the formation of the complex heteropoly acid. The ascorbic acid component reduces the complex *in situ* and the antimony allows the rapid formation of bluish-purple colour with an absorbance maximum at 882 nm.

3. Safety note

3.1. Antimony compounds can cause irritation of the skin and mucous membranes and care should be taken in the handling of potassium antimonyl tartrate.

4. Reagents

Use analytical grade reagents and distilled water or equivalent throughout.

4.1. Ammonium molybdate solution

Dissolve 15± 0.1 g of ammonium molybdate tetra-hydrate $(NH_4)_6$ $Mo_7O_{24}.4H_2O$ in 500± 5 ml of water and store in a polythene bottle.

4.2. Sulphuric acid 4.9N

Add 140± 2 ml of concentrated sulphuric acid to 900± 5 ml of water, cool and store in a polythene bottle.

4.3. Ascorbic acid solution

Dissolve 1.35± 0.02 g of ascorbic acid in 38± 0.5 ml of water and 12± 0.5 ml of acetone. Prepare fresh daily.

4.4. Potassium antimonyl tartrate solution

Dissolve 0.34± 0.02 g of potassium antimonyl tartrate KSbO $C_4H_4O_6$ in 250± 2 ml of water and store in a polythene bottle.

4.5. Mixed reagent

Mix together in the following order 50± 1 ml of ammonium molybdate solution (4.1.), 125± 2 ml of 4.9N sulphuric acid (4.2.), 50± 1 ml of ascorbic acid solution (4.3.) and 25± 1 ml of potassium antimonyl tartrate solution (4.4.). Keep no longer than 6 h.

4.6. Standard phosphate solution (stock)

Dissolve 0.340 g of potassium hydrogen phosphate KH_2PO_4 in 500 ml of water in a graduated flask and mix well. Add 1 ml of chloroform as a preservative and store in a dark glass bottle. This solution is stable for many months in the absence of evaporation.

4.7. Standard phosphate solution (dilute)

Dilute 2.00 ml of stock standard phosphate solution to 100 ml with water in a graduated flask and mix well. Prepare fresh daily.

4.8. Standard phosphate solution (working)

See calibration 7.2.

5. Apparatus

5.1. Analytical apparatus

A segmented stream analyser (Technicon AA II) is used. The colorimeter uses 880 nm interference filters and a 50 mm flowcell.

6. Sampling and samples

6.1. Samples from coastal waters (this includes the North Sea, Irish Sea and English Channel) are filtered through a glass fibre filter (Whatman GF/C or equivalent) and kept in glass bottles and 1 ml of chloroform is added as preservative. Samples are analysed as soon as possible, but until then are kept cool and in the dark. Use 4 ml polystyrene sample cups that have been previously acid washed. Do not use sample cups that have been washed in phosphate-containing detergent.

7. Procedure

7.1. Pump distilled water through all lines and set colorimeter control to suit range of samples.

Absorbance range	Concentration range	
0 - 0.3	0 - 3.0 µg at PO_4-P l^{-1}	) 50 mm
0 - 1.0	0 - 10 µg at PO_4-P l^{-1}	) flowcell

Allow system to equilibrate for 15 min and set baseline. Introduce the mixed reagent into the pump line and 30 min later change the sample line into sea water wash and allow to run for 45 min. Load samples into tray with 6 mid-range standards at the beginning and end of each run. At the end of each run reverse the order of operations until the system is pumping distilled water in all lines.

When reagent is introduced into the sample stream containing distilled water or sea water wash it will be noticed that the chart trace exhibits a drift indicating increasing absorbance, which at first is considerable but slows to a small steady drift after about 30 min. This is probably due to coating out within the flowcell and will continue throughout a run of samples. (It is absolutely essential that the reagent stream is not allowed to run out while a run of analysis is proceeding). As the run-down sequence proceeds it will be observed that there is a drift in the opposite direction indicating decreasing absorbance in the sea water wash plus reagent and distilled water wash plus reagent phases and particularly in the distilled water only phase. This behaviour points to there being a relationship between 'coating-out' and concentration of phosphate and some considerable period of time is needed to reach an equilibrium state. It is therefore seen that it is unwise to carry out a run of analysis where there may be individual samples of high concentration among a set of predominantly low concentration samples. Also great care must be taken in the interpretation of the calibration data.

