COMMONWEALTH OF AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

Division of Fisheries and Oceanography

REPORT 51

LABORATORY TECHNIQUES IN MARINE CHEMISTRY

A MANUAL

by

G. A. Major, G. Dal Pont, J. Klye and B. Newell

Marine Laboratory
Cronulla, Sydney
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CONTENTS.

I. INTRODUCTION

This manual assembles the methods used for the more routine chemical analyses performed by the Division at the present time.

Although most of the basic parameters which are considered of importance to oceanographers are included, the contents merely reflect the current interests of the Division, and in no way include all the components in seawater which may be of interest. A more comprehensive coverage is available elsewhere[1].

While intended as a work manual for use within the Division, it is directed also to other workers who may have no easy access to these analytical procedures.

The techniques are all standard methods which, as a result of extensive experience in their use, have been adopted - in some cases with a few modifications - for their reliability, relative simplicity and convenience.

The high salt content and the presence of other complex organic and inorganic materials in seawater not only preclude most fresh-water analytical methods, but have resulted in an excessive number of methods being devised in the course of attempting to overcome such interferences. Besides describing the more acceptable procedures which have been developed to date, it is hoped this manual may help to standardize analytical practice in Australia.

For those techniques involving the development of a coloured solution, instructions are given for the preparation of standards. To avoid repetition, no mention is made of the construction of calibration graphs. It is assumed that users of this manual will be familiar with this standard method of calculating results.

Some terms and symbols used in the manual are defined as follows:

$\mu g = 10^{-6}$ g
$nm = 10^{-9}$ metre
‰ = parts per thousand (g/kg)
p.p.m= parts per million
= µg/g or µg/ml
= µl/litre or mg/litre
p.p.b = parts per billion
= µg/litre or mg/m^3
µ mole/litre = µg-at/litre (or µg-molecule/litre, or µg-formula/litre)
= µg-atom/litre
$= \frac{\mu g}{\text{atomic weight}}$/litre
C.O.D.= Chemical Oxygen Demand

$$\text{absorbance} = \text{extinction} = \text{optical density} = \log_{10}\left(\frac{\text{incident light intensity}}{\text{transmitted light intensity}}\right)$$

"cuvette" and "absorption cell" are equivalent terms

"NH_3-N" (for instance) means "the mass of nitrogen in the ammonia present".

The normal pH of seawater is 8.2.

Reference

(1) Strickland, J.D.H. and Parsons, T.R. (1968).- A Practical Handbook of Seawater Analysis. Bull. Fish. Res. Bd Can., 167.

Further references to analytical methods are given in Riley, J.P., and Skirrow, G. (eds), (1965), Chemical Oceanography, Volumes 1 and 2, Academic Press, London, especially Vol. 2. This is a basic reference on the chemistry of the sea.

2. SALINITY - INSTRUMENTAL METHODS ONLY

Introduction

In this laboratory the older method involving the estimation of chloride by titration has been replaced by instrumental techniques. These instruments measure the conductivity of the water which, at a given temperature, is a function of the chlorinity.

Salinity = 0.03 + 1.805 x chlorinity

Method 1 - DETERMINATION IN THE FIELD

Reagents and Apparatus

(a) Portable salinity-temperature (S-T) meter. Model 602, manufactured by Auto-Lab Industries Pty Ltd, Chatswood, N.S.W.

(b) International standard salinity phials. Obtained from the Standard Sea-water Service, Charlottenlund Slot, 2920, Charlottenlund, Denmark.

(c) Glass storage bottle (20 litre capacity) for sub-standard.

(d) Filtered seawater substandard. Standardized against international standard seawater and stored under liquid paraffin.

(e) For laboratory use of this instrument, see Method 2 for extra apparatus required.

Procedure

The instrument consists of a battery-operated unit with a 50 or 100 metre length of cable leading from the instrument to the conductivity-measuring head.

The manual supplied with the instrument provides adequate procedural instructions.

The temperature and salinity of the water may be read off directly from the instrument dials.

Although primarily designed for use in estuarine field work, such instruments have also proved useful in the laboratory when large numbers of samples have to be analysed quickly in programmes where the highest accuracy is not required.

Reference

(1) Lockwood, D.R. (1970).- Portable temperature-chlorinity bridge (S-T meter) instruction manual. CSIRO Aust. Div. Fish. Oceanogr. Rep. 47.

2. SALINITY

Method 2 - DETERMINATION IN THE LABORATORY

Reagents and Apparatus

(a) Inductively coupled salinometer. Units manufactured by both Auto-Lab (Chatswood, Sydney) and by I.M.E. (Petersham, Sydney), are used in this laboratory.

(b) International standard salinity phials. Obtained from the Standard Sea-water Service, Charlottenlund Slot, 2920 Charlottenlund, Denmark.

(c) Filtered seawater substandard. Standardize against international standard seawater and store under liquid paraffin.

(d) Glass storage bottle (20 litre capacity) for sub-standard.

(e) Sample bottles. Approximately 200 ml capacity. Either standard type salinity bottles with caps clipped down by wire, or screw-capped bottles with plastic caps incorporating rubber (neoprene) liners.

Procedure

In general the instructions as laid down in the manuals supplied are adequate, but experience has shown that the error due to contamination by previous samples is unacceptably large unless samples are very close in salinity.

Consequently a larger sample than previously is now taken - about 200 ml. This allows thorough rinsing of the cell, the first rinsing being allowed to run to waste. The remainder of the sample is then drawn into the cell and the conductivity ratio read. This sample is then drained back into the bottle and the cell refilled without removing the sample bottle. This should be repeated until consecutive readings give a difference in ratio of less than 0.00005.

When collecting samples, the bottles (including caps) should be rinsed twice with about 50 ml of sample water before filling with the sample for the determination.

3. DISSOLVED OXYGEN

WINKLER'S METHOD

Reagents and Apparatus

(a) 40% manganese sulphate solution (w/v). Dissolve 200 g of manganese sulphate, $MnSO_4.4H_2O$, (A.R.) in distilled water to 500 ml. total volume.

(b) Alkaline potassium iodide solution. Dissolve 150 g of potassium iodide, KI, (A.R.) in 150 ml of distilled water, and dissolve 180 g of sodium hydroxide, NaOH, (A.R.) separately in 200 ml distilled water. After cooling the hydroxide solution, mix both solutions and make up with distilled water to 500 ml total volume. Store away from light.

(c) Concentrated sulphuric acid, H_2SO_4, A.R. quality.

(d) 0.1N sodium thiosulphate solution. Weigh out accurately 24.82 g of A.R. sodium thiosulphate, $Na_2S_2O_3.5H_2O$, into a 100 ml beaker, add about 50 ml of distilled water, and stir with a glass rod until solution is complete.

Rinse out a 1000 ml volumetric flask with distilled water and transfer with washings the contents of the beaker to the flask and make up to the mark. Shake well and store in a dark glass container.

(e) 0.01 N sodium thiosulphate solution. Carefully dilute 100 ml of the above solution to 1000 ml with distilled water. Shake well before use.

(f) 0.01N potassium iodate solution. Weigh out accurately in a 100 ml beaker, 0.178 g of A.R. grade KIO_3; dissolve in about 50 ml of distilled water, transfer with washings to a 500 ml volumetric flask. Make up to the mark, shake well and keep in a dark glass container.

(g) 1% starch solution. Weigh out 2.5 g of soluble starch and make a suspension in 25 ml of distilled water. Bring to the boil 225 ml of distilled water in a 400 ml beaker, and then,

with stirring, add the starch suspension. Continue to boil for one minute, allow to cool, and bottle. The insoluble residue, if any, gradually sinks to the bottom of the bottle and the solution should be used from the clear upper layer. This starch solution should be renewed after 2 days; its stability is increased by the addition of several drops of carbon disulphide, CS_2. If the starch solution gives a green instead of a blue colour with iodine, or seems to be lacking in sensitivity, discard and prepare a fresh solution.

(h) 250 or 300 ml capacity (8 or 10 oz) reagent bottles with well-fitting ground glass stoppers; thoroughly washed and dried.

(i) Reagent dispensers, 0.5 ml, 1.0 ml and 2.0 ml capacities.

(j) Pipette - 10 ml capacity, and burette - 10 ml capacity.

Procedure

(a) Collection of samples

Polythene tubing of sufficient length to reach easily to the bottom of the reagent bottle is fitted to the tap on the collecting bottle filled with sample. The tap is opened to fill the tube which is then inserted carefully right to the bottom of the reagent bottle, and the tap slightly opened to allow a gentle flow of water into the reagent bottle with a minimum disturbance of the surface. The tap can be progressively opened to full flow, and the water is allowed to overflow by an amount equal to half the capacity of the reagent bottle. The tube is slowly removed with the tap still open so that the reagent bottle is completely filled, and the stopper inserted carefully so that no air is trapped.

As soon as possible afterwards 0.5 ml of $MnSO_4$ reagent followed by 1.0 ml of NaOH - KI reagent is added to the sample, the stopper carefully replaced as before, and the bottle shaken at least 20 times. When the precipitate has settled to about $\frac{1}{2}$ the volume of the bottle, 2 ml of conc. H_2SO_4 is added carefully to ensure no loss of precipitate, and the bottle shaken again to effect complete solution.

(b) Titration

Standardize the 0.01N $Na_2S_2O_3$ solution by the following procedure:

(i) pipette 10 ml of 0.01N KIO_3 into a 250 ml beaker;

(ii) add 90 ml of distilled water, washing down quantitatively the KIO_3;

(iii) add 1 ml of KI - NaOH solution and stir well;

(iv) immediately before titration add slowly 2 ml of conc. H_2SO_4(A.R.) with gentle stirring;

(v) titrate the liberated iodine against 0.01N $Na_2S_2O_3$ to a starch endpoint. Repeat until the titres of successive determination agree to within 0.05 ml.

After standardization of the thiosulphate, titrate 100 ml aliquots of the samples. Run a duplicate titration on at least every tenth sample.

(c) Reagent blank

When all the reagents have been prepared and only the best quality chemicals used, it is still recommended that a test be performed on interfering substances which they might contain. Add to 100 ml of distilled water all reagents in reverse order in which they are employed in the actual determination of dissolved oxygen. That is, 2 ml sulphuric acid is added to the water followed by 1 ml of the alkali-iodide reagent. At this point the solution should be distinctly acid. Then 0.5 ml of manganous sulphate is introduced. Allow to stand for 10 minutes, add starch indicator and titrate any liberated iodine with the thiosulphate. This is a test for oxidizing iodate, hypoiodate in alkali-iodide reagent, ferric salts in manganese sulphate or nitrogen oxides in sulphuric acid. If no free iodine shows at this stage, add a drop (or more if required) of KIO_3 solution to test for reducing sulphur dioxide which could be contained in the sulphuric acid. The sulphuric acid is of the required purity if the blue starch-iodine colour shows immediately after the first drop of KIO_3.

(d) Calculation of result

Dissolved oxygen = $\frac{A}{S}$ x 5.64 ml/litre

where A = ml of $S_2O_3^{2-}$ required to titrate 100 ml sample

S = ml of $S_2O_3^{2-}$ required to titrate 10 ml .01N IO_3^-

References

1. Thompson, T.G. and Robinson, R.J. (1939).- Notes on the determination of dissolved oxygen in seawater. J. mar. Res., 2 : 1-8.

2. Jacobsen, J.P. (1950).- A review of the determination of dissolved oxygen in seawater by the Winkler Method. Publs scient. Ass. Oceanogr. phys., 11.

3. Green, E.T. and Carritt, D.E. (1967).- New tables for oxygen saturation of seawater. J. mar. Res., 25, 140-7.

4. DISSOