CONTENTS

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
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have been introduced over the past 10-15 years and, whereas large numbers of samples are to be processed, have largely superseded the manual techniques.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® 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 Earlier published work was concerned with methodology adapted to the AutoAnalyser I, a system which has now been replaced by the AutoAnalyser II.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 analyses (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, analysts 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 auto-
mation, 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 mani-
pulate 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 accom-
modate 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 absorp-
tion 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 plateaus as
displayed on the recorder. A recent modification as sug-
gested by Stainton (1974) has been incorporated into our
system and consists of a length of cadmium wire inserted
into a length of 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 plateaus 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. Lidickcost, Tibbiits 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 develop-
ment in this method involves heating to 300C and irradia-
tion 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 concen-
trations that allow a greater sample/reagent volume ratio,
an overall sensitivity increase of approximately x 3.5 is pos-
ible. 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
Koroellef (1976).

For the various methods used estimates of standard devia-
tion 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
interuption 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 NH₄⁺-N 1⁻¹ (0-1050 μg NH₄⁺-N 1⁻¹)

1.5. Calibration Linear to at least 75 μg at NH₄⁺-N 1⁻¹

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

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

4.3. Sodium Hydroxide 1.5 M

Dissolve 3.9±0.05 g of Phenol C₆H₅OH in 100±2 ml of 37 v/v ethanol (4.1.). Prepare fresh daily.

4.4. Trisodium Citrate

Dissolve 390±2 g of trisodium citrate dihydrate Na₃C₆H₅O₇·2H₂O 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 K₄Fe(CN)₆·3H₂O in water and make up to 100 ml with water. Prepare fresh daily.

4.7. Standard ammonia solution (stock) 100μg at NH₄⁺-N ml⁻¹

Dissolve 3.3030 g of ammonium sulphate (NH₄)₂SO₄ 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 NH₄-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.

<table>
<thead>
<tr>
<th>Absorbance range</th>
<th>Concentration range</th>
</tr>
</thead>
</table>
| 0 - 0.5          | 0 - 10 μg at NH₄-N 1⁻¹ |} flowcell
| 0 - 1.0          | 0 - 15 μg at NH₄-N 1⁻¹ |
| 0 - 1.0          | 0 - 75 μg at NH₄-N 1⁻¹ |} flowcell

4.12. 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.

<table>
<thead>
<tr>
<th>Absorbance range</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.5</td>
<td>Dilute 0.05 ml of dilute standard ammonia solution (4.8.) to 100 ml with low ammonia sea water. Added ammonia = 5 μg at NH₄-N 1⁻¹</td>
</tr>
<tr>
<td>0 - 1.0</td>
<td>Dilute 1.00 ml of dilute standard ammonia solution (4.8.) to 100 ml with low ammonia sea water. Added ammonia = 10 μg at NH₄-N 1⁻¹</td>
</tr>
<tr>
<td>0 - 1.0</td>
<td>Dilute 2.50 ml of dilute standard ammonia solution (4.8.) to 100 ml with low ammonia sea water. Added ammonia = 25 μg at NH₄-N 1⁻¹</td>
</tr>
</tbody>
</table>

7.3. Calculation

See general notes on automatic analysis.
Table 1  Automated determination of ammonia in sea water. Statistical data

<table>
<thead>
<tr>
<th>Level  ( \mu g ) at NH(_4)-N (1^{-1})</th>
<th>Colorimeter setting</th>
<th>Absorbance range</th>
<th>Flowcell length mm</th>
<th>Standard deviation S.D.</th>
<th>Precision ( \pm 2 ) S.D. ( \mu g ) at NH(_4)-N (1^{-1})</th>
<th>Detection limit: 3 S.D. ( \mu g ) at NH(_4)-N (1^{-1})</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>Std. cal. 300</td>
<td>0-0.5A</td>
<td>50</td>
<td>0.02</td>
<td>( \pm 0.04 )</td>
<td>0.06</td>
<td>( \equiv 0.2A )</td>
</tr>
<tr>
<td>2.50</td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td>( \pm 0.04 )</td>
<td></td>
<td>( \equiv 0.2A )</td>
</tr>
<tr>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td>( \pm 0.04 )</td>
<td></td>
<td>( \equiv 0.2A )</td>
</tr>
<tr>
<td>7.50</td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
<td>( \pm 0.12 )</td>
<td></td>
<td>( \equiv 0.2A )</td>
</tr>
<tr>
<td>10.00</td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>( \pm 0.10 )</td>
<td></td>
<td>( \equiv 0.2A )</td>
</tr>
<tr>
<td>2.50</td>
<td>Std. cal. 100</td>
<td>0-1.0A</td>
<td>50</td>
<td>0.02</td>
<td>( \pm 0.04 )</td>
<td>0.06</td>
<td>( \equiv 0.2A )</td>
</tr>
<tr>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>( \pm 0.10 )</td>
<td></td>
<td>( \equiv 0.2A )</td>
</tr>
<tr>
<td>7.50</td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>( \pm 0.08 )</td>
<td></td>
<td>( \equiv 0.2A )</td>
</tr>
<tr>
<td>10.00</td>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
<td>( \pm 0.14 )</td>
<td></td>
<td>( \equiv 0.2A )</td>
</tr>
<tr>
<td>12.50</td>
<td></td>
<td>0-1.0A</td>
<td>10</td>
<td>0.04</td>
<td>( \pm 0.08 )</td>
<td>0.12</td>
<td>( \equiv 0.29A )</td>
</tr>
<tr>
<td>25.00</td>
<td></td>
<td></td>
<td></td>
<td>0.08</td>
<td>( \pm 0.16 )</td>
<td></td>
<td>( \equiv 0.29A )</td>
</tr>
<tr>
<td>50.00</td>
<td></td>
<td></td>
<td></td>
<td>0.08</td>
<td>( \pm 0.16 )</td>
<td></td>
<td>( \equiv 0.29A )</td>
</tr>
<tr>
<td>75.00</td>
<td></td>
<td></td>
<td></td>
<td>0.15</td>
<td>( \pm 0.30 )</td>
<td></td>
<td>( \equiv 0.29A )</td>
</tr>
</tbody>
</table>
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 0.5-1 µg at NO₂⁻N 1⁻¹ (0.70 µg, NO₂⁻N 1⁻¹)

1.5. Calibration Linear to 10 µg at NO₂⁻N 1⁻¹

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

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) ethylenediamine 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) ethylenediamine solution (4.3.) to 180±5 ml with water. Prepare fresh daily.

4.5. Standard nitrite solution (stock) 5 µg at NO₂⁻N ml⁻¹

Dissolve 0.3450 gm of sodium nitrite NaNO₂ 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 NO₂⁻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 MgSO₄.7H₂O and 0.50±0.01 g of sodium bicarbonate NaHCO₃.H₂O in 10 l of distilled water.
5. Apparatus

5.1. A segmented stream analyzer (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 polystyrene 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.

<table>
<thead>
<tr>
<th>Absorbance range</th>
<th>Concentration range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.2</td>
<td>0 - 0.5 µg at NO₂-N 1⁻¹</td>
</tr>
<tr>
<td>0 - 0.3</td>
<td>0 - 1.0 µg at NO₂-N 1⁻¹</td>
</tr>
<tr>
<td>0 - 0.5</td>
<td>0 - 2.0 µg at NO₂-N 1⁻¹</td>
</tr>
<tr>
<td>0 - 1.0</td>
<td>0 - 5.0 µg at NO₂-N 1⁻¹</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Absorbance range</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.2</td>
<td>Dilute 1.00 ml of dilute standard nitrite solution (4.6.) to 100 ml with synthetic sea water (4.8.).</td>
</tr>
<tr>
<td></td>
<td>Added nitrite = 0.5 µg at NO₂-N 1⁻¹</td>
</tr>
<tr>
<td>0 - 0.3</td>
<td>As above</td>
</tr>
<tr>
<td>0 - 0.5</td>
<td>Dilute 1.00 ml of dilute standard nitrite solution (4.6.) to 50 ml with synthetic sea water (4.8.).</td>
</tr>
<tr>
<td></td>
<td>Added nitrite = 1.0 µg at NO₂-N 1⁻¹</td>
</tr>
<tr>
<td>0 - 1.0</td>
<td>Dilute 5.00 ml of dilute standard nitrite solution (4.6.) to 100 ml with synthetic sea water (4.8.).</td>
</tr>
<tr>
<td></td>
<td>Added nitrite = 2.5 µg at NO₂-N 1⁻¹</td>
</tr>
</tbody>
</table>

7.3. Calculation

See general notes on automatic analysis.
<table>
<thead>
<tr>
<th>Level µg at NO₂-N 1⁻¹</th>
<th>Colorimeter setting</th>
<th>Absorbance range</th>
<th>Flowcell length mm</th>
<th>Standard deviation S.D.</th>
<th>Precision ± 2. S.D. µg at NO₂-N 1⁻¹</th>
<th>Detection limit: 3. S.D. µg at NO₂-N 1⁻¹</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>Std. cal. 750</td>
<td>0-0.2A</td>
<td>50</td>
<td>0.004</td>
<td>±0.008</td>
<td>0.012</td>
<td>0.5 µg at NO₂-N 1⁻¹ ≤ 0.08A</td>
</tr>
<tr>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td>0.005</td>
<td>±0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>Std. cal. 500</td>
<td>0-0.3A</td>
<td>50</td>
<td>0.008</td>
<td>±0.016</td>
<td>0.024</td>
<td>1 µg at NO₂-N 1⁻¹ ≥ 0.15A</td>
</tr>
<tr>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td>0.005</td>
<td>±0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td>0.006</td>
<td>±0.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>Std. cal. 300</td>
<td>0-0.5A</td>
<td>50</td>
<td>0.005</td>
<td>±0.010</td>
<td>0.015</td>
<td>1 µg at NO₂-N 1⁻¹ ≥ 0.35A</td>
</tr>
<tr>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td>0.005</td>
<td>±0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td>0.007</td>
<td>±0.014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
<td>±0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.50</td>
<td>Std. cal. 100</td>
<td>0-1.0A</td>
<td>50</td>
<td>0.018</td>
<td>±0.036</td>
<td>0.054</td>
<td>2.5 µg at NO₂-N 1⁻¹ ≥ 0.37A</td>
</tr>
<tr>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
<td>±0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color Combination</td>
<td>Flow Rate</td>
<td>Description</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red/Red</td>
<td>0.80 ml min⁻¹</td>
<td>Sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black/Black</td>
<td>0.32 ml min⁻¹</td>
<td>Air</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange/Green</td>
<td>0.10 ml min⁻¹</td>
<td>Sulphanilamide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange/Green</td>
<td>0.10 ml min⁻¹</td>
<td>N-1-Naphthyl ethylenediamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange/Orange</td>
<td>0.42 ml min⁻¹</td>
<td>De bubbler</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red/Red</td>
<td>0.80 ml min⁻¹</td>
<td>Flowcell pull through</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2a**  Arrangement of pump tubes

![Flow diagram](image)

**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 1$^{-1}$
(0-700 μg NO$_3$-N 1$^{-1}$)

1.5. Calibration
Linear to 50 μg at NO$_3$-N 1$^{-1}$

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 1$^{-1}$ 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 (approximately 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 whose absorbance 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

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

Dissolve 175± 5 g of ammonium chloride NH$_4$Cl in 500± 5 ml of water and store in a glass or plastic bottle.

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

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

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

Dilute 2± 0.1 ml of 35% w/v ammonium chloride (4.1) to 100± 2 ml with water. Store in a dark glass bottle.
4.4. Copper sulphate solution (2% w/v)
Dissolve $2 \pm 0.1$ g of copper sulphate pentahydrate CuSO$_4 \cdot 5$H$_2$O in 100$\pm 2$ ml of water.

4.5. Hydrochloric acid 1M.

4.6. Sulphanilamide solution (stock)
Dissolve $5 \pm 0.1$ g of sulphanilamide in a mixture of 50$\pm 1$ ml of concentrated hydrochloric acid HCl and 300 ml of water. Make up to 500$\pm 5$ ml with water.

4.7. Sulphanilamide solution (working)
Dilute 30$\pm 1$ ml of stock sulphanilamide solution (4.6.) to 180$\pm 5$ ml with water. Prepare fresh daily.

4.8. N-(1-naphthyl) ethylenediamine solution (stock)
Dissolve $0.5 \pm 0.02$ g of N-(1-naphthyl) ethylenediamine dihydrochloride in 500$\pm 5$ 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$\pm 1$ ml of stock N-(1-naphthyl) ethylenediamine (4.8.) to 180$\pm 5$ ml with water. Prepare fresh daily.

4.10. Standard nitrate solution (stock) 12.5 $\mu$g 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 $\mu$g 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 $310 \pm 2$ g of sodium chloride NaCl, $100 \pm 1$ g of magnesium sulphate heptahydrate MgSO$_4 \cdot 7$H$_2$O and $0.50 \pm 0.01$ g of sodium bicarbonate NaHCO$_3 \cdot$H$_2$O 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.

5.4. Sampling and samples
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.

6. Procedure
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: 0 - 0.5 corresponds to 0 - 10 μg at NO₃-N 1⁻¹
Absorbance range: 0 - 1.0 corresponds to 0 - 20 μg at NO₃-N 1⁻¹
Absorbance range: 0 - 2.0 corresponds to 0 - 30 μg at NO₃-N 1⁻¹
Absorbance range: 0 - 2.0 corresponds to 0 - 40 μg at NO₃-N 1⁻¹

Allow system to equilibrate for 45 min and set baseline as required on the chart recorder. Introduce sulfanilamide 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 cell 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.

7.3. Calculations

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

<table>
<thead>
<tr>
<th>Level 1μg at NO₃-N 1⁻¹</th>
<th>Columnarimeter setting</th>
<th>Absorbance range</th>
<th>Flowcell length mm</th>
<th>Standard deviation S.D.</th>
<th>Precision ±2. S.D. 1μg at NO₃-N 1⁻¹</th>
<th>Detection limit:3 S.D. 1μg at NO₃-N 1⁻¹</th>
<th>Sensitivity 5μg at NO₃-N 1⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>Std. cal. 300</td>
<td>0.05A</td>
<td>15</td>
<td>0.02</td>
<td>±0.04</td>
<td>0.06</td>
<td>5μg at NO₃-N 1⁻¹</td>
</tr>
<tr>
<td>2.50</td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td>±0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
<td>±0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.00</td>
<td></td>
<td></td>
<td></td>
<td>0.11</td>
<td>±0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.50</td>
<td>Std. cal. 100</td>
<td>0-1.0A</td>
<td>15</td>
<td>0.07</td>
<td>±0.14</td>
<td>0.21</td>
<td>10μg at NO₃-N 1⁻¹</td>
</tr>
<tr>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
<td>0.03</td>
<td>±0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.00</td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>±0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.00</td>
<td></td>
<td></td>
<td></td>
<td>0.08</td>
<td>±0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>Std. cal. 000</td>
<td>0-2.0A</td>
<td>15</td>
<td>0.05</td>
<td>±0.10</td>
<td>0.15</td>
<td>20μg at NO₃-N 1⁻¹</td>
</tr>
<tr>
<td>10.00</td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
<td>±0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.00</td>
<td></td>
<td></td>
<td></td>
<td>0.17</td>
<td>±0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40.00</td>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
<td>±0.14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 3a  Arrangement of pump tubes

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

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 heteropoly acid is reduced \textit{in situ} to give a blue solution whose absorbance is measured at 880 nm.

1.4. Range of application
0.10 \( \mu \)g at PO\(_4\)-P \( \cdot \)1\(^{-1} \)
(0.310 \( \mu \)g at PO\(_4\)-P \( \cdot \)1\(^{-1} \))

1.5. Calibration
Linear to 28 \( \mu \)g at PO\(_4\)-P \( \cdot \)1\(^{-1} \)

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 \( \mu \)g at Si \( \cdot \)1\(^{-1} \) is reported to interfere equivalent to 0.04 \( \mu \)g at PO\(_4\)-P \( \cdot \)1\(^{-1} \). Arsenate produces a colour similar to that of phosphate, but concentrations of arsenate encountered in sea water are unlikely to produce any significant interference. 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 stabilisation 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 \textit{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\( \pm \) 0.1 g of ammonium molybdate tetrahydrate \((\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}\) in 500\( \pm \) 5 ml of water and store in a polystyrene bottle.

4.2. Sulphuric acid 4.9N

Add 140\( \pm \) 2 ml of concentrated sulphuric acid to 900\( \pm \) 5 ml of water, cool and store in a polystyrene bottle.

4.3. Ascorbic acid solution

Dissolve 1.35\( \pm \) 0.02 g of ascorbic acid in 38\( \pm \) 0.5 ml of water and 12\( \pm \) 0.5 ml of acetone. Prepare fresh daily.

4.4. Potassium antimonyl tartrate solution

Dissolve 0.34\( \pm \) 0.02 g of potassium antimonyl tartrate \(\text{K}_2\text{SbO}_4\cdot \text{C}_4\text{H}_6\text{O}_6\) in 250\( \pm \) 2 ml of water and store in a polystyrene bottle.

4.5. Mixed reagent

Mix together in the following order 50\( \pm \) 1 ml of ammonium molybdate solution (4.1.), 125\( \pm \) 2 ml of 4.9N sulphuric acid (4.2.), 50\( \pm \) 1 ml of ascorbic acid solution (4.3.) and 25\( \pm \) 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 \(\text{KH}_2\text{PO}_4\) 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.

<table>
<thead>
<tr>
<th>Absorbance range</th>
<th>Concentration range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.3</td>
<td>0 - 3.0 μg at PO₄-P l⁻¹</td>
</tr>
<tr>
<td>0 - 1.0</td>
<td>0 - 10 μg at PO₄-P l⁻¹</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Absorbance range</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.3</td>
<td>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 PO₄-P l⁻¹</td>
</tr>
<tr>
<td>0 - 1.0</td>
<td>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 PO₄-P l⁻¹</td>
</tr>
</tbody>
</table>

7.3. Calculation

See general notes on automatic analysis.
<table>
<thead>
<tr>
<th>Level (µg at PO₄-P l⁻¹)</th>
<th>Colorimeter setting</th>
<th>Absorbance range</th>
<th>Flowcell length (mm)</th>
<th>Standard deviation (S.D.)</th>
<th>Precision ± 2. S.D. (µg at PO₄-P l⁻¹)</th>
<th>Detection limit: 3. S.D. (µg at PO₄-P l⁻¹)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>Std. cal. 500</td>
<td>0.3 A</td>
<td>50</td>
<td>0.01</td>
<td>±0.02</td>
<td>0.03</td>
<td>1 µg at PO₄-P l⁻¹</td>
</tr>
<tr>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
<td>±0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
<td>±0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>±0.08</td>
<td></td>
<td>≈ 0.08 A</td>
</tr>
<tr>
<td>1.25</td>
<td>Std. cal. 100</td>
<td>0.10 A</td>
<td>50</td>
<td>0.01</td>
<td>±0.02</td>
<td>0.03</td>
<td>5 µg at PO₄-P l⁻¹</td>
</tr>
<tr>
<td>2.50</td>
<td></td>
<td></td>
<td></td>
<td>0.03</td>
<td>±0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td>±0.04</td>
<td></td>
<td>≈ 0.35 A</td>
</tr>
<tr>
<td>10.00</td>
<td></td>
<td></td>
<td></td>
<td>0.13</td>
<td>±0.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 SiO₂-Si 1⁻¹ (0-1680 μg SiO₂-Si 1⁻¹)

1.5. Calibration Linear to about 150 μg at SiO₂-Si 1⁻¹

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 seawater there should be no significant interference from any of these substances. Phosphates in amounts greater than 5 μg at PO₄-P 1⁻¹ 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 H₂SO₄ to 900 ml of water and dilute to 1 l.

4.2. Ammonium molybdate solution

Dissolve 18.25± 0.05 g of ammonium molybdate trihydrate (NH₄)₂Mo₇O₂₄.4 H₂O 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 H₂C₂O₄ 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 C₆H₈O₆ 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 SiO₂-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 indefinitely.

4.6. Standard silicate solution (dilute) 1 μg at SiO₂-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.

<table>
<thead>
<tr>
<th>Std. cal.</th>
<th>Absorbance range</th>
<th>Concentration range</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0 - 1.0</td>
<td>0 - 60 µg at SiO₂-Si 1⁻¹</td>
</tr>
<tr>
<td>300</td>
<td>0 - 0.5</td>
<td>0 - 30 µg at SiO₂-Si 1⁻¹</td>
</tr>
<tr>
<td>500</td>
<td>0 - 0.3</td>
<td>0 - 20 µg at SiO₂-Si 1⁻¹</td>
</tr>
<tr>
<td>750</td>
<td>0 - 0.2</td>
<td>0 - 10 µg at SiO₂-Si 1⁻¹</td>
</tr>
</tbody>
</table>

Allow system to equilibrate for 30 min and set baseline 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:

<table>
<thead>
<tr>
<th>Std. cal.</th>
<th>Absorbance range</th>
<th>Concentration range</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0 - 1.0</td>
<td>0 - 60</td>
<td>20</td>
</tr>
<tr>
<td>300</td>
<td>0 - 0.5</td>
<td>0 - 30</td>
<td>10</td>
</tr>
<tr>
<td>500</td>
<td>0 - 0.3</td>
<td>0 - 20</td>
<td>10</td>
</tr>
<tr>
<td>750</td>
<td>0 - 0.2</td>
<td>0 - 10</td>
<td>5</td>
</tr>
</tbody>
</table>

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 SiO₂-Si 1⁻¹.

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 SiO₂-Si 1⁻¹.

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

<table>
<thead>
<tr>
<th>Level µg at SiO₂-Si 1⁻¹</th>
<th>Colorimeter setting</th>
<th>Absorbance range</th>
<th>Flowcell length mm</th>
<th>Standard deviation S.D.</th>
<th>Precision ± 2. S.D. µg at SiO₂-Si 1⁻¹</th>
<th>Detection limit: 3. S.D. µg at SiO₂-Si 1⁻¹</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>Std. cal. 750</td>
<td>0-0.2A</td>
<td>50</td>
<td>0.02</td>
<td>±0.04</td>
<td>0.06</td>
<td>5 µg at SiO₂-Si 1⁻¹</td>
</tr>
<tr>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>±0.10</td>
<td>±0.075A</td>
<td>±0.14A</td>
</tr>
<tr>
<td>10.0</td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>±0.10</td>
<td>±0.18</td>
<td>±0.15A</td>
</tr>
<tr>
<td>2.5</td>
<td>Std. cal. 500</td>
<td>0-0.3A</td>
<td>50</td>
<td>0.02</td>
<td>±0.04</td>
<td>0.06</td>
<td>10 µg at SiO₂-Si 1⁻¹</td>
</tr>
<tr>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
<td>±0.04</td>
<td>±0.08</td>
<td>±0.26A</td>
</tr>
<tr>
<td>10.0</td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>±0.10</td>
<td>±0.22</td>
<td>±0.14A</td>
</tr>
<tr>
<td>20.0</td>
<td>Std. cal. 300</td>
<td>0-0.5A</td>
<td>50</td>
<td>0.03</td>
<td>±0.06</td>
<td>0.09</td>
<td>10 µg at SiO₂-Si 1⁻¹</td>
</tr>
<tr>
<td>2.5</td>
<td>Std. cal. 100</td>
<td>0-1.0A</td>
<td>50</td>
<td>0.09</td>
<td>±0.18</td>
<td>0.27</td>
<td>20 µg at SiO₂-Si 1⁻¹</td>
</tr>
<tr>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
<td>±0.12</td>
<td>±0.075A</td>
<td>±0.15A</td>
</tr>
<tr>
<td>10.0</td>
<td></td>
<td></td>
<td></td>
<td>0.03</td>
<td>±0.12</td>
<td>±0.18</td>
<td>±0.26A</td>
</tr>
<tr>
<td>20.0</td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
<td>±0.06</td>
<td>±0.14A</td>
<td>±0.14A</td>
</tr>
<tr>
<td>40.0</td>
<td></td>
<td></td>
<td></td>
<td>0.08</td>
<td>±0.16</td>
<td>±0.26A</td>
<td>±0.15A</td>
</tr>
<tr>
<td>60.0</td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>±0.08</td>
<td>±0.26A</td>
<td>±0.14A</td>
</tr>
</tbody>
</table>
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\(^{-1}\) De bubbler
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
References


The reference to proprietary products in this report does not imply official endorsement of those products, nor is any criticism implied of similar products not mentioned.