7.2. Calibration

Calibration is always carried out by addition of known amounts of phosphate to low phosphate sea water. 6 samples of this base water are placed in the sample tray followed by 6 samples of the spiked base water. As the concentration/absorbance relationship is linear over the range of concentration used, single mid-range standards are used as detailed below:–

Absorbance range	Standard
0 - 0.3	Dilute 1.00 ml of dilute standard phosphate solution (4.7.) to 100 ml with low phosphate sea water in a graduated flask and mix well. Added phosphate = 1.0 µg at PO_4-P l^{-1}
0 - 1.0	Dilute 5.00 ml of dilute standard phosphate solution (4.7.) to 100 ml with low phosphate sea water in a graduated flask and mix well. Added phosphate = 5.0 µg at PO_4-P l^{-1}

7.3. Calculation

See general notes on automatic analysis.

Table 4 Automated determination of phosphate in sea water. Statistical data.

Level µg at PO_4-P l^{-1}	Colorimeter setting	Absorbance range	Flowcell length mm	Standard deviation S.D.	Precision ± 2. S.D. µg at PO_4-P l^{-1}	Detection limit: 3. S.D. µg at PO_4-P l^{-1}	Sensitivity
0.25	Std. cal. 500	0-0.3A	50	0.01	±0.02	0.03	1µg at PO_4-P l^{-1}
0.50				0.01	±0.02		
1.00				0.01	±0.02		≡ 0.08A
2.00				0.04	±0.08		
1.25	Std. cal. 100	0-1.0A	50	0.01	±0.02	0.03	5µg at PO_4-P l^{-1}
2.50				0.03	±0.06		
5.00				0.02	±0.04		≡ 0.35A
10.00				0.13	±0.26		

o	Red/Red	0.80 ml min^{-1}	Sample
o	Black/Black	0.32 ml min^{-1}	Air
o	Orange/Yellow	0.16 ml min^{-1}	Mixed reagent
o	Orange/Orange	0.42 ml min^{-1}	Debubbler
o	White/White	0.60 ml min^{-1}	Flowcell pull through

Figure 4a Arrangement of pump tubes

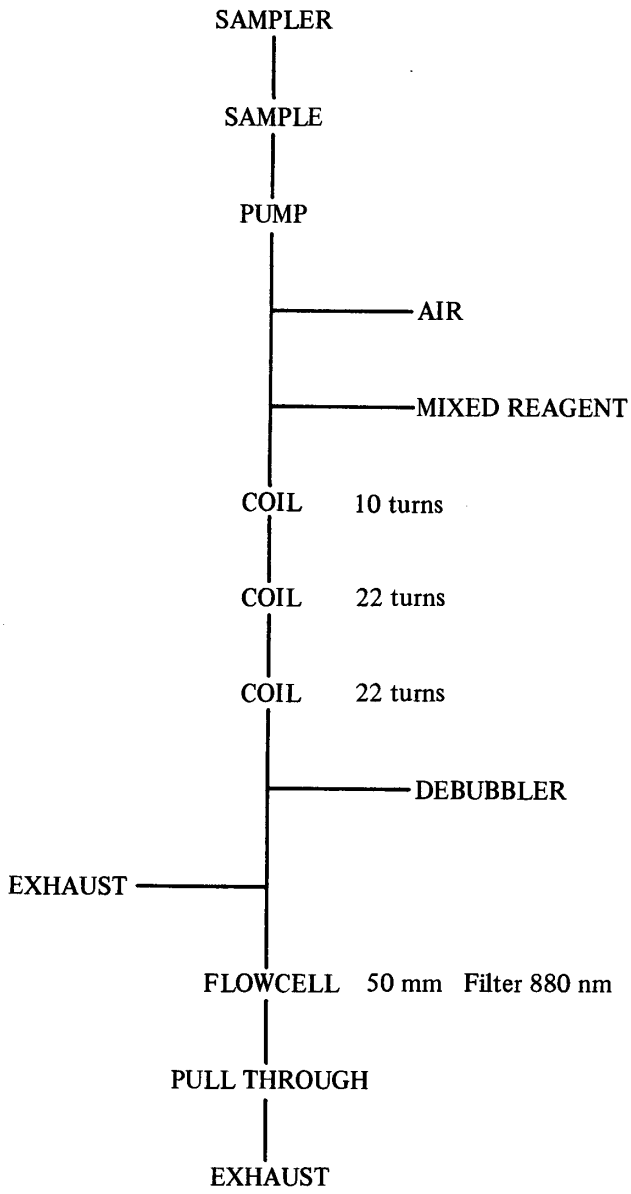

Figure 4b Flow diagram of the automated determination of phosphate in sea water

Automated determination of silicate in sea water

1. Performance characteristics of the method

1.1.	Substance determined	Dissolved reactive silicate
1.2.	Type of sample	Sea water
1.3.	Basis of method	Silicate ions are reacted with ammonium molybdate in acidic solution and the resulting silicomolybdate is reduced to 'molybdenum blue' by ascorbic acid.
1.4.	Range of application	0-60 µg at SiO_3-Si l^{-1} (0-1680 µg SiO_3-Si l^{-1})
1.5.	Calibration	Linear to about 150 µg at SiO_3-Si l^{-1}
1.6.	Statistical data	See Table 5
1.7.	Limit of detection	See Table 5
1.8.	Sensitivity	See Table 5
1.9.	Bias	No bias detected
1.10.	Interferences	A number of substances interfere including tannin, large amounts of iron, colour, turbidity and sulphide, but with filtered sea water there should be no significant interference from any of these substances. Phosphates in amounts greater than 5 µg at PO_4-P l^{-1} may interfere.
1.11.	Time required for analysis	Samples are run at 50 h^{-1} with a sample to wash ratio of 6:1. Set-up and run-down time (approximately 30 min each) must be added to give an overall estimate.

2. Principle

2.1. Silicate ions are reacted with ammonium molybdate in acidic solution to form silicomolybdate. Before the addition of ascorbic acid to reduce the silicomolybdate to 'molybdenum blue', oxalic acid is added to eliminate phosphate interference.

3. Safety note

Oxalic acid is poisonous and care should be taken in the handling of it or reagents incorporating it.

4. Reagents

Use analytical grade reagents and distilled water or equivalent throughout.

4.1. Sulphuric acid 0.55N

Add 15± 0.1 ml of concentrated sulphuric acid H_2SO_4 to 900 ml of water and dilute to 1 l.

4.2. Ammonium molybdate solution

Dissolve 18.25± 0.05 g of ammonium molybdate tetrahydrate $(NH_4)_6\ Mo_7O_{24}.4\ H_2O$ in 1000± 5 ml of sulphuric acid 0.55N (4.1.). Filter and store in a polythene bottle. Prepare fresh monthly or when the solution begins to show a slight turbidity.

4.3. Oxalic acid solution

Dissolve 70± 1 g of oxalic acid $H_2C_2O_4$ in 900 ml of water and make up to 1000± 5 ml. Store in a polythene bottle.

4.4. Ascorbic acid solution

Dissolve 3.2± 0.1 g of ascorbic acid $C_6H_8O_6$ in 80 ml of water, add 10± 0.5 ml of acetone and make up to 100± 1 ml with water. Prepare fresh daily.

4.5. Standard silicate solution (stock) 10 µg at SiO_3-Si ml^{-1}

Dissolve 1.880 g of sodium silicofluoride in water and make up to 1000 ml in a graduated flask, mix well and transfer immediately to a tightly sealed polythene bottle. This solution keeps indefintely.

4.6. Standard silicate solution (dilute) 1 µg at SiO_3-Si ml^{-1}

Dilute 10± 0.02 ml of stock standard silicate solution (4.5.) to 100 ml in a graduated flask and mix well. Use immediately or transfer to a polythene bottle. Prepare fresh daily.

4.7. Standard silicate solution (working)

See calibration 7.2.

5. Apparatus

5.1. A segmented stream analyser (Technicon AA II) is used. The colorimeter is fitted with 660 nm interference filters and a 50 mm flowcell.

6. Sampling and Samples

6.1. Samples from coastal waters (this includes the North Sea, Irish Sea and English Channel) are filtered through a glass fibre filter (Whatman GF/C or equivalent) without apparent uptake of silicate and transferred to polythene bottles and kept cool and dark. No preservatives are added. Analysis is performed as soon as possible.

7. Procedure

7.1. Pump distilled water through all lines and set colorimeter control to suit concentration range of samples.

Std. cal.	Absorbance range	Concentration range
100	0 - 1.0	0 - 60 µg at SiO_3-Si l^{-1}
300	0 - 0.5	0 - 30 µg at SiO_3-Si l^{-1}
500	0 - 0.3	0 - 20 µg at SiO_3-Si l^{-1}
750	0 - 0.2	0 - 10 µg at SiO_3-Si l^{-1}

Allow system to equilibrate for 30 min and set base-line on recorder. Introduce reagents and 10 min later change sample line from distilled water to sea water. After a further 10 min introduce a tray of samples with 4 mid-range standards at the beginning and end of each run. At the end of the run reverse the order of operations until the system is pumping distilled water in all lines.

7.2. Calibration

Calibration is always carried out by addition of known amounts of silicate to low silicate sea water. 4 samples of this base water are placed in the sample tray followed by 4 samples of the spiked base water. As the concentration/absorbance relationship is linear over the range of concentration used, single mid-range standards are used as detailed below:-

Std. cal.	Absorbance range	Concentration range	Standard
100	0 - 1.0	0 - 60	20
300	0 - 0.5	0 - 30	10
500	0 - 0.3	0 - 20	10
750	0 - 0.2	0 - 10	5

Dilute 1.00 ml of dilute standard silicate solution (4.6.) to 200 ml with low silicate sea water in a graduated flask and mix well.
Added silicate = 5 µg at SiO_3-Si l^{-1}.

Dilute 1.00 ml of dilute standard silicate solution (4.6.) to 100 ml with low silicate sea water in a graduated flask and mix well.
Added silicate = 10 µg at SiO_3-Si l^{-1}.

Dilute 1.00 ml of dilute standard silicate solution (4.6.) to 50 ml with low silicate sea water in a graduated flask and mix well.

7.3. Calculation

See general notes on automatic analysis.

Table 5 Automated determination of silicate in sea water. Statistical data

Level µg at SiO_3-Si l^{-1}	Colorimeter setting	Absorbance range	Flowcell length mm	Standard deviation S.D.	Precision ±2. S.D. µg at SiO_3-Si l^{-1}	Detection limit: 3. S.D. µg at SiO_3-Si l^{-1}	Sensitivity
2.5	Std. cal. 750	0-0.2A	50	0.02	±0.04	0.06	5 µg at SiO_3-Si l^{-1}
5.0				0.05	±0.10		
10.0				0.05	±0.10		≡0.075A
2.5	Std. cal. 500	0-0.3A	50	0.02	±0.04	0.06	10 µg at SiO_3-Si l^{-1}
5.0				0.01	±0.02		
10.0				0.05	±0.10		≡ 0.14A
20.0				0.06	±0.12		
2.5	Std. cal. 300	0-0.5A	50	0.03	±0.06	0.09	10 µg at SiO_3-Si l^{-1}
5.0				0.04	±0.08		
10.0				0.11	±0.22		≡ 0.15A
20.0				0.11	±0.22		
2.5	Std. cal. 100	0-1.0A	50	0.09	±0.18	0.27	20 µg at SiO_3-Si l^{-1}
5.0				0.06	±0.12		
10.0				0.03	±0.06		≡ 0.28A
20.0				0.06	±0.12		
40.0				0.08	±0.16		
60.0				0.04	±0.08		

o	Orange/White	0.23 ml min^{-1}	Ammonium molybdate
o	Black/Black	0.32 ml min^{-1}	Air
o	Red/Red	0.80 ml min^{-1}	Sample
o	Orange/White	0.23 ml min^{-1}	Oxalic acid
o	Orange/White	0.23 ml min^{-1}	Ascorbic acid
o	Orange/Orange	0.42 ml min^{-1}	Debubbler
o	Grey/Grey	1.00 ml min^{-1}	Flowcell pull through

Figure 5a Arrangement of pump tubes

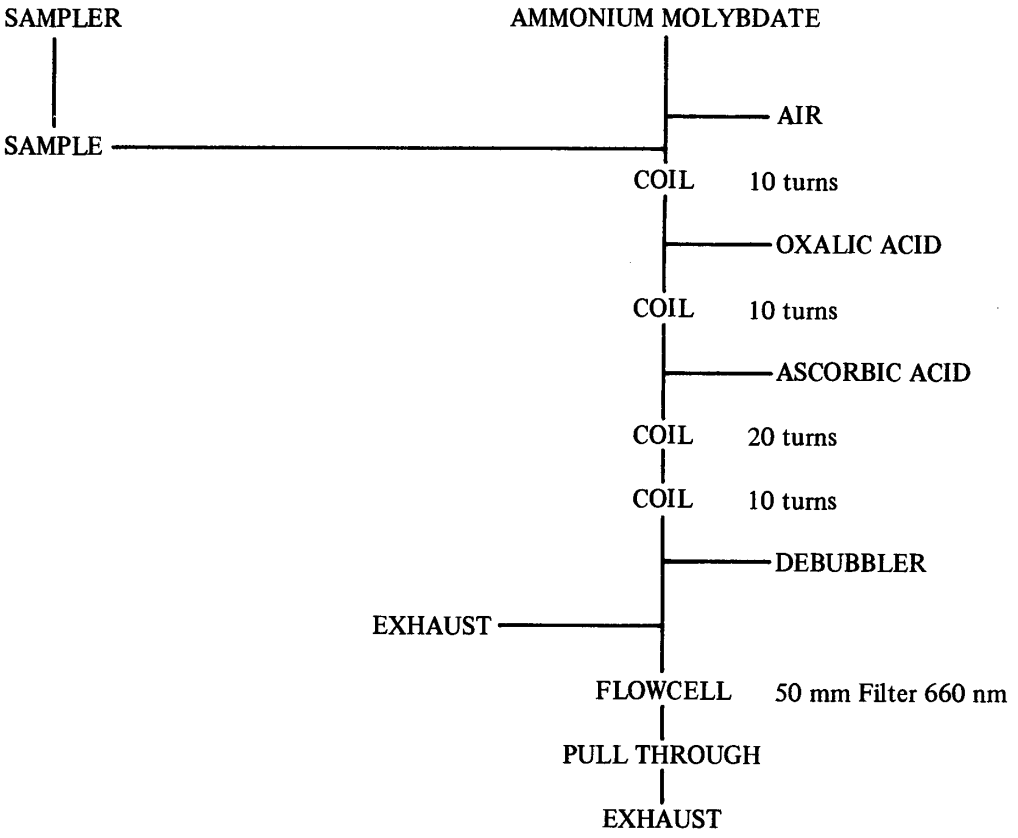

Figure 5b Flow diagram of the automated determination of silicate in sea water

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