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CHEMICAL METHODS FOR USE IN MARINE ENVIRONMENTAL MONITORING

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PREFACE

This Manual was prepared by the Intergovernmental Oceanographic Commission (IOC) of Unesco, with the collaboration of a consultant, Mr. Stig R. Carlberg, of the Institute of Hydrographic Research, National Swedish Board of Fisheries, Gothenburg, at the request of the United Nations Environment Programme (UNEP), for its Co-ordinated Mediterranean Pollution Monitoring and Research Programme (MED POL), whose support in this regard is gratefully acknowledged.

The Manual contains descriptions of chemical methods for analysis of parameters of general interest in programmes for chemical oceanography, as well as for marine environmental monitoring. It is intended for use by marine science institutes that are or will become involved in such activities, particularly within the Regional Seas Programme of UNEP.

For preparation of practical manuals a supply of good background material is absolutely essential. In this regard, the use of three earlier publications (see Carlberg 1972, FAO 1975 and Grasshoff 1976 in the list of references) is gratefully acknowledged.

The methods presented in this report were produced for the purpose of serving as reference methods for work performed under MED POL. However, since they have much wider applicability than for just one regional area, they are published for the use of interested marine scientists throughout the world.

1. POTENTIOMETRIC DETERMINATION OF pH

1.1. SCOPE AND FIELD OF APPLICATION

The determination of pH described herein is performed electrometrically, using a glass electrode, with calomel electrode as reference. For the determination of pH with the highest precision and accuracy, which represents normal practice in oceanography, the full details of this methods should be followed. If the demands are less stringent (precision and accuracy of ± 0.1 pH unit or less) a simplified procedure, omitting the closed measuring cell and the constant temperature bath, may be applied. The calibration procedure is , however, generally applicable.

1.2. PRINCIPLE

pH is defined as the negative logarithm of the hydrogen ion activity (a_{H^+}) and thus pH = $-\log a_{H^+}$ is a direct measure of acidity according to the Brönsted definition of acids as substances that are able to donate hydrogen ions, and bases as substances that are able to combine with hydrogen ions : acids $\rightleftharpoons$ base + hydrogen ion.

The principle for the potentiometric determinations is that the potential of the electrode chain consisting of an indicator electrode sensitive to the hydrogen ion activity according to the Nernst equation - and a pH-independent reference electrode, both immersed in the test solution, is measured by means of a suitable galvanometer (pH meter). The measured potential is then compared with the electrode chain potential obtained when a buffer solution of known pH is used as test solution. The actual pH value is found using the formula for "Operational pH" :

$$pH(X) = pH(S) + \left[E(X) - E(S)\right] / R'T$$

where :

pH(X) and pH(S) = pH of test solution and buffer solution respectively
E(X) and E(S) = electrode chain potential in test solution and buffer respectively. R'T = the Nernst factor
(R' = a constant, T = the absolute temperature).

A correctly calibrated pH meter automatically converts the measured potential to give direct reading in pH units.

1.3. REAGENTS

As pH values are temperature dependent, the pH values of the following buffer solutions are given in Table I. For a proper calibration at least two of the solutions are required. The choice of solutions is governed by the pH range of the samples.

1.3.1. Bates 1:1 buffer. Potassium dihydrogen phosphate, KH_2PO_4; 3.39 g and disodium hydrogen phosphate, Na_2HPO_4, 3.53 g are dissolved in distilled water free of carbonates and diluted to 1 000 ml.

1.3.2. Bates 1 : 3.5 buffer. Potassium dihydrogen phosphate, KH_2PO_4; 1.179 g and disodium hydrogen phosphate, Na_2HPO_4, 4.30 g are dissolved in distilled water free of carbonates and diluted to 1 000 ml.

1.3.3. Palitzsch borax buffer Sodium tetraborate, $Na_2B_4O_7 . 10H_2O$, 9.554 g, boric acid, H_3BO_3, 6.202 g, and sodium chloride, NaCl, 1.462 g are dissolved in distilled water free of carbonates and diluted to 1 000 ml.

1.3.4. Bates 0.01 M borax buffer. Sodium tetraborate, $Na_2B_4O_7 . 10H_2O$, 3.814 g is dissolved in distilled water free of carbonates and diluted to 1 000 ml.

The sodium and potassium salts should be dried before weighing. It is always advisable to keep a supply of these salts in a desiccator containing silica gel; thus, time-consuming drying in an oven is avoided. Some authors recommend recrystallization of borax before use. This should be done from water below 50°C. To prevent partial dehydration of borax, it should be kept over a saturated solution of sugar or sodium chloride or slightly moist sodium bromide, in a closed container. The boric acid could be kept in the same manner.

Distilled water free of carbonates is prepared by boiling distilled water for a while and, preferably, passing nitrogen gas through during the coling. The water should then immediately be used for the preparation of solutions.

Table I

Standard pH values of the buffer solutions

Temp. °C	Bates 1:1 (1.3.1)	Bates 1:3.5 (1.3.2)	Palitzsch borax[a] (1.3.3)	Bates 0.01 M borax (1.3.4)
0	6.984			
5	6.951			
10	6.923	7.472		9.332
15	6.900	7.448		9.275
20	6.881	7.429	8.510	9.225
25	6.865	7.413	8.459	9.180
30		7.400		9.139
35				9.068

a/ For Palitzsch buffer only two values are given because at other temperatures the values are uncertain, as found in e.g. ICES intercalibration exercises(Gieskes 1969).

1.4. APPARATUS AND EQUIPMENT

1.4.1. Water bath, thermostated, with pump for external circulation of the thermosted water. Should be capable of holding a selected temperature to within ± 0.5°.

1.4.2. Measuring cell and heat exchanger, of glass, constructed as in Figure 1. These items and connecting tubes should be as small as possible in order to reach thermal equilibrium quickly, and should not require too much sample water for washing and measurement. The electrodes should be kept in sea-surface water between measurements. Only during longer storage should distilled water be used.

1.4.3. Electrode (glass electrode and calomel reference electrode) any commercial type of good quality may be used. (W. Ingold A.G., Zürich, Switzerland, has a glass electrode called LOT 201, which may be a good choice because it has a fast reponse and works properly even at low temperature).

1.4.4. pH meter of good quality. An instrument with a galvanometer should be provided with scale expansion facilities. For shipboard use an instrument with digital display is preferred, as the galvanometer needle is influenced by the movements of the ship. The pH value must be displayed with at least two decimals.

1.5. SAMPLING PROCEDURE

It is important to minimize interaction between the sample and the carbon dioxide in the air. Hence the same care and precautions as in oxygen sampling are advocated. Because of this statement O_2 analyses probably should preceed pH. The sample is drawn via a hose from the water sampler (immediately after the oxygen sample) into a 100 ml glass bottle with a ground stopper. The sample should be analysed within one hour after sampling.

1.6. ANALYTICAL PROCEDURE

1.6.1. Calibration

For the exact handling of the pH meter, the instruction manual supplied with the instrument shoud be consulted.

The pH meter is standardized with Bates 1:1 phosphate buffer (1.3.1.) at the chosen constant temperature (20 or 25°C). Because most electrode pairs are not entirely linear over the entire pH scale, the slope (mV/pH) of the electrodes should be corrected. Immerse the electrodes in Bates 0.01 M borax buffer (1.3.4.) (rinse well). If a slope correction is provided on the instrument, this is used to get the proper pH reading according to the values in Table I. (In this case the temperature compensation is set at the measuring temperature and not shifted after calibration of the instrument). If no slope correction is available, the temperature compensator can be used because it works similarly. As a final check Bates 1:3.5 (1.3.2), buffer can be recommended.

Instead of Bates 0.01 M borax buffer (1.3.4), Palitzsch borax buffer (1.3.3) can be used. The advantage of this is that the pH value at room temperature is close to the maximum pH value of sea water.

Rinse the measuring cell with sea water several times to remove all traces of buffer solutions. Keep the cell filled up with surface sea water.

1.6.2. Measurement

The sample is brought through the heat exchanger into the cell with the aid of a siphon, a rubber tube connected to the heat exchanger. Rinse the cell three to four times with the sample. Fill the cell completely and let the sample reach constant temperature. (A small thermometer should extend into the cell as in Figure 1). For high precision and accuracy, the temperature difference between calibration and measurement should not exceed 0.5°C. Make a note of the pH and the measurement temperature, and remove the sample.

Experience has shown that no problem is encountered when going from a sample with a high pH to a sample with a low pH (as is the case at the halocline). However, after measurement of a sample with a low pH, additional rinsing may be required.

1.7. CALCULATION AND EXPRESSION OF RESULTS

The measured pH value is not the true pH value (in situ value) of the sampled water. The measured -or apparent- value has to be corrected for the temperature difference between measurement conditions and those at the sample depth in the water. If the sample was obtained from about 500 m or deeper, a further correction for the difference in hydrostatic pressure must be applied.

The formula for calculation of the $pH_{\underline{in\ situ}}$ is then :

$$pH_{\underline{in\ situ}} = pH_{measured} + 0.0118(t_2 - t_1)$$

where : t_1 = temperature in situ

t_2 = measurement temperature

This formula is valid for work in areas with water depths not exceeding 500-1 000 m (depending on the accuracy desired). The complete correction formula is :

$$p^H_{\underline{in\ situ}} = pH_{measured} + 0.0118(t_2 - t_1) - B.. Z$$

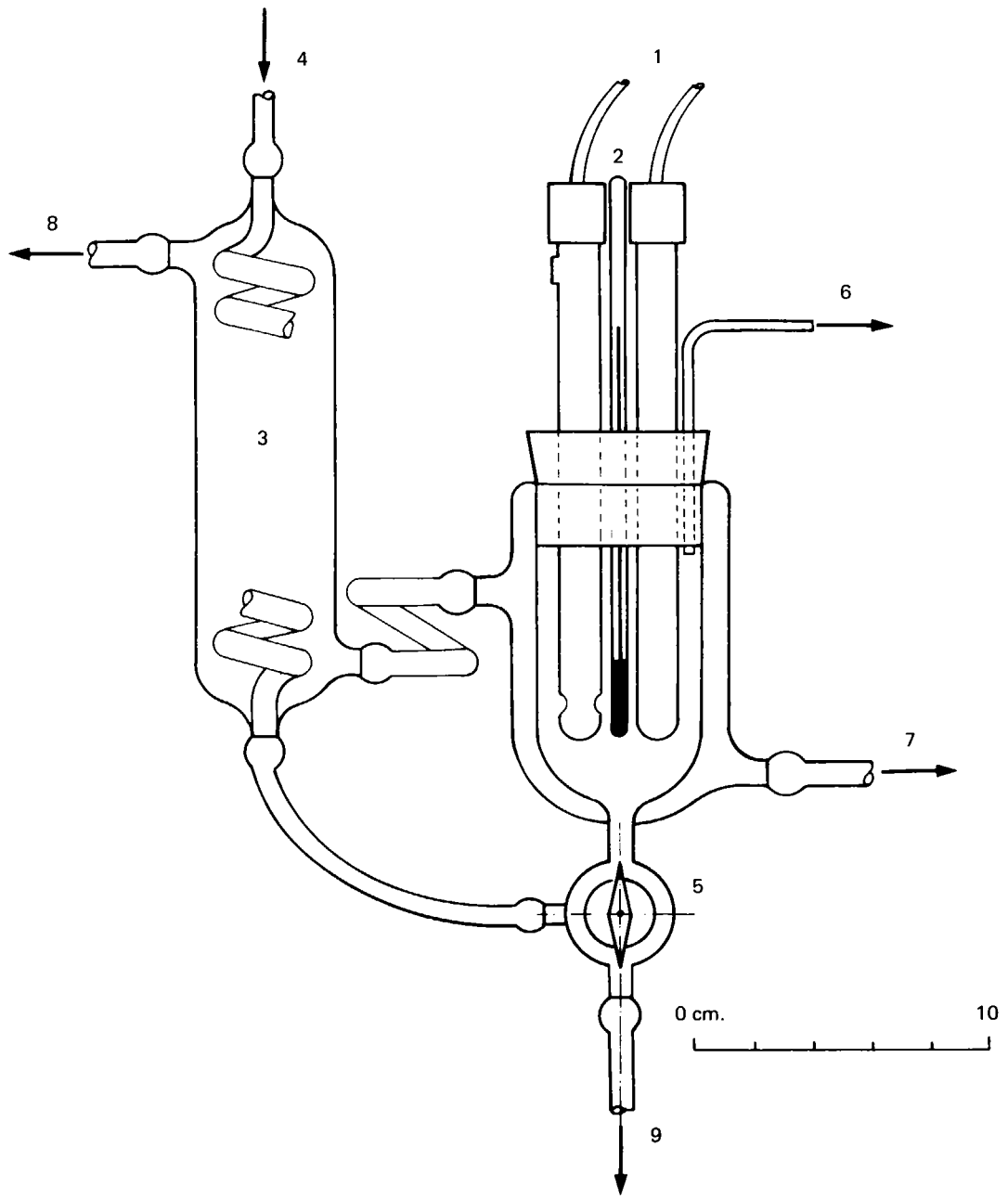

Figure 1. Thermostated pH-cell and heat-exchanger

(1) electrodes
(2) thermometer
(3) heat exchanger (about 3mm bore)
(4) sample inlet
(5) teflon valve (3 way-key)
(6) sample overflow
(7) thermostated water inlet
(8) thermostated water outlet
(9) to waste

The last term B. Z is a correction factor for the difference in hydrostatic pressure between the sampling depth and the laboratory at the sea surface. Z is the sampling depth in metres and the 8 -value is obtained from Table II.

Table II

$pH_{measured}$	B	$pH_{measured}$	B
7.5	35.10^{-6}	8.0	22.10^{-6}
7.6	31.10^{-6}	8.1	21.10^{-6}
7.7	28.10^{-6}	8.2	20.10^{-6}
7.8	25.10^{-6}	8.3	20.10^{-6}

1.8. ESTIMATION OF PRECISION AND ACCURACY

It is obvious that precision and accuracy are dependent not only on the handling of the samples and the quality of buffer solutions. The pH meter and its electrodes are important factors. It is absolutely essential that these are of good quality and that they are maintained and used according to instructions given by the manufacturer. With good quality equipment the reproducibility can be as good as ±0.002 pH units and standard deviation between different instruments may be as low as ±0.005 pH units (Gieskes 1969). For normal routine work a precision and accuracy around 0.02 pH units may be expected with proper equipment.

2. · DETERMINATION OF DISSOLVED OXYGEN AND OXYGEN SATURATION

2.1. SCOPE AND FIELD OF APPLICATION

The method described is the common Winkler method, modified for sea water by Carritt and Carpenter.

2.2. PRINCIPLE

The method is based on the following principle : Manganous ions are precipitated in alkaline medium, forming manganous hydroxide. This is oxidized by the dissolved oxygen : $2Mn(OH)_2 + O_2 \longrightarrow 2MnO(OH)_2$. The manganese hydroxide is dissolved with acid and reduced by iodide ions : $MnO(OH)_2 + 4H_3O^+ + 3I^- \longrightarrow Mn^{2+} + I_3^- + 7H_2O$. The I_3^- ions produced are determined by titration with thiosulphate ions :

$$2S_2O_3^{2-} + I_3^- \longrightarrow S_4O_6^{2-} + 3I^-$$

2.3. REAGENTS

2.3.1. Manganous sulphate, $MnSO_4.H_2O$. 500 g is dissolved in distilled water and diluted to one litre with distilled water. Store in a plastic bottle. ($MnSO_4.4H_2O$, 670 g, or $MnCL_2$ in equivalent amount may be used.).

2.3.2. Sodium iodide, NaI. 600 g is dissolved by warming in the smallest possible volume of distilled water.

2.3.3. Sodium hydroxide, NaOH. 320 g is dissolved in the smallest possible volume of distilled water. Great care should be exercised in doing this as the hydroxide produces considerable heat when it dissolves. Add the hydroxide pellets, portion by portion, into the water, stirring continuously.

2.3.4. Sodium azide, NaN_3. 10 g is dissolved in the smallest possible volume of distilled water. (This reagent is used in order to destroy nitrite in stagnant waters).

2.3.5. Alkaline iodide reagent

These three sodium reagents (2.3.2. - 2.3.4) are mixed together and diluted to one litre with distilled water. Store in a plastic bottle. These reagents are very concentrated and generally will not become clear until they are diluted to the full volume. If crystals are formed, the clear liquid is decanted into another vessel.

2.3.6. Sulphuric acid, H_2SO_4 (1 + 1) solution about 9 moles/liter. Concentrated acids mixed with the same volume of distilled water. The acid is slowly poured into the water under constant mixing and cooling.

The reason for using sulphuric acid instead of the commonly recommended concentrated phosphoric acid is because during field work there is a great risk of contaminating samples for the phosphate analysis when phosphoric acid is used.

2.3.7. Sodium thiosulphate, $Na_2S_2O_3.5H_2O$, 0.02 moles/liter. 25 g is dissolved in distilled water. 50 ml isobutylalcohol is added and the solution is diluted to 5 litres.

2.3.8. Starch solution. 1 g soluble starch is dissolved in 100 ml distilled water by warming until the solution becomes clear. The solution may be preserved by the addition of 0.1g salicylic acid ($HOCOC_6H_4COOH$).

2.3.9. Thyodene indicator (Campbell Williams & Co, 14 St. Neots Road, Abbotsley, Hunts, England). This indicator is better than the starch solution, because the sample remains clear during the whole titration and the end point is easily detected. When using the starch solution the sample mostly becomes turbid. This indicator also can be used as a solution, prepared according to the instructions on the container

2.4. APPARATUS AND EQUIPMENT

2.4.1. Calibrated burette, for instance a 12 ml Derona burette with an automatic zero adjuster, or a motor-driven piston burette.

2.4.2. Automatic syringe pipettes, piston pipette or similar. As the reagents have to be added swiftly, common types of pipettes with or without suction devices should be avoided.

2.4.3. Winkler bottles, volume about 100-130 ml, carefully calibrated. These may be substituted by common 50 ml glass bottles with ground stoppers, if these stoppers are long enough to extend through the bottle neck and into the body of the bottle, thus eliminating the risk of trapping air bubbles in the bottle.

2.5. SAMPLING AND PRE-TREATMENT

The sample should if possible be drawn from water samplers of plastic or plastic-coated metal. This should be done as soon as possible after the hydrographic cast is brought on board (sampling). The oxygen sample should be the first sample drawn from the water sampler. For this purpose a rubber tubing is fitted to the outlet of the sampler. A glass tube about 10 cm long is connected to the tubing.The glass tube is inserted to the bottom of the Winkler bottle and at least one volume of sample water is allowed to flow through the bottle before filling with the sample. The water should flow at a moderate speed, avoiding air bubbles. (Air bubbles trapped in the rubber tubing are removed by squeezing). The tube is then carefully removed from the bottle which is filled to overflowing. With automatic syringe pipettes the reagents manganous sulphate (2.3.1) and alkaline-iodide (2.3.5) are added simultaneously. The syringes should have long plastic tips, extending deep into the bottle. 1 ml of each reagent is added to the sample volume 100-130 ml. (To the smaller bottle 0.5 ml of each is added). The winkler bottle is carefully closed with the stopper avoiding the trapping of air bubbles, and the bottle is vigorously shaken with a snapping motion of the wrist.

2.6. ANALYTICAL PROCEDURE

2.6.1. Analysis

When the precipitate has settled to at least about half way down the bottle, 1 ml sulphuric acid (2.3.6) is added with an automatic syringe. The bottle is closed again, avoiding air bubbles, and shaken until the precipitate is dissolved. Now the bottle should be stored in a dark place and titrated after a maximum time of one hour. If the analysis has to be delayed, the bottles should be stored with the oxygen precipitated. The acid should by no means be added if the bottles have to be stored for a long time. If dissolved, the iodine will evaporate, passing the glass stopper. If the samples have to be stored for several days, or until the ship arrives at the home port, the Winkler bottles should be stored in glass or plastic jars filled with sample water. The bottles should be completely submerged, and the jars closed (it is a good idea to store the bottles upside-down in the jars).

The dissolved sample is quantitatively transferred into a wide-necked 300 ml Erlenmeyer flask and titrated with standardized thiosulphate solution (2.3.7.), until a very pale straw colour remains. The titration must be carried out at an even speed. Three drops of starch solution (2.3.8.) - freshly prepared - (or Thyodene indicator (2.3.9) are added and the titration is continued until the solution is colourless. If the thiosulphate is added too slowly at the equivalent point, there will always be a slight colour in the sample due to oxidation.

2.6.2. Standardization of the thiosulphate solution.

The exact titre of this solution can be determined by titration agains iodate ions in acid solution :

$$IO_3^- + 5I^- + 6H_3O^+ \longrightarrow 2I_3^- + 9H_2O$$

$$6S_2O_3^{2-} + 2I_3^- \longrightarrow 3S_4O_6^{2-} + 6I^-$$

Thus 1 mole iodate is equivalent to 6 moles thiosulphate.

Analytical grade potassium iodate (KIO_3, molecular weight 214.04) , is dried at 180°C to constant weight. Exactly 1.1891 g KIO_3 is dissolved in distilled water and diluted to 1 000 ml. 5 ml of this 0.00555 moles/litre solution is added to a glass-stoppered Erlenmeyer flask (250 ml) containing 40 ml distilled water, 1 ml of the sulphuric acid (2.3.6) solution, 1 ml manganous and 1 ml sodium reagent. After five minutes the solution is titrated with the thiosulphate solution as described for the samples. The reagent blank is obtained by omitting the addition of iodate and then repeating the procedure. The addition of starch should give no, or only a faint, blue colour.

M = the concentration of the thiosulphate solution, moles/litre

a = ml KIO_3 solution

y = the concentration of the KIO_3 solution, moles/litre

b = ml thiosulphate solution consumed, corrected for blank

$$M = \frac{a.y}{b.6}$$

2.7. Calculation and Reporting of Results

To calculate the concentration of dissolved oxygen, use the formula below.

a ◘ volume of the thiosulphate consumed in ml

M ◘ concentration of the thiosulphate solution, moles/litre

V = volume of the Winkler bottle in ml

$$\text{then } O_2 \text{ ml/l} = \frac{a.M.22393}{4\,(V - 2)}$$

for the calculations it may be practical to calculate the term $\frac{M.22393}{4}$ as a constant factor (which is close to 112 for a solution that contains 0,02 moles/litre

If the small sample bottle (50-60ml)is used the volume of each added reagent is 0.5 ml. In this case the (V-2) in the formula is replaced by (V-1).

At any given temperature and salinity a water is saturated with oxygen when it has dissolved a certain, specified quantity. This quantity is referred to as the saturation concentration or 100 percent saturation. Thus, any oxygen concentration found by analysis can be expressed as a percentage of the saturation value instead of ml/l. The saturation concentration of the water is obtained from a standard table when the temperature and salinity of the sample are known.

2.8. Estimation of precision and Accuracy

The main source for systematic errors in analysis of dissolved oxygen is in the sampling. If this is left out of the discussion the individual reproducibility can be $\pm$ 0.02 ml/litre for oxygen contents below 2 ml/litre and $\pm$0.04 ml/litre at higher concentrations. Intercalibration exercises between five different laboratories, each using its own modification of the method, demonstrated a standard deviation of $\pm$ 0.03 ml/litre (Grasshoff 1976).

2.9. Remarks

The concentration of sodium iodide has to be at least 600 g/l in the reagent, according to Carritt and Carpenter. Potassium iodide may as well be used, but may be more difficult to dissolve in the concentration used.

Potassium hydrogen iodate, $KH(IO_3)_2$, may as well be used in equivalent concentration as standard titre substance.

It is very important that the entire sample volume is titrated. Due to the losses of iodine, it is impossible to take out a representative aliquot for the titration as described in some procedures.

3. DETERMINATION OF HYDROGEN SULPHIDE

3.1. SCOPE AND FIELD OF APPLICATION

Sulphide is found in anoxic waters, where it is formed by microbiological reduction of sulphate ions. The method described here, depending on the formation of methylene blue from dimethyl p-phenylene diamine, is a simple application of a well established colorimetric method for sulphide. The method is as nearly sensitive as is theoretically possible, and is for direct determination applicable to concentrations up to about 100 moles/liter. At higher concentrations of hydrogen sulphide the sample has to be diluted with oxygen-free distilled water prior to the analysis.

3.2. PRINCIPLE

The method is based on the following principle : the acidified sample is allowed to react with dimethyl p-phenylene diamine, with ferric ions as catalyst. A complex oxidation and substitution takes place, resulting in the quantitative incorporation of any sulphide-sulphur present into a heterocyclic dye called methylene blue. The absorption of light by the sample is measured before or after dilution in 1,5 or 10 cm cell.

3.3. REAGENTS

3.3.1. N,N-dimethyl p-phenylene diamine dihydrochloride, p.a., $(CH_3)_2NC_6H_4.NH_2 2HCl$ (1,4). 1 g is dissolved in 500ml 6M hydrochloric. This acid may be prepared by diluting concentrated HCl (37 percent, density 1.19) with an equal amount of distilled water.

3.3.2. Ferric chloride, p.a., $FeCl_3$. 8 g is dissolved in hydrochloric acid (6 moles/liter) (prepared as above) to 500 ml.

3.3.3. Oxygen-free distilled water

A suitable volume of distilled water is boiled for 30 minutes Nitrogen gas may be bubbled through the water during the boiling. As the water cools down to room temperature, nitrogen gas must be continually bubbled through the water.This is continued as long as water is needed for the calibration.

3.3.4. Sulphide stock solution.

(About 3.12 µmoles/ml S^{2-}). . A solution of sodium sulphide is prepared with oxygen-free distilled water. Crystals of $Na_2S.9H_2O$ p.a. are quickly washed with distilled water by squirting from a washing bottle. The crystals are dried with filter paper and placed in a pre-weighed, glass stoppered, weighing glass. 0.750 g is weighed on an analytical balance and dissolved in oxygen-free distilled water (added with aid of a siphon) to 1 000 ml in a volumetric flask.

3.3.5. Sulphide working solution (about 0,156 µmoles/ml S^{2-})

Siphon a suitable volume of oxygen-free distilled water into a 500 ml volumetric flask. Into this is pipetted 25 ml of the sulphide stock solution and oxygen-free distilled water is added to the mark.For the greatest accuracy, the concentration is determined by titration as described below. The solution is stable for only 15-30 minutes.

3.3.6. Sodium thiosulphate solution, 0.02M

The same solution as for the determination of dissolved oxygen. 5 g $Na_2S_2O_3.5H_2O$ p.a. is dissolved in 1 000 ml distilled water. Add 5 ml isobutyl alcohol before diluting to the full volume of the volumetric flask.

3.3.7. Potassium iodate solution (0.00555 moles/litre)

Weigh out accurately 1.1891 g KIO_3 analytical grade (molecular weight 214.04), dried at 180°C for one hour. Dissolve in distilled water and dilute to 1 000 ml. This solution is the same as used for the oxygen determinations and it is very stable.

3.3.8. Sulphuric acid (1 + 1) solution

The same solution as for the dissolved oxygen determination. One volume of acid is carefully mixed with one volume of distilled water under constant cooling and mixing.

3.3.9. Starch solution

1 g per 100ml distilled water or Thyodene indicator as for the dissolved oxygen determination.

3.3.10. Potassium iodide

KI, p.a. crystals.

3.4. APPARATUS AND EQUIPMENT

3.4.1. Winkler bottles, volume calibrated or volumetric flasks of 100ml volume.

3.4.2. Spectrophotometer of filterphotometer with filter at or close to 670 nm. 1cm and 5cm or 10cm cells.

3.4.3. Automatic syringe pipettes.

3.5. SAMPLING

The samples must be obtained with plastic water samplers. If these are not available, metallic ones may be used provided that the inner surfaces are plastic-coated.

3.6. ANALYTICAL PROCEDURE

3.6.1. Calibration

3.6.1.1. Standardization of the thiosulphate solution.

Proceed as described in section 2.6.2. above.

3.6.1.2. Standardization of the sulphide working solution.

This is carried out within minutes after preparation of the working solution (3.3.5) and at the same time as the preparation of the photometric standard samples (3.6.1.3).

Take six Erlenmeyer flasks with ground glass stoppers and add to each about 10 ml distilled water and 1-2 g potassium iodide (3.3.10). Pipette into each flask 10.00 ml iodate solution (3.3.7). Add 1.0 ml sulphuric acid (3.3.8) into each flask. Into three of the flasks pipette 50 ml of the sulphide working solution and to the other three add about 50 ml distilled water. Set all flasks aside in a cool place whilst the colorimetric standardization is begun then titrate the contents of the flasks with thiosulphate using the starch indicator (3.3.9).

A = mean of the titrations of three solutions with no added sulphide, in ml.

B = mean of the titrations of the three solutions containing sulphide, in ml.

M = concentration of the thiosulphate solution, moles/litre

(the individual titrations constituting a triplicate should agree to within 0.05 ml).

$$\frac{221.40 \times M \times (A-B)}{22.14 \times 0.02 \times 50} = 10 \times M \times (A-B)$$

3.6.1.3. Photometric standard samples

From the working solution (3.3.5) the following standard series is prepared.

Too 100 ml volumetric flasks or volume calibrated Winkler bottles the following volumes of working solution are added by means of a pipette or burette (Table III) :

Table III

20 ml =	31.2 µmoles/liter	S^{2-}
16 ml =	25.0	"	"
12 ml =	18.7	"	"
8 ml =	12.5	"	"
4 ml =	6.3	"	"
0 ml =	0.0	"	"

These concentrations correspond to a sulphide working solution with precisely 0,156 µmoles/ml. Thus they have to be corrected according to the true value found by titration.

If Winkler bottles are used the concentration values given above have to be recalculated accordingly for the calibration graph described below.

With the aid of a siphon the bottles are filled up with oxygen-free distilled water to the 100 ml mark, or in case of calibrated Winkler bottles, to the neck. In the latter case a correction for the volume has to be made for each bottle.

As soon as a bottle is filled, 1 ml of each reagent is added with an automatic syringe pipette and the contents of the bottle are mixed well. After 60 minutes, the samples are measured against the blank, at 670 nm in 1 cm and/or 5 cm cells, as suitable. From the results a calibration graph for each of the cell lengths is prepared on millimeter paper. The graph should be a straight line and go through origin.

If higher concentrations are analysed, the graph will start deviating from the straight line at 40-50 µmoles/liter. The exact point for the start of the deviation depends on the quality of the amine solution. However, if this is taken into account when calculating the analytical results, the graph - and thus the working range of the method - can be extended up to about 100 µmoles/liter.

3.6.2. Analysis

An ordinary Winkler bottle is filled with the sample in exactly the same manner as described for the dissolved oxygen determination (2.5.). The reagents diamine and ferric (3.3.1. and 3.3.2) chloride are immediately added with pipettes (preferably automatic syringe pipettes) to the sample. Let the pipette tips extend deep into the sample bottle. The stopper is now inserted avoiding air bubbles. The blue colour starts developing in a few minutes and the sample is ready for measurement in a photometer after 30 minutes. However, if the sample contains high concentrations of hydrogen sulphide, one hour must be allowed for full colour development. The colour intensity may be regarded as constant for at least 24 hours.

The colour intensity of the sample is measured against distilled water (or, if necessary, compensated for by reagent blanks, as described in the note below) at 670 nm using 1 or 5 cm cells as required.

If the sample contains higher concentrations of hydrogen sulphide than 100 µmoles/liter, it has to be diluted prior to the analysis. This is done by pipetting a suitable volume of the sample into a measuring flask or Winkler bottle containing some oxygen-free distilled water. The pipette tip should extend below the surface of the water. Then more of the diluting water is added by means of a siphon up to the calibrated volume of the flask or bottle. The reagents are added and the sample is then thoroughly mixed. Account has to be taken of the dilution factor when calculating the result of the analysis.

According to Strickland and Parsons (1968) it may be necessary to compensate the absorption value of each sample for the absorption value of a reagent blank. This latter value is obtained by adding reagents to filtered surface water and measuring this against the same filtered water containing no reagents. The absorption value should not exceed 0.5 in a 10 cm cell and should preferably be less than 0.25. However, the blank values are usually negligible, even if a discoloured amine solution is used.

Note : It is difficult to prepare a standard solution of sulphide with a high degree of accuracy. Therefore a systematic error may be included in the analysis. Using the method described, this error will be below 2%.

3.7. CALCULATION AND REPORTING OF RESULTS

For the routine analysis a calibration factor is calculated. Select from the calibration graph a corrected absorbance value (e.g. 0,500) and find its corresponding concentration figure (e.g. 20,2 µmoles/litre) and calculate the factor F for the cell length that was used for the measurement :

$$F = \frac{20,2}{0,500} \qquad \text{which gives } F = 40,4$$

Then the concentration of the sample, in µmoles/litre, is obtained by multiplying its abosrbance with the factor F.

3.8. ESTIMATION OF PRECISION AND ACCURACY

At a concentration level of 25 µmoles/liter, the precision can be expressed as $\pm 0.3\sqrt{\text{number of replicate samples}}$.

3.9. REMARK

It seems impossible to obtain a diamine that is not more or less discoloured. However, this does not seem to affect the results. Occasional tests using a one-year old brownish diamine solution produced results which differed only slightly from the results obtained with a freshly prepared solution.

4. DETERMINATION OF DISSOLVED INORGANIC PHOSPHATE

4.1. SCOPE AND FIELD OF APPLICATION

This is a modification of the well known single solution method by Murphy and Riley (1962). The use of two different solutions makes the reagents more stable.

No error from salinity will occur in the determinations as this deviation is less than 1 percent. No interferences from copper, iron and silicate occur at concentrations several times higher than those in sea water. Arsenate reacts giving a similar colour, but is regarded as existing in minimal amounts in sea water. Recent results by Johnson (1971) indicate, however, that the concentration of arsenate is higher than was believed. In periods of high primary production - and hence low concentrations of phosphate - arsenate and phosphate levels are said to be equal and sometimes arsenate may be dominating?

The determination, on an unfiltered sample, gives the concentration of dissolved inorganic phosphate ions in true solution and probably also includes a small fraction of these ions which are absorbed into particles and are subsequently dissolved by the acid in the mixed reagent. The latter fraction is not included if the analysis is performed on a filtered sample.

4.2. PRINCIPLE

The phosphate in water is allowed to react with ammonium molybdate, forming a complex heteropoly acid. This acid is reduced by ascorbic acid to a blue-coloured complex, the light absorption of which is measured in a photometer. Normally, this reduction is slow, but by adding a catalyst - in this case antimonyl tartrate - the reduction proceeds swiftly.

This is a modification of the well known single solution method by Murphy and Riley (1962). The use of two different solutions makes the reagents more stable. According to experience from intercalibrations it is necessary to measure the samples of sea water against water from the same depths in order to compensate for the varying turbidity. This turbidity is somewhat by the acid in the molybdate reagent. The turbidity blank should for this reason be of the same acidity as the sample. Therefore it is convenient to use two reagent solutions - one containing all components except the ascorbic acid and a second which is the ascorbic acid solution.

4.3. REAGENTS

4.3.1. Sulphuric acid, about 4,75 moles/litre

253 ml of concentrated sulphuric acid (density 1.84) is, under cooling and continuous mixing, carefully poured into a suitable volume (600 ml) of distilled water. After cooling the volume is adjusted to 1 000 ml with distilled water.

4.3.2. Ammonium heptamolybdate solution, 0.073 moles/litre

Dissolve 9.0 g of the salt $(NH_4)_6Mo_7O_{24}.4H_2O$ in distilled water and dilute to 100 ml. A new solution should be made if any precipitation occurs.

4.3.3. Potassium antimonyltartrate solution, 0.1 moles/litre

Dissolve 3.25 g of the salt $K(SbO)C_4H_4O_6$ in distilled water and dilute to 100 ml. The solution should be renewed if a precipitation occurs.

4.3.4. Acid - molybdate reagent

200 ml of the sulphuric acid (4.3.1) is mixed under continuous stirring with 45 ml of the molybdate solution (4.3.2). Finally, 5 ml of the tartrate solution (4.3.3) is added. If stored cold in a dark bottle the reagent is stable for several months.

4.3.5. Ascorbic acid solution, 0.4 moles/litre

7.0 g of ascorbic acid, $C_6H_8O_6$, is dissolved in distilled water and diluted to 100 ml. If stored cold in a dark bottle, the reagent is stable for some weeks.

For preservation, 0.05 g of EDTA (ethylene diamine tetraacetic acid) and 1 ml formic acid, HCOOH, may be added.

4.3.6. Phosphate stock solution

Potassium dihydrogen phosphate is dried over concentrated sulphuric acid or at 100°C. 0.3403 g of KH_2PO_4 p.a. is dissolved in distilled water and diluted to 1 000 ml in a volumetric flask. Some drops of chloroform are added for preservation. This solution contains 2.5 µmoles/ml PO_4-P. The solution is stable for many months if kept in a refrigerator.

4.3.7. Phosphate working solution

10.0 ml of the stock solution is diluted with distilled water to 1 000 ml in a volumetric flask. 1 ml contains 0.025 µmole PO_4-P. This solution should not be stored for future use.

4.4. APPARATUS AND EQUIPMENT

4.4.1. Spectrophotometer or filter photometer with filter at or close to 882 nm. Cells of 5 cm and/or 10 cm length.

4.4.2. Glass stoppered test tubes, 30-50 ml volume, or other suitable containers.

4.4.3. Kipp pipette (Figure 2) of 35 ml or 25 ml volume for dispensing of samples. This handy pipette is marketed under a variety of names e.g. pour-out dispenser, TIPET, automatic transfer pipette, repeater pipette.

- Automatic syringe pipettes of 1 ml or 2 ml volume for reagent additions.

These last two types of pipettes are not necessary, but they make the routine handling of a great number of samples much easier.

4.5. SAMPLING AND PRETREATMENT

The analysis should be commenced as soon as possible after sampling, preferably within half an hour, and definitely before two hours. Samples should be kept in a cool dark place and not warmed to room temperature until the time of analysis. If the analysis has to be delayed for more than about one hour, store the samples in the refrigerator. On short trips it may be possible to add the reagents to the samples and perform the measurements ashore. There is conflicting evidence concerning the effect of different preservation methods and the fate of phosphate molecules in glass and plastic bottles. No entirely satisfactory preservation method is known (for samples collected for nutrient analysis). However, quick freezing in a dry ice/acetone bath has often been used when there is no alternative to storing samples.

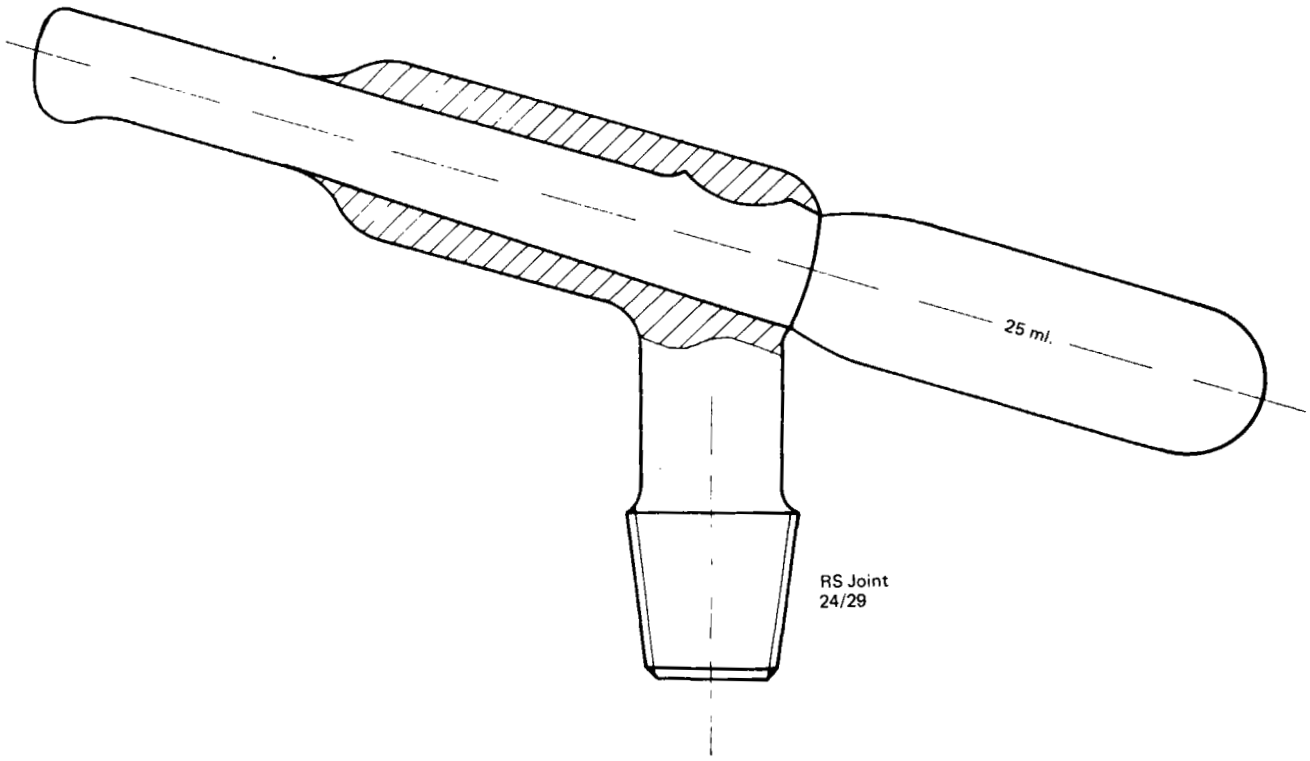

Figure 2 Kipp Pipette

4.6. ANALYTICAL PROCEDURES

4.6.1. Calibration

A series of working standards are prepared from the phosphate working solution by dilution with distilled water. If 50 ml volumetric flasks are used, Table IV can be used.

Table IV

0.00	ml	of	working	solution	(4.3.7)/50	ml	= 0.00	µmoles/litre PO_4-P
0.40	"	"	"	"	"	"	= 0.20	"
0.80	"	"	"	"	"	"	= 0.40	"
1.20	"	"	"	"	"	"	= 0.60	"
1.60	"	"	"	"	"	"	= 0.80	"
2.00	"	"	"	"	"	"	= 1.00	"
4.00	"	"	"	"	"	"	= 2.00	"
8.00	"	"	"	"	"	"	= 4.00	"

As described in (4.6.2) below, 35 ml (or 25 ml) portions of these solutions are transferred to test tubes and reagents are added. For the calibration no turbidity blanks (samples with only the acid - molybdate reagent) are needed. A reagent blank is prepared from the same volumes of distilled water and reagents.

After measurement the absorbances - corrected for the reagent blank - are plotted versus concentration on millimetre paper. This calibration should give a straight line up to at least 10 µmoles/litre. of PO_4-P.

4.6.2. Analysis

All glassware to be used must be absolutely clean and should not be used for other purposes. Powdered synthetic detergents contain phosphate (large amounts) in most cases, and such products should not be used. A solution of distilled water containing the detergent used for cleaning must be tested for phosphate content. This is the only safe way to avoid unnecessary complications.

Sea water for the different analyses is preferably poured directly from the water samplers into stoppered glass bottles of suitable volume. For routine analysis of a great number of samples it is very convenient to measure the different subsamples with an automatic dispensing pipette (Kipp pipette) with a ground glass joint fitting to the glass sample bottle.

From each sample two 35-ml portions are transferred to test tubes. One of the portions is regarded as the sample and the other is the turbidity blank. To each of the portions 1 ml of the acid - molybdate solution (4.3.4) is added and to the sample also 1 ml of the ascorbic acid solution (4.3.5). Mix well between the additions. In case 25 ml of sample is analyzed use 0.7ml portions of the reagents.

After five minutes each sample is measured against its turbidity blank in the photometer at 882 nm. If this wave-length is not provided, it is possible to measure at a shorter wave-length but this results in decreased sensitivity.

The colour of the samples is stable for at least 24 hours. However, the reduction of the arsenate molybdate complex is complete after one hour (see also paragraph 4.6.3.2.).

NOTE : The use of a turbidity blank may be omitted in case of high concentrations of phosphate and - at the same time - insignificant turbidity. However, to test the significance of the turbidity, it is recommended to analyse at least some samples with and without a turbidity blank.

If 5-cm cells are used instead of 10 cm, the sample volumes may be reduced to 25 ml and the reagent volumes to 0.7 ml.

4.6.3. Disturbances

4.6.3.1. Disturbance from silicate

It is a well known fact that the blue phosphomolybdenum complex formed with ascorbic acid is stable for hours. If great amounts of silicate are present, especially in some types of river and lake waters and stagnant sea water that contains hydrogen sulphide a blue silicomolybdate complex is gradually formed. With the stated concentrations of sulphuric acid and molybdate, and by measuring the absorption after five minutes, up to 10 mg of SiO_4-Si per litre can be present without disturbance.

4.6.3.2. Disturbance from arsenate

The arsenate ion gives with sulphuric acid, molybdate and ascorbic acid, a blue complex. However, the reaction is rather slow, being complete after one hour. By measuring the absorption after five minutes the disturbance from arsenate is avoided. It may be mentioned that with stannous chloride as the reductant the blue arsenate complex is formed immediately.

4.6.3.3. Influence from the concentration of sulphuric acid

To achieve a rapid colour development and to depress the interference from silicate, it is of fundamental importance that the final reaction takes place at a pH lower than 1 and that the ratio between sulphuric acid (in moles/liter) and molybate (in per cent) is kept between 2.0 - 2.5. With the reagents described the concentration of sulphuric acid in the sample is about 0.1 mole/liter. It has been shown experimentally that with the concentration of antimony ions used here (0.00068 per cent) the acid concentration may vary between 0.06 and 0.15 moles/liter whereas at 0.18 moles/liter the absorbance decreases by about 30 per cent.

4.7. CALCULATION AND REPORTING OF RESULTS

The corrected absorbance is used for evaluation of the result, using the calibration graph described in paragraph 4.6.1).

For routine analyses a calibration factor is calculated. Select from the graph an absorbance value (e.g. 0.170) and find its corresponding concentration figure (e.g. 1.65 umoles/litre) and calculate.

$$F_{5cm} = \frac{1.65}{0.170} \quad : \quad \text{which gives} \quad F_{5cm} = 9.71$$

Then the concentration of a sample, in moles/litre, is obtained by multiplying its absorbance with the factor F.

4.8. ESTIMATION OF PRECISION AND ACCURACY

From a nutrient intercalibration reported by Koroleff and Palmork (see Grasshoff 1976) accuracy was given as ± 15 per cent at low level (0.2 umole/litre), ±5 per cent at an intermediate level (0.9 umole:Liter) and ±2 per cent at a high level (2.8 umoles/litre).

5. DETERMINATION OF TOTAL PHOSPHORUS

A great number of sensitive analytical procedures for determination of total phosphorus have been published during recent years (see e.g. Koroleff 1976). All of these methods involve the use of peroxodisulfate as an oxidant to transform the various phosphorus fractions to dissolved orthophosphate.

However, in most marine chemistry programmes not only total phosphorus but also total nitrogen are determined. In 1977 Koroleff presented a practical method for the simultaneous determination of these parameters from the same sample and in 1981 Valderrama published a slightly modified variant of the same method. Therefore, no separate procedure for the determination of total phosphorus is presented here, but the analyst is guided to the technique described in section 10 "Simultaneous persulfate oxidation of phosphorus and nitrogen compounds in seawater".

6. DETERMINATION OF REACTIVE SILICATE

6.1 SCOPE AND FIELD OF APPLICATION

Depending on pH the yellow silicomolybdate complex that is formed in this analysis exists in two isomeric forms, which differ only in their hydration. The -silicomolybdic acid which is formed below a pH value of 2.5 is rather unstable, but has a higher molar absorbance than the -acid.

In early work silicate was determined by visual or photometric estimation of the -complex. The literature has been reviewed by Mullin and Riley (1955), and in 1968 a careful study of the optimum conditions for this technique was performed by a research group at the Sagami Chemical Center in Japan. In their modified method the colour of the complex is stable from 5 to 20 minutes at room temperature, in both distilled water and sea water; the salt effect is diminished to 3 percent.

Grasshoff (1984) has developed a method based on the formation of the -isomer at a pH of 3.7 to 4.0, and states that the colour in sea water is stable (after two hours) for days.

6.2 PRINCIPLE

The determination of dissolved silicon compounds in natural waters is based on the formation of a yellow silicomolybdic acid, when a more or less acidic sample is treated with a molybdate reagent. Only silicic acid and its dimer react with molybdate at any appreciable speed and the methods therefore give only the amount of "reactive" silicate, which is probably a reasonable measure of the silicate available to growing phytoplankton.

Since both of the yellow silicomolybdic acid isomers are rather weak in colour, several methods have been developed in which they are reduced to intensely coloured, blue complexes. A wide range of inorganic and organic reducing agents have been used. A mixture of metol and sulphite is commonly used. In the method described here ascorbic acid acts as the reductant.

The molar absorption of the coloured complex is about 22 200 litres/mole/cm. This implies that if the concentrations are given as µmoles/litre, the absorbance 1.000 is equal to 45 µmoles/litre Si with a 1 cm cell used for the measurement. With this cell length up to about 80 µmoles/litre of Si can be determined (absorbance about 1.7). With a 10 cm cell, 0.045 µmoles/litre of Si can be determined. As this corresponds to an absorbance of 0.010, it can be regarded as the limit for direct determination.

6.3 REAGENTS

All solutions should be prepared from reagent grade (pro analysi) chemicals, using good quality distilled water. The solutions should be stored in plastic bottles rather than glass bottles.

6.3.1 Ammonium heptamolybdate solution 0.16 mole/litre

Disssolve 49.5 g $(NH_4)_6Mo_7O_{24}.4H_2O$ and dilute to 250 ml. As the salt dissolves slowly, moderate heating may be applied.

6.3.2 Sulphuric acid solution about 3,7 moles/litre

198 ml H_2SO_4 (concentrated sp.gr. 1.82) is carefully poured, under constant cooling and mixing, into distilled water and finally diluted to 1000 ml.

6.3.3 Molybdate reagent

Add a measured volume of the molybdate solution (6.3.1) to an equal volume of the sulphuric acid solution (6.3.2) and mix.

6.3.4 Oxalic acid solution 0.7 mole/litre

Dissolve 63 g $C_2O_4H_2$ and dilute to 1000 ml.

6.3.5 Ascorbic acid solution 0.1 mole/litre

Dissolve 4.4 g $C_6H_8O_6$ and dilute to 250 ml. Stored cold in a brown bottle, the reagent is stable for weeks.

6.3.6 Silicate stock solution

Sodium silicofluoride, Na_2SiF_6 p.a. is dried over concentrated sulphuric acid to constant weight. Weigh in 0.4701 g of the salt and dissolve in distilled water in a plastic beaker. Transfer to a 500-ml volumetric flask and dilute to the specified volume with distilled water.

Finally, transfer to a plastic bottle. 1 ml contains 5.0 umoles Si. The solution is stable for several months.

6.3.7 Silicate working solutions

50 ml of the stock solution (6.3.6) is diluted with distilled water or synthetic sea water of appropriate salinity (see below) to a final volume of 500 ml in a volumetric flask made of plastic materials. 1 ml contains 0.50 µmole/litre Si. This solution should be used at once.

6.3.8 Synthetic sea water

25 g sodium chloride, NaCl p.a., and 8 g magnesium sulphate heptahydrate, $MgSO_4.7H_2O$ p.a. are dissolved in distilled water for every litre of solution. This water must be stored in a plastic bottle. For analytical purposes it is equivalent to a salinity of 28.10^{-3}. For calibration work it may be diluted to the desired salinity. the silicate concentration should be below 1-2 umoles Si/litre.

6.4 APPARATUS AND EQUIPMENT

6.4.1 Test tubes. Stoppered tubes of glass may be used but plastic ones are preferred (inexpensive tubes made from polystyrene are commercially available). Small plastic bottles (25-50 ml) may also be used. If glass tubes are used they must initially be carried through the analytical procedure at least five times, otherwise they will yield too low results.

6.4.2 Automatic syringe pipettes (3 pcs.), capacity 2 ml.

6.4.3 Spectrophotometer, or filter photometer with the filter at or close to 810 nm. If this is not provided, use a filter having a maximum transmission above 700 nm; in this case the sensitivity is reduced and a slight deviation from the Lambert-Beer's law may occur. Cells of 1,5 and 10 cm length as required.

6.5 SAMPLING AND PRETREATMENT

Samples of sea water for silicate determination should not be stored in glass bottles for more than a short while prior to analysis and it is best, therefore, to transfer samples directly into containers of polyethylene or waxed glass. To minimize the effects of diatom multiplication, store samples in the dark and for nc longer than a day prior to analysis. Conflicting opinions and evidence exist as to whether samples can be stored for long periods or not, and if stored, whether they should be deep frozen or not. If deep frozen, the silicate concentration can be expected to be acceptable only for samples containing less than 50 µmoles/litre. When stored frozen, silicate tends to polymerize. Therefore, samples must be allowed to stand for at least three hours at room temperature after thawing. Thus it is recommended to perform the

analysis as soon as possible after sampling. If the sampler have to be stored, the tolerable storage time ought to be investigated and the possible size of the error estimated.

6.6 ANALYTICAL PROCEDURES

6.6.1 Calibration

From the working solution (6.3.7) a series of working standards are prepared by diluting with distilled water or synthetic sea water (6.3.8) in volumetric flasks. Table V below may be used.

For routine analysis in sea water it is normally sufficient to prepare the calibration solutions in synthetic sea water of a salinity representing a mean value of the salinities in the working area. However, for the greatest accuracy, it should be more convenient to calibrate in distilled water and then correct for the salt error. This is of particular importance in areas where the silicate concentrations in surface water may become very low as a result of phytoplankton primary production.

TABLE V

Preparation of working standards for silicate

ml of working solution/100 ml (dilute with synthetic sea water or distilled water)	µmoles/litre Si
0.0	0.0
0.5	2.5
1.0	5.0
2.0	10.0
5.0	25.0
10.0	50.0
20.0	100.0
25.0	125.0
30.0	150.0

Some of the standards are chosen depending on the expected concentration range in the samples. Samples are pipetted from the working standards to the test tubes. The distilled water or synthetic sea water of the chosen salinity respectively, should be used as the blank. The reagent blank must be prepared together with every calibration.

The blank sample compensates for the silicate content in the reagents, as well as in the distilled water (or synthetic sea water) used for the dilution of the standards. However, the same blank value cannot be used for the analysis of the natural water samples. In this latter case a reagent blank has to be used. Preparation of reagent blank and analysis of samples is described in the section below.

The absorbance is plotted versus concentration on millimetre paper. The result should be a straight line over the entire range of concentration.

6.6.2 Analysis

To 35 ml of the sample in a plastic test tube 1.0 ml of the molybdate reagent (6.3.3) is added. After 10 to 20 minutes (for distilled water) or 5 to 10 minutes (for sea water), 1.0 ml of the oxalic acid solution (6.3.4) is added followed immediately by 1.0 ml of the reductant ascorbic acid (6.3.5). Swirl gently during the reagent additions. After at least 30 minutes the absorbance of the sample is measured against distilled water in a cell of suitable length at a wave-length of 810 nm. Correct the measured absorbancy of the sea water samples by substracting that of the reagent blank. The reference sample is used for correcting the absorbances of the calibration samples.

The reagent blank is prepared by carrying out the above procedure using 35 ml of distilled water. The absorbance is denoted A_1. Repeat the determination, but with only 0.5 ml of the molybdate reagent. In this case measure the absorbance after one hour (A_2).

Absorbance of reagent blank = $2(A_1 - A_2)$

The absorbance caused by the silicate content of the reagents only is thereby obtained. This reagent blank should be performed every time any of the reagent solutions are prepared. In addition, it is advisable to check the reagent blank from time to time.

For the most precise estimate of low amounts of silicate using a 10 cm cell, a reference absorbance should be measured for every sample to compensate for its natural turbidity. The reference solution is prepared by adding 3 ml of 0.25 mole/litre sulphuric acid solution (e.g., prepared by diluting 7 ml of the 3.7 moles/litre solution to 100 ml with distilled water) to 35 ml of sample. Thus the absorbance of the sample is:

$$A_{sample} = A_{measured} - A_{reference} - A_{reagent\ blank}$$

6.7 CALCULATIONS AND REPORTING OF RESULTS

For the routine analyses a calibration factor is calculated. Select from the graph (described in section 6.6.1) an absorption value, corrected as described in section 6.6.2 (e.g., 0.730) and find its corresponding concentration figure (e.g., 37,8 umoles/litre) and calculate:

$$F_{1\ cm} = \frac{37,8}{0,730} \qquad \text{which gives } F_{1\ cm} = 58,8$$

Then the concentration of a sample, in µmoles/litre, is obtained by multiplying its absorbance with the factor F. However, for the most accurate work in marine waters a correction must be applied for the salt error. It is known that the dissolved salts of sea water reduce the final colour intensity to some extent. It has been found that the variation of the salt error can be expressed as a linear function of the sea water salinity:

$$A_{\text{true, distilled water}} = A_{\text{observed}} \cdot (1 + 0.0045 \cdot \text{sample salinity})$$

Thus, if the calibration factor F determined for distilled water is F_{dist}:

$$F_{\text{sample}} = F_{\text{dist.}} \cdot (1 + 0.0045 \cdot \text{sample salinity})$$

Note that these figures cannot be guaranteed for general use. Because of the varying quality of reagents, etc., every laboratory should construct a correction formula of its own, by calibrating at several salinities and then calculating from the observed variations of the absorbances.

6.8 ESTIMATION OF PRECISION AND ACCURACY

From a nutrient intercalibration reported by Koroleff and Palmork (see Grasshoff 1976) accuracy was given at ± 4 per cent at a low level (4.5 µmoles/litre), ± 2,5 per cent at an intermediate level (45 µmoles/litre) and ± 6 per cent at a high level (150 µmoles/litre).

6.9 REMARKS

6.9.1 Effect of the oxalic acid. Oxalic acid is added for two reasons:

(a) To avoid the reduction of any excess molybdate reagent. The stability of the silicomolybdic acid in the presence of oxalic acid is limited, and the reductant is therefore added immediately after the acid;

(b) to eliminate the influence of any phosphate present. Trials have been made with up to 10 umoles/litre of P and no increase in colour intensity was observed.

6.9.2 Time for colour development and colour stability

For up to about 30 µmoles/litre of Si, the colour reaches its maximum after about 30 minutes and is then - provided any evaporation is prevented - stable for several days. However, for practical purposes it may be advisable to regard the coloured complex as being stable for several hours. For higher concentrations, up to an absorbance of 1.7 (1 cm cell), the intensity increases extremely slowly after 30 minutes, only about 0.1 percent per hour.

7. DIRECT DETERMINATION OF AMMONIA WITH THE INDOPHENOL BLUE METHOD

7.1 SCOPE AND FIELD OF APPLICATION

This method, which is specific for ammonia, is applicable to all kinds of natural waters and is the recommended method for sea water. It is four times as fast as the Nessler reaction. "Ammonia" here refers to the sum of ammonia and ammonium ions, because the original proporitons of them in a water sample are pH dependent.

7.2 PRINCIPLE

In a weakly alkaline solution ammonia reacts with hypochlorite to form monochloramine, which in the presence of phenol, catalytic amounts of nitroprusside ions and an excess of hypochlorite yields indophenol blue. By increasing the concentrations of the reagents used in this method, labile organic nitrogen compounds may also react, while the reaction time is decreased. The reaction mechanism leading to the coloured complex is complicated and not yet fully understood. Probably, a quinone chlorimide is formed in an intermediate step. The intensity of the coloured complex is measured in a photometer.

7.3 REAGENTS

7.3.1 "Ammona-free" water

There is not a standard procedure for the preparation of water with a very low ammonia content, as the results obtained depend on the equipment and working facilities as well as the quality of the water used. Each laboratory has therefore to find the method that gives the most satisfactory result.

Deionized water may sometimes be used, with or without subsequent distillation, but it must be remembered that some ion exchange resins may leach out ammonia containing organic substances.

Ordinary distilled water may be used after a second distillation. In this second step, 2 ml concentrated sulphuric acid and 1 g potassium peroxodisulphate ($K_2S_2O_8$) are added per litre. Freshly distilled, such water should contain less than 0.3 µmole of nitrogen per litre.

Another alternative is to use surface water samples in an open sea area, preferably shortly after a plankton bloom. This water usually contains very little ammonia and it should be stored in tightly closed plastic containers with thick walls.

7.3.2 Reagent A, phenol - nitroprusside solution

Dissolve 35 grams of analytical grade phenol, C_6H_5OH, (a slightly pink quality may be accepted) and 400 mg of sodium nitroprusside dihydrate ($Na_2Fe(CN)_5NO.2H_2O$) in ammonia free distilled water and dilute to 1 litre. If the solution is stored in a dark bottle in a refrigerator it is

stable for several months. When the reagent becomes greenish it should be discarded. (It is usually a good idea to store also the pure phenol in the refrigerator).

7.3.3 Reagent B, alkaline solution with free chlorine, variant I

Prepare a solution of hypochlorite containing 0.14 per cent of free chlorine in 0.5 µmole/litre sodium hydroxide in the following way.

Commercial bleaching substance or concentrated sodium hypochlorite, NaClO, can be used as stock solution. the hypochlorite content is tested as follows. Dissolve 0.5 g potassium iodide (KI) in 50 ml distilled water and acidify with 5 ml sulphuric acid, 9 µmoles/litre (as used in the determination of oxygen) and titrate the liberated iodine with thiosulphate solution, 0.1 µmole/litre, using starch as indicator. 1 ml of the thiosulphate solution is equivalent to 3.54 mg active chlorine.

Prepare the reagent B by diluting the required volume with sodium hydroxide, 0.5 µmole/litre (20 g NaOH dissolved in 50 ml ammonia-free distilled water and then made up to 1 litre with the same water to yield a solution with 140 mg chlorine per litre. Store the reagent in the refrigerator in a dark bottle. It is stable for weeks.

7.3.4 Reagent B, variant II

The reagent can be conveniently prepared from a solid substance instead of the hypochlorite solution. Trion is the commercial name for dichloroisocyanuric acid (dichloro-s-triazine-2,4,6 (1H, 3H, 5H)-trione). Trion is available in at least technical grade. The product has to be tested for its actual content of available chlorine using the procedure described above. A typical figure may be that 1 g dissolved in the sodium hydroxide, 1 µmole/litre, produces a soluton containing 0.6 per cent chlorine. Thus, roughly 2 g Trion should be dissolved per litre of the sodium hydroxide solution (prepared as in variant I) to yield a solution with 140 mg chlorine per litre. The reagent should be stored in a dark bottle in the refrigerator and it is stable for weeks.

The advantage with variant is that the solution is easy to prepare without having to analyze the Trion at every occasion. However, the reactions in the analysis will proceed somewhat slower with this reagent.

7.3.5 Buffer solution

Dissolve 66.7 g trisodium citrate dihydrate, $Na_3C_6H_5O_7.2H_2O$, 34 g boric acid, H_3BO_3, and 19.4 g citric acid dihydrate, $C_6H_8O_7.H_2O$ in ammonia free distilled water and dilute to 1 litre.

7.3.6 Ammonia stock solution

As standard substance, either ammonium sulphate or ammonium chloride of p.a. quality can be used. Before weighing, the salts must be dried to constant weight at 100°C and then preferably stored in a dessicator.

Dissolve 0.1320 g of $(NH_4)_2SO_4$ or 0.0535 g NH_4Cl in ammonia-free water and dilute to 100 ml. Add a drop of chloroform as preservant. The solutions should be stored in glass bottles. They are stable if protected from direct sunlight and evaporation. Each solution contains 10.0 µmoles of NH_3-N per ml.

7.4 APPARATUS AND EQUIPMENT

7.4.1 Test tubes with ground glass (or plastic) stoppers and a volume of 30 ml or more

7.4.2 Automatic syringe pipettes of 2 ml capacity (3 pcs.)

7.4.3 Spectrophotometer or filter photometer with a filter having maximum transmission at (or close to) 630 nm. Photometric cells of 1, 5 or 10 cm length as required.

7.5 SAMPLING AND PRETREATMENT

The samples may not be preserved with sublimate ($HgCl_2$). Acid preservation is possible, but in that case every sample must be carefully neutralized with sodium hydroxide before the analysis is started, and corrections made for the ammonia content in acid and hydroxide. This makes the analysis uncertain and the acid preservation should be avoided if at all possible. Preferably, the samples should be analyzed within a short time after the sampling, and any necessary storage should be in a dark, cold place.

Polluted waters are often turbid and may be filtered through washed glass wool filters. However, such waters frequently contain high concentrations of ammonia and may therefore be diluted before the analysis. Any residual turbidity can be compensated with a similarly diluted sample in the reference cell. Thus, filtration should be avoided, if possible.

7.6 ANALYTICAL PROCEDURES

7.6.1 Calibration

All work with ammonium analysis must be carried out where smoking is not permitted, in order to avoid disturbances from ammonium. Before the test tubes can be used for calibration or analysis, they must be carefully cleaned; it is not sufficient just to wash them, but the following subsequent cleaning procedure must be carried out. To every tube about 25 ml distilled water (not necessarily ammonia-free water) and reagents are added as described in paragraph 7.6.2. Let the reaction proceed for at least two hours and shake the tubes from time to time during the reaction period. All ammonia contained in the tubes (dissolved in the water or adhered to the glass wall) will react. then the tubes are rinsed with ammonia-free water and kept stoppered when not in use. The tubes should not be washed between the different sets of calibrations or analyses, but just rinsed with ammonia-free water.

The analysis of ammonia is under the influence of disturbances from variations in pH and salinity of the samples. To account for this, the calibration can be carried out in either of two ways. For work in true oceanic areas, where the salinity variations are small, the ammonia working solution and the working standards should be diluted with ammonia-free sea surface water instead of distilled water. For work in e.g., estuaries where the brackish water displays large salinity variations, a calibration in distilled water, followed by corrections for the salinity of each sample, is preferred.

With the guidance of Table VI, the given volumes of ammonia working solution are pipetted into 100 ml volumetric flasks and diluted to the mark with distilled water, or sea surface water, that is free from ammonia.

TABLE VI

Preparation of working standards for ammonium

ml of working solution to be diluted to 100 ml	resulting concentration µmoles/litre
0.0	0.00
0.5	0,25
1.0	0.5
2.0	1.0
6.0	3.0
10.0	5.0
20.0	10.0

Note that the concentration µmole/litre refers to NH_3-N, which means nitrogen coming from ammonia (and not the concentration of ammonia itself).

From each of the standard concentration solutions above, 26 ml (preferably three portions of 25 ml) transferred to the test tubes. These samples are then analyzed as described in paragraph 7.6.2.

The following is an example of a calibration series (measured in a 1 cm cell) where the absorbances have not been corrected for the blank value:

µmoles/litre	0	7.14	17.85	35.7	53.6
A	0.022	0.171	0.397	0.772	1.105

The blank sample here corrects for the absorbance caused by the ammonia in the reagents and also the residual ammonia in the ammonia-free water. After subtraction of the blank absorbance, a calibration graph is constructed, showing corrected absorbance versus concentration.

The calibration graph is reproducible within the entire range for direct determination and it is linear up to a concentration of about 36 µmoles/litre. For concentrations up to 14 µmoles/litre, a 5 cm cell can be used, and with this cell, concentrations down to 0.04 µmole/litre can be determined.

A new calibration is required every time any of the reagents is exchanged for a new solution.

The reagents, especially the buffer solution (7.3.5), contain ammonium ions and hence the abosrbance caused by the ammonium in the reagents - the reagent blank - must be determined. To 22 ml of ammonia-free water (preferably three portions of 22 ml), the normal reagent volumes are added - 1 ml of each. To a second set of 22 ml portions, double the reagent volumes - 2 ml of each - are added. After two hours, 3 ml of ammonia-free water is added to each sample in the first set, and the absorbances of all samples are measured immediately. The difference between the mean values of the two sets is the reagent blank. This difference is caused only by the ammonia content of single volumes of the reagents.

For example: the mean of the set with 2 ml of each reagent is A = 0.040 and the mean of the second set is A = 0.022. The difference - the reagent blank - is then 0.018 (for 1 cm cell).

If this is compared with the blank value given in the calibration series it can be noticed that the nitrogen content in the water used for dilution of the calibration samples gives an absorbance of 0.022-0.018 = 0.004, which corresponds to a concentration of about 0.2 µmole/litre.

7.6.2 Analysis

Before the analysis and between analysis of different sets of samples the test tubes must be cleaned as described in the beginning of section 7.6.1. For each sample a volume of 25 ml is measured and transferred to a test tube. Add 1 ml of the buffer solution (7.3.5) and 1 ml each of the reagents A (7.3.2) and B (7.3.3 or 7.3.4). Mix properly, e.g., by swirling the test tube, after each addition. Stopper the tubes and keep them in a dark place during the reaction period (2 to 6 hours; see also below in paragraph 7.9.2).

Measure the abosorbances of the samples in the photometer using cells of suitable length depending on the colour intensity of the samples. Use distilled water as reference and take the measurements at the wavelength 630 nm.

Depending on how the calibration was done, the result of the analysis has to be calculated according to one of the procedures in the following section (7.7).

7.7 CALCULATION AND REPORTING OF RESULTS

For routine analysis a calibration factor is calculated. Select from the graph an absorption value (e.g., 0.240) and find its corresponding concentration figure (e.g., 2.85 µmoles/litre and calculate:

$$F_{5cm} = \frac{2.85}{0.240} \quad \text{which gives} \quad F_{5\ cm} = 11.88$$

How this calibration factor should be used in the calculations is shown below.

The absorbance should be corrected with the absorbance of the reagent blank that is described in paragraph 7.6.1. If the calibration has been made, using sea surface water of about the same salinity as the sample has, the calculation is made using the calibration factor F, thus:

$$NH_4\text{-N}\ \mu\text{moles/litre} = F \cdot (A_{sample} - A_{reagent\ blank})$$

If, however, the calibration was made with solutions of distilled water it is necessary to correct for the actual salinity of each sample. Choose for each sample the salinity correction factor, F_s, from the table in paragraph 7.9.1. Thus we have:

$$NH_4\text{-N}\ \mu\text{moles/litre} = F \cdot (A_{sample} - A_{reagent\ blank}) \cdot F_s$$

7.8 ESTIMATION OF PRECISION AND ACCURACY

High quality analysis of ammonia with good precision and accuracy is highly dependent on how successful one can avoid contamination of samples by tobacco smoke and other airborne ammonia as well as contamination of reagents and glassware.

An intercalibration exercise described by Dahl (see Grasshoff 1976) yielded a precision of $\pm$ 4.8 per cent and an accuracy of $\pm$ 5.5 per cent.

7.9 INFLUENCING FACTORS AND DISTURBING SUBSTANCES

7.9.1 Salinity and pH

It can be noted from the calibration factors found for distilled water and sea water solutions that in sea water the blue colour produced will be less intense for any given concentration of ammonia. This depends on the fact that in the final sample solution the pH will be a function of the sample salinity. The reaction is complete and quantitative in the pH-range between 10.4 and 11.3. Thus, for each sample a correction has to be made with respect to its salinity and the resulting pH. The correction factor is selected from Table VII.

TABLE VII

Salinity correction factors for ammonia and analysis

Salinity ($\cdot 10^3$)	0-8	11	14	17	20	23	27	30	33	36
pH	11.4-10.8	10.6	10.5	10.4	10.3	10.2	10.0	9.95	9.90	9.80
F_s	1.00	1.01	1.02	1.03	1.04	1.05	1.06	1.07	1.08	1.09

7.9.2 Reaction time

At room temperature the reaction is complete after two hours in distilled water and sea water with salinity below 15 if the reagent B used contains hypochlorite instead of Trion.

In ocean water the reaction requires four hours. If the reagent B with Trion is used the reaction will proceed slower and another two hours reaction time should be added. However, as the blue colour produced is stable for many hours it is convenient in routine analysis to let the samples react over night.

The reaction time may be shortened by using a higher concentration of hypochlorite. This cannot be recommended as some organic substances containing nitrogen may decompose and form monochloramine, thus giving too high results.

7.9.3 Disturbing substances

With the recommended concentration of hypochlorite the method is specific for ammonia and other nitrogen compounds do not react. Among inorganic substances, Hg-ions are capable of impeding the reaction. Consequently, samples should never be preserved with sublimate ($HgCl_2$). Likewise it has been observed that iron, in concentrations higher than 2 mg/l causes disturbance.

Stagnant water ofen contains hydrogen sulphide and if its concentration exceeds 2 mg/l a disturbing effect is obtained. In such waters the concentration of ammonia is normally rather high, and thus the disturbance may be eliminated by diluting the samples.

8. DETERMINATION OF NITRITE

8.1 SCOPE AND FIELD OF APPLICATION

This method is applicable for the determination of nitrite nitrogen (NO_2-N) in most types of waters (including sea water and waste water). It constitutes a modification of the well known Bendschneider and Robinson method (Koroleff, 1973).

The colorimetric reaction is almost specific for nitrite ions. Interferences may be caused by aromatic amines, copper (more than 0.5 mg/l), iodide ion (more than 0.1 mg/l). Suspended matter and strong colour in the sample also interfere.

This method is not appreciably affected by salinity, small changes in reagent concentration or volume, or by temperature. Of the interfering compounds, none are normally present in significant amounts in the ocean, inshore or estuarine waters unless excessive pollution from land drainage is encountered.

8.2 PRINCIPLE

The determination of nitrite is based on the classical Griess' reaction, in which the nitrite ion at pH 1.5-2.0 is diazotized with sulphanilamide, resulting in a diazo compound, which in turn is coupled with N-(1-naphthyl)-ethylenediamine to form a highly coloured azo dye with an absorption maxima at 545 nm whichis measured colorimetrically.

8.3 REAGENTS

8.3.1 Magnesium sulphate solution, about 2 moles/litre

Dissolve 50 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 100 ml.

8.3.2 Sodium hydroxide solution 4 moles/litre

Dissolve 16 g NaOH in distilled water and dilute to 100 ml.

8.3.3 Sulphanilamide reagent

Dissolve 8 g sulphanilamide, $NH_2C_6H_4SO_2NH_2$, in a mixture of 80 ml concentrated hydrochloric acid (HCl, 37 percent sp. gr. 1.19) and 400 ml water. Dilute to 500 ml. The reagent is stable for several months.

8.3.4 Diamine solution

N-(1-naphthyl)-ethylenediamine dihydrochloride. Dissolve 0.8 g of $C_{10}H_7NHCH_2CH_2NH_2.2HCl$ in distilled water and dilute to 500 ml. Store the solution in a dark bottle. Renew the solution about once a month, or as soon as it develops a strong brown colour.

8.3.5 Nitrite stock solution

Anhydrous sodium nitrite, $NaNO_2$ p.a. is dried at 110°C for several hours. Dissolve 0.3449 g of the dry salt in distilled water and dilute to 1,000 ml. Store the solution in a dark bottle with 1 ml chloroform as preservant. The solution is stable for at least 1-2 months. 1 ml contains 5 umoles as NO_2-N.

8.3.6 Nitrite working solution

10 ml of the stock solution is transferred to a volumetric flask and diluted to 1,000 ml with distilled H_2O. The solution must be used the same day. 1 ml contains 0.05 umole NO_2-N.

8.4 APPARATUS AND EQUIPMENT

8.4.1 Test tubes with glass or plastic stoppers and with a capacity of 25 ml or more.

8.4.2 Automatic syringe pipettes or piston pipettes for dispensing of reagents (not necessary, but very handy equipment).

8.4.3 Colorimetric equipment, one of the following is required:

(i) Spectrophotometer for use at 545 nm, with 1 cm cells and longer as required;

(ii) Filter photometer, equipped with a yellow-green filter having maximum transmittance near 545 nm. 1 cm cells or longer as required.

8.5 SAMPLING AND PRETREATMENT

Nitrite is an intermediate compound in the simplified redox chain ammonia - nitrite - nitrate. Samples for analysis of nitrite cannot be properly preserved. Storage in a dark and cold place can be tolerated for up to about five hours. If possible, samples should be analyzed without delay.

Avoid filtering the turbid samples, and use coprecipitation as pretreatment in extreme cases: Add 0.2 ml magnesium sulphate solution and 0.2 ml 4 moles/litre sodium hydroxide solution per 100 ml sample; agitate, and after 30 minutes, take the decanted part of the sample for analysis.

Samples that are slightly turbid and contain no other disturbing substances, as sea water from near-shore areas, may be analyzed together with turbidity blanks instead of being pretreated.

8.6 ANALYTICAL PROCEDURES

8.6.1 Calibration

As there is no salinity effect in the formation of the azo dye, the calibration can be done in solutions made with distilled water.

Using the nitrite working solution (1 ml = 0.05 µmole) a series of standard solutions is prepared by diluting with distilled water. If 100-ml volumetric flasks are used Table VIII below can be followed:

TABLE VIII

Preparation of working standards for nitrite

0.10	ml working solution/100 ml		0.05	µmole/litre	NO_2-N
0.20	"	"	0.10	"	"
0.50	"	"	0.25	"	"
1.00	"	"	0.50	"	"
2.00	"	"	1.00	"	"

From the flasks, 25 ml samples are transferred to stoppered test tubes. Distilled water is used as the blank. The reagents are added and the analysis is performed as described below. The absorbances (corrected for the blank) are plotted versus concentration on a millimetre paper. In a 10 cm cell, the concentration 1 µmole/litre NO_2-N gives an absorbance of about 0.500.

8.6.2 Analysis

For precise analysis of low concentrations of nitrite in sea water, any turbidity in the sample must either be removed (as described in section 8.5) or compensated for by a turbidity blank.

25 ml of the possibly pretreated sample is transferred to a stoppered test tube. If a turbidity blank is to be analyzed, another 25 ml is transferred to a second tube. Add 0.5 ml of the sulphanilamide solution (8.3.3) to the sample and turbidity blank, then mix well. After not less than three minutes, but not longer than eight minutes, 0.5 ml of the diamine solution (8.3.4) is added to the sample but not to the turbidity blank. Mix the sample once again.

After ten minutes, the absorbances of the sample and the turbidity blank are measured in a photometer at 545 nm in a cell length as required. The colour intensity is constant for two hours. The concentration of the sample is evaluated from the calibration graph, when the abosrbance of the turbidity blank has been subtracted from that of the sample.

As the addition of acid to a water sample usually changes its turbidity, it is important that the acidic sulphanilamide solution is added not only to the sample, but to the turbidity blank as well.

8.7 CALCULATION AND REPORTING OF RESULTS

For routine analysis a calibration factor is calculated. Select from the graph an absorption value (e.g., 0.230) and find its corresponding concentration figure (.e.g., 0.904 µmole/litre) and calculate:

$$F_{5\ cm} = \frac{0.904}{0.230} \qquad \text{which gives } F_{5cm} = .9$$

Then the concentration of a sample, in µmoles/litre, is found by multiplying its absorbance, corrected for absorbance caused by turbidity and the reagent blank, by the factor F.

8.8 ESTIMATION OF PRECISION AND ACCURACY

Turbidity of the sample may introduce a considerable systematic error, particularly at low concentration levels. For highest accuracy and turbidity should, therefore, be removed as described in section 8.5 or compensated for as described in section 8.6.2.

In routine analytical work the precision can be expected to be about $\pm$ 0.02 µmole/litre.

9. DETERMINATION OF NITRATE

9.1 SCOPE AND FIELD OF APPLICATION

This method based on a previous publication (Koroleff, 1973) is applicable for the determination of the sum of nitrate and nitrite, in natural waters.

If the sample contains considerable amounts of nitrite, a separate determination of nitrite must be conducted and the result subtracted from that obtained with this method.

Nitrate concentrations up to about 40 µmoles/litre of nitrogen (NO_3-N) can be determined without prior dilution of the samples.

The colorimetric reaction is almost specific for nitrite ions. Interferences may occur, caused by aromatic amines, copper (more than 0.5 mg/l), and iodide ion (more than 0.1 mg/litre). Suspended matter and a strong colour in the sample also interfere. Instead of filtering the sample, use the precipitation technique. However, moderate amounts of suspended matter can be tolerated as it will be trapped in the reduction column.

At concentrations higher than about 15 µmoles/litre of NO_3-N in sea water, the dissolved salts cause a deviation from the linear relationship between concentration and the intensity of the coloured nitrite complex.

9.2 PRINCIPLE

Nitrate is reduced to nitrite almost quantitatively (about 90-95 percent) by amalgamated cadmium. The nitrite is then determined according to the classical Griess' reaction as described in the method for nitrite analysis (section 8).

The reduction is carried out at a pH of about 8.5. An ammonium chloride buffer is added to the sample to control the pH and to complex the liberated cadmium ions.

9.3 REAGENTS

Generally, the ordinary distilled or de-ionized water of the laboratory should be suitable for the analysis of nitrite as well as nitrate. However, if problems are encountered with the quality of the water, "nitrogen-free" water has to be prepared.

Distilled (or de-ionized) water is distilled in all-glass equipment. For this distillation 2 ml concentrated sulphuric acid and 1 g potassium peroxodisulphate ($K_2S_2O_8$) are added per litre.

All chemicals should be of reagent grade (pro analysi).

9.3.1 Hydrochloric acid, about 0.5 moles/litre

Dilute 10 ml concentrated HCl (density 1.19/ml) to 250 ml with water.

9.3.2 Mercuric chloride solution, 1 percent. Dissolve 1 g $HgCl_2$ in 100 ml distilled water.

9.3.3 Hydrochloric acid, 2 moles/litre. Dilute 166 ml concentrated HCl (density 1.19 g/ml) to 1 litre with distilled water.

9.3.4 Concentrated buffer solution

Dissolve 250 g ammonium chloride, NH_4Cl, in distilled water, add 25 ml concentrated ammonium hydroxide, NH_4OH (density 0.91 g/ml) and dilute to 1 litre.

9.3.5 Dilute buffer solution

Dilute 20 ml of the concentrated buffer to 1 litre with distilled water. Renew daily.

9.3.6 Acid washing solution

Dissolve 4 g NH_4Cl in distilled water, add 15 ml hydrochloric acid, 2 moles/litre, and dilute to 1 litre with water.

9.3.7 Sulphanilamide reagent (same reagent as for nitrite determinations)

Dissolve 8 g sulphanilamide, $NH_2C_6H_4SO_2NH_2$, in a mixture of 80 ml concentrated hydrochloric acid (sp. gr. 1.19) and 400 ml water. Dilute to 500 ml with water. The reagent is stable for several months.

9.3.8 N-(1-naphthyl)-ethylenediamine dihydrochloride solution (same reagent as for nitrite determinations)

Dissolve 0.8 g $C_{10}H_7NHCH_2CH_2NH_2.2HCl$ in water and dilute to 500 ml. Store the solution in a dark bottle. Renew the solution about once a month, or immediately if it develops a strong brown colour.

9.3.9 Magnesium sulphate solution, about 2 moles/litre

Dissolve 50 g $MgSO_4.7\ H_2O$ in water and dilute to 100 ml.

9.3.10 Sodium hydroxide solution 4 moles/litre

Dissolve 160 g NaOH in water and dilute to 1 litre.

9.3.11 Nitrate stock solution

Dissolve 1.0111 g potassium nitrate, KNO_3, in distilled water and dilute to 1,000 ml. Add 1 ml chloroform as preservant. The solution is stable for several months if evaporation is prevented. 1 ml = 10.0 ugat (140 ug) NO_3-N.

9.3.12 Diluted nitrate solution

10 ml of the stock solution is diluted with distilled water to 1,000 ml. 1 ml = 0.1 µgat (1.4 µg) NO_3-N.

9.3.13 Synthetic sea water

Dissolve 310 g sodium chloride, NaCl, 100 g magnesium sulphate, $MgSO_4.7H_2O$ and 0.50 g sodium hydrogen carbonate, $NaHCO_3.H_2O$ in 10 litres of distilled water. The salinity of this water is about 33.7 percent.

9.3.14 Amalgamated cadmium

WARNING: Cadmium is a poisonous metal. For reasons of occupational health and safety it should be handled with care. Perform all operations on the dry metal, particularly the granules, in a well ventilated area e.g., a fume cupboard. Never inhale the dust. If granulated cadmium (for instance, Merck Cadmium grob gepulvert, zur Füllung von Reduktoren) is not available, one has to prepare it in the following way:

File sticks of pure cadmium metal (reagent grade) with a coarse metal hand file (about second cut) and collect the fraction which passes a sieve with 1-mm openings and is retained on a sieve with 0.5-mm openings[1]. Calculate the required amount of cadmium about 35 g per reductor column (see Figure 3). Rinse the filings quickly with 0.5 mole/litre hydrochloric acid (9.3.1) and then with distilled water until the rinse water does not give the chloride reaction with silver nitrate.

Transfer about 35 g of the washed metal into a 100 ml glass bottle and fill the bottle with the mercuric chloride solution (9.3.2) so that all air is excluded from the bottle. Seal the bottle with a glass stopper. After this step avoid all contact between air and the metal. Rotate the bottle for 90 minutes in a horizontal position with suitable equipment. Open the bottle and rinse out the turbid sublimate solution with distilled water which is introduced through a glass tube a' the bottom of the bottle. Collect the sublimate solution.

If a rotary evaporator is available the treatment can be conveniently carried out. Transfer the cadmium and the sublimate solution to a 100 ml or 250 ml round bottom flask that is aligned on the evaporator where it is allowed to turn for the required time.

Do not pour the used mercuric chloride (sublimate) solution into the sewer! When a suitable volume is collected, add 25 ml concentrated HCl per litre and precipitate with hydrogen sulphide (H_2S) or sodium sulphide (Na_2S). Filter the liquid. Store the precipitate and discard the clear filtrate.

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1) There are different sieves available commercially. Table IX gives the suitable sieves.

TABLE IX

Comparison of sieve dimensions according to different systems

Opening:	mm	DIN	Mesh/cm	Mesh/inch	Sieve No.
Upper sieve	1	6	7	17.2	18
Lower sieve	0.5	12	13	32	35

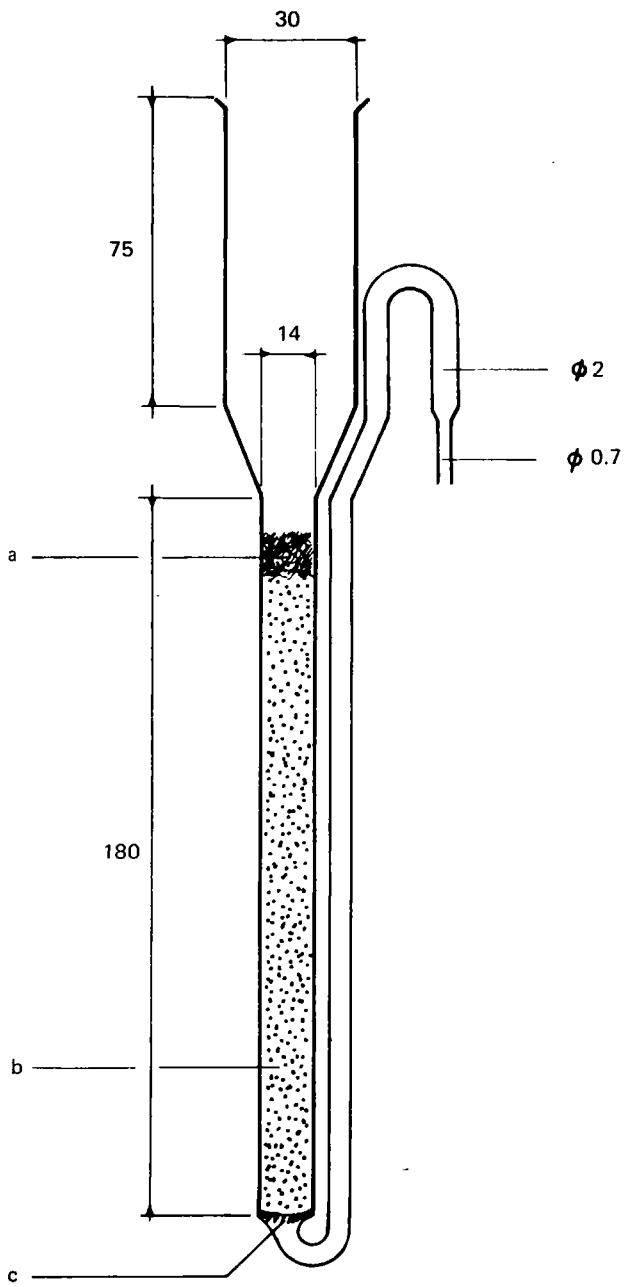

Figure 3. Reduction column for analysis of nitrate and total nitrogen (all measures given in millimetres)

(a) glass wool
(b) Cd-amalgam
(c) copper wire

9.4 APPARATUS AND EQUIPMENT

9.4.1 Reduction columns (see Figure 3)

9.4.2 Volumetric flasks or test tubes with a capacity of 25 ml.

The tubes should either be graduated or marked at the 25 ml volume.

9.4.3 Colorimetric equipment; one of the following is required:

(a) spectrophotometer, for use at 545 nm and provided with cells of 1 cm (and 5 cm) length, or

(b) filter photometer, equippped with a filter having maximum transmittance at (or near) 545 nm, with cells of 1 cm (and 5 cm) length.

9.5 SAMPLING AND PRETREATMENT

The analysis should preferably be undertaken immediately after the sampling. If this is not possible, the samples may be preserved by the addition of 1 ml sulphuric acid 4 moles/litre per litre. The sample has to be neutralized again before the analysis of this preservation is applied.

Suspended matter and a strong colouration in the sample are removed by using a precipitation technique: Add 0.2 ml of magnesium sulphate solution and 0.2 ml of sodium hydroxide solution per 100 ml sample. Agitate, and after 30 minutes, use the decanted solution for analysis.

9.6 ANALYTICAL PROCEDURES

9.6.1 Preparation of the reduction columns

Place a small ball of thin copper wire at the bottom of the reduction column and fill it entirely with water. Open the bottle containing the amalgamated cadmium fillings and put a finger (or a rubber plate) on the opening. turn the bottle obliquely downward and place the opening under the water surface in the reductor column. The metal should flow down into the reductor column without coming into contact with air. Make sure that no cavities are formed in the reductor. Fill the column with the metal to about 1 cm below the reservoir and plug the column with glass wool. This operation should be carried out so that all liquid that flows from the column etc. can be collected and precipitated as described above.

Flush the column several times with the acid washing solution (9.3.6) and with the dilute buffer solution (9.3.5) until the effluent shows a weakly alkaline reaction (for instance, blue colouration with bromothymol blue). The column should never be left with the acid washing solution. Flush the column weekly when it is used regularly or more often if the flow decreases through the column. A newly prepared column reduces nitrate almost immediately with an efficiency of 95-100 percent (see section 9.7 for further details). If the reduction efficiency decreases below 85 percent, empty the column, wash the filings quickly with 2 moles/litre HCl and rinse very thoroughly with distilled water. Dry the filings, sieve again, and reamalgamate as described above.

If a column does not give a proper reduction when it is newly prepared it may be "started" by running through a nitrate standard solution, e.g., 10 µmoles/litre, or common tap water. In most cases, less than one litre will suffice.

9.6.2 Calibration

A series of standard concentrations is prepared from the nitrate stock solution (9.3.11) and the diluted nitrate solution (9.3.12) by diluting with distilled water or synthetic sea water. As there is no salt error at concentrations below about 15 µmoles/litre the synthetic sea water will probably seldom be required.

As several columns are likely to be calibrated at the same time, the standard solutions are prepared in at least 1 litre quantities according to Table X.

TABLE X

Preparation of working standards for nitrate

ml diluted nitrate solution/1,000 ml	µmoles/litre
1.00	0.10
5.00	0.50
10.00	1.00
50.00	5.00
ml nitrate stock solution/1,000 ml	**µmoles/litre**
1.00	10.0
2.00	20.0
3.00	30.0
4.00	40.0

The standard solutions are analyzed as described below. Duplicates of each solution must be analyzed.

The absorbances are plotted versus concentration for each of the reduction columns. If the results are very uniform from column to column, one calibration graph may be used for the subsequent evaluation of the samples. However, most probably one graph will be required for every column.

If samples have been treated according to the precipitation procedure in paragraph 9.5, then the calibration solutions and blank samples should be treated in the same way.

9.6.3 Analysis

9.6.3.1 Reduction of nitrate

Neutralize acid preserved samples with 0.1 or 0.5 mole/litre NaOH after addition of two drops of bromothymol blue indicator. (Calculate prospective dilution).

(1) Fill the reservoir of the reduction column with the dilute buffer solution (9.3.5) and let it flow through. Discard the solution.

(2) Fill the reservoir again with 50 ml distilled water and 1 ml concentrated buffer solution (9.3.4). Discard the first 20 ml which flows through the column and collect 25 ml in a volumetric flask or test tube. Proceed in the same manner with all the columns in order to obtain a blank value. These blank samples are then analyzed for nitrite as described in (9) and onward. If samples have been treated according to the precipitation procedure in paragraph 9.5, then blank samples and the calibration solutions should be treated in the same way.

(3) The reduction efficiency of each column must be controlled for every analytical batch. The standard concentration used for this control depends on the types of concentration range of the samples. Analyze duplicates of this control standard as described in (4) to (10).

(4) Take a 50 ml sample or less containing up to 15 µmoles/litre NO_3-N) and adjust the volume to 50 ml.

(5) Add 1 ml of concentrated buffer solution (9.3.4) and mix; use an automatic pipette if possible.

(6) Draw off the excess solution in the reductor by using a capillary-tipped glass tube connected to a suction pump and proceed immediately with (7).

(7) Transfer the entire sample (4) to the reservoir of the reductor. Discard the first 10 ml flowing through the column and use 10 ml to rinse a 25 ml volumetric flask (or test tube). Then collect 25 ml in the flask or tube. 50 ml should flow through the column in ten to twelve minutes. Proceed as described in (9). (The determination of nitrite should be carried out within one hour.)

(8) Draw off the remaining sample from the reductor and begin directly with the next sample.

The reductor column must be washed immediately after every analytical batch. Fill the reservoirs twice with the dilute buffer solution (9.3.5). Cover the reductors and make sure that they do not dry.

9.6.3.2 Determination of nitrite

(9) To 25 ml of the reduced sample, coming from the reductor, add to samples, standard solutions and blank samples 0.5 ml sulphanilamide solution (9.3.7) and mix. After not less than 3 minutes, and not longer than 8 minutes, add 0.5 ml of N-(1-naphthyl)-ethylenediamine solution (9.3.8) and mix again.

(10) The absorbance of the coloured sample is measured after 10 minutes, but within two hours, against the reference sample at 545 nm. Use a cell of suitable length depending on the intensity of the colour. (The absorbance of the blank sample against water should not exceed 0.010, measured in a 1 cm cell.)

9.7 CALCULATION AND REPORTING OF RESULTS

Subtract the absorbance of the blank sample from the absorbances of the samples and evaluate their concentrations from the calibration graph.

For routine analysis a calibration factor is calculated. Select from the graph an absorption value (e.g., 0.715) and find its corresponding concentration figure (e.g., 13.9 µmoles/litre) and calculate:

$$F_{1cm} = \frac{13.9}{0,715} \qquad \text{which gives } F_{1cm} = 19.44$$

Then the concentration of a sample, in µmoles/litre, is found by multiplying its absorbance, corrected for the absorbance caused by the blank, by the factor F.

NOTE: If the nitrate determination is part of the determination of total nitrogen the calculation is carried out as described in section 10.7.

The result obtained is the sum of the concentrations of nitrite and nitrate nitrogen in each sample. If a sample contains a significant concentration of nitrite, this must be compensated for by subtracting the result found from a separate determination for nitrite according to the previous method (Section 8).

(If the sample was diluted prior to the reduction this must be taken into account in the calculation:

$$\text{µmoles/litre } NO_3\text{-N} = \frac{A \times B}{V}$$

where:

A = nitrate concentration, µmoles/litre, as obtained from the calibration graph.
V = ml of original sample before dilution
B = final volume, after dilution.

For example: A 25 ml sample was diluted to 100 ml and of this 50 ml was analyzed. The result ob ained from the calibration graph was 26 umoles.

$$\text{Then} \quad \frac{26 \times 100}{25} = 104 \text{ µmoles/litre } NO_3\text{-N}$$

To check the reduction efficiency of the reductor (4) a nitrate standard solution of suitable concentration is analyzed. For instance, a solution of 1 µmole/litre is analyzed and an absorbance of 0.241 is obtained when using a 5 cm cell. An analysis of the nitrite solution with the same concentration gives the absorbance 0.258 under the same conditions. The reduction efficiency is then: $\frac{0.241 \times 100}{0.258}$ □ 93 percent

9.8 ESTIMATION OF PRECISION AND ACCURACY

Deviation between duplicate samples analyzed with the aid of one and the same reductor is ± 0.1 µmole/litre in the range 0 - 5 µmoles/litre, ±0.2 µmole/litre in the range 5 - 10 µmoles/litre and 0.5 µmole/litre at higher concentrations. Deviation of results from different columns depend on the reduction efficiency (yield factors) of the columns.

When systematic errors are avoided an accuracy of ± 3 per cent can be assumed in the range between 0 - 10 µmoles/litre.

10. SIMULTANEOUS PERSULPHATE OXIDATION OF PHOSPHORUS AND NITROGEN CONTAINING COMPOUNDS IN WATER

10.1 SCOPE AND FIELD OF APPLICATION

Commonly used names of the parameters determined with this method are total phosphorus and total nitrogen. Earlier methods published for their determination have been designed for either of these parameters. In 1977 Koroleff presented a method for a simultaneous determination of total phosphorus and total nitrogen in the same sample. The method was published in 1981 by Valderrama in a slightly modified version. The method exhibits improved reliability and precision and it allows for the storage of samples to be analyzed later.

The yields obtained by oxidation of various nitrogen compounds depend on the form of nitrogen linkage. Thus, for example, nitrate, nitrite, ammonia, urea, some aliphatic amino acids and some proteins give yields over 92%. Pronouncedly poor yields have been obtained from compounds containing nitrogen-to-nitrogen bonds, while a double bond between nitrogen atoms seems to prevent their oxidation to nitrate entirely (Nydahl, 1978). The digestion time, a function of the autodecomposition of peroxodisulphate, needs to be no longer than 30 minutes, provided that the temperature attained in the pressure cooker is 110-115°C.

10.2 PRINCIPLE

To allow nitrogen compounds to become oxidized, it is necessary to use an alkaline medium, otherwise nitrate is not produced in quantifiable amounts. Conversely, the oxidation of phosphorus compounds must be performed on an acidified sample. In the simultaneous oxidation the reaction starts at pH 9.7 and ends at pH 5-6. These conditions are obtained by a boric acid-sodium hydroxide system. In seawater samples no precipitate is formed when the oxidation reagent is added. At elevated temperature (100-120°C) a precipitate is formed, which, however, almost dissolves as oxidation proceeds. After the oxidation the remaining small amount dissolves upon swirling. The free chlorine, which is formed in seawater samples, is reduced by adding ascorbic acid before the molybdate reagent (Koroleff, 1977).

After oxidation the nitrogen compounds are determined as nitrate according to the procedure in Section 9 and the phosphorus compounds are determined as inorganic phosphate according to the procedure in Section 4.

10.3 REAGENTS

In addition to the reagents needed for determintion of dissolved inorganic phosphate (Section 4) and nitrate (Section 9) the following items are needed.

10.3.1 Dilute hydrochloric acid, about 0.2 umoles/litre

Dilute 17 ml concentrated hydrochloric acid, HCl (density 1.19 g/ml) with distilled water to 1 litre.

10.3.2 Sodium hydroxide, 1 umole/litre

Dissolve 40 g sodium hydroxide, NaOH, in about 50-60 ml distilled water and then dilute to 1 litre.

10.3.3 Oxidation reagent

Dissolve 50 g sodium peroxodisulphate, $K_2S_2O_8$, and 30 g boric acid, H_3BO_3, in 350 ml of sodium hydroxide (1 mole/litre) and make up to 1 litre with distilled water.

The peroxodisulphate has to have a very low content of nitrogen compounds. The product with No. 5092 from Merck is usually of very satisfactory quality.

If the oxidation reagent is stored in dark glass bottle and protected from direct light in room remperature it is stable for at least six to eight months.

10.3.4 Concentrated standard solution

Dissolve 0.4505 g dried glycine, $C_2H_5O_2N$ (reagent grade) and 0.1361 g of potassium dihydrogen phosphate, KH_2PO_4 (reagent grade), previously dried at 110°C and kept in a desiccator, with distilled water and make up to a final volume of 100 ml. 1 ml contains 10.0 µmoles of P and 60.0 µmoles of N.

10.3.5 Dilute standard solution

Pipette 1.00 ml of the concentrated standard solution (10.3.4) to a 100 ml volumetric flask and make up to the mark with distilled water. 1 ml contains 0.10 µmoles of P and 0.60 µmoles of N.

10.4 APPARATUS AND EQUIPMENT

In addition to the equipment needed for the determination of dissolved inorganic phosphate (Section 4) and nitrate (Section 9) the following items **are needed.**

10.4.1 Oxidation bottles of alkali resistent glass with screw caps of non-nitrogen containing material and about 50 ml volume. (Suitable bottles are Sovirel graduated bottles with screw caps. However, as these caps, contain nitrogen compounds they have to be replaced. The replacement must not be of polyethylene as they will become brittle and crack as a result of the free chlorine that is developed in the oxidation. Suitable replacements are polypropylene caps from Nalgene Labware, Nalge Corporation).

10.4.2 Stainless steel pressure cooker of any ordinary kitchen type that will maintain an internal temperature of 110-115°C.

10.5 SAMPLING AND PRETREATMENT

Soon after the sampling, e.g., in connection with the analysis of the other nutrient parameters, 30 ml portions of the samples are transferred to oxidation bottles. To each portion 4 ml of the oxidation reagent (10.3.3) are added with an automatic syringe pipette and the bottles tightly stoppered.

Boil the samples for 30 minutes at 110-115°C in suitable batches (e.g., 30-40 bottles) to fill the pressure cooker. Together with each batch boil two oxidation reagent blank samples consisting of 4 ml reagent and no extra distilled water.

After boiling, the samples should be gently swirled to promote dissolution of the precipitate thay may have formed. Then the bottles are allowed to cool down to room temperature. The bottles should not be opened until the analysis of phosphate and nitrate is started.

It has been shown (Valderrama, 1981) that it is preferential to add the oxidation reagent and boil the samples soon after sampling and then store the samples in oxidized form. If samples are stored before oxidation instead the analyses will receive a higher coefficient of variation. The storage has been successfully tested for at least three months.

10.6 ANALYTICAL PROCEDURES

10.6.1 Calibration

Using the dilute standard solution, prepare a series of standards with the concentrations given in Table XI, below:

TABLE XI

Preparation of working standards for total phosphorus and total nitrogen

ml dilute standard/200 ml	phosphorus µmoles/litre	nitrogen µmoles/litre
14.0	7.0	42.0
10.0	5.0	30.0
6.0	3.0	18.0
2.0	1.0	6.0

Analyze 30 ml portions in duplicate from each standard according to the description in section 10.5 (oxidation) and then section 10.6.2 (procedure).

Note that for the calibration the blank samples must consist of 30 ml of the distilled water used with addition of 4 ml of oxidation reagent (10.3.3) to each blank. This will compensate for any phosphorus and nitrogen present in the distilled water and the oxidation reagent.

Correct the two absorbance figures obtained for each solution with the corresponding blank absorbance and construct calibration graphs as described previously in paragraph 13.6 for nitrate and paragraph 8.5 for phosphate. The calibration factors, F, can also be calculated as described.

As the original sample taken for analysis (30 ml) is diluted to 35 ml in the procedure, the calibration factors will be about 1.167 times (35/30) higher than those for nitrate and phosphate. Thus a factor, F_N, of about 24 for nitrogen and F_P, 11 for phosphorus can be considered as representative.

Note that although the oxidized nitrogen sample is diluted from 10 ml to 50 ml in the analysis this is compensated for by using a 5 cm cell for the photometric reading as compared with the 1 cm cell that is (usually) used for nitrate. Thus the difference in the calibration factors becomes rather small.

10.6.2 Analysis

Open the bottles containing the oxidized, cool, samples. As each sample is to be used for two determinations it has to be divided into two separate parts. Transfer each sample to a measuring cylinder, preferably a stoppered one, and adjust the volume to 35 ml and mix well. Continue as described below for each analysis.

After each batch of samples that have been oxidized it is convenient to fill the oxidation bottles with the diluted hydrochloric acid (10.3.1) and let them stand for a couple of hours before rinsing them with distilled water.

10.6.2.1 Procedure for nitrogen compounds (total nitrogen)

Prepare the reduction columns for nitrate analysis as described in steps (1) and (2) of section 9.6.3 Nitrate analysis.

Pipette 10 ml of the sample, adjusted to volume as described above in section 14.7, and continue the analysis as for a nitrate sample according to the steps (4) through (10) in section 9.6.3 Nitrate analysis. In most cases a 5 cm cell is suitable for the photometric determination as indicated above concerning calibration.

10.6.2.2 Procedure for phosphorus compounds (total phosphurus)

The remaining 25 ml from the sample in section 10.6.2 is used for the analysis of phosphorus compounds. Transfer the sample to a test tube and add 0.7 ml of the ascorbic acid solution (4.3.5). Mix well and let the sample rest 1-2 minutes so any remaining free chlorine, that was liberated during the oxidation, is completely destroyed. Finally, add 0.7 ml of the acid-molybdate reagent (4.3.4) and mix the sample well again. (The preparation of these reagents were described in Section 4.3).

After five minutes read the absorbance of the sample in a 5 cm cell at 882 nm using distilled water as a reference. See also paragraphs 4.6.2 and 4.6.3 concerning the stability of the coloured complex and possible disturbances.

10.7 CALCULATION AND REPORTING OF RESULTS

The absorbance measured for each sample, A_s has to be corrected for the absorbance of the blank that contains the oxidation reagent, A_{ox}. The corrected absorbance is then used for evaluation of the result from the calibration graph.

If a calibration factor, F , is used for the calculation use the formulas:

$$\text{Total nitrogen, } \mu\text{moles/litre} = F_N \times (A_{s,N} - A_{ox.N})$$

$$\text{Total phosphorus, } \mu\text{moles/litre} = F_p \times (A_{s,P} - A_{ox,P.})$$

10.8 ESTIMATION OF PRECISION AND ACCURACY

The actual capability of the method is summarized in Table XII below (from Valderrama 1981).

TABLE XII

Accuracy and precision

	Total nitrogen			Total phosphorus		
True concentration values (μmol l^{-1})	1.5	6.0	30.0	0.25	1.00	5.0
No. measurements	8	8	8	8	8	8
Measured concentrations Mean values in (μmol l^{-1})	1.8	6.7	31.2	0.27	1.02	4.94
Range	1.7—1.9	6.4—6.9	29.9—32.7	0.25—0.29	0.98—1.04	4.82—4.98
Standard deviation	0.063	0.217	0.988	0.014	0.020	0.056
Variation coefficient (%)	3.5	3.2	3.2	5.2	2.0	1.1
Relative error (%)	20.0	11.7	4.0	8.0	2.0	−1.2

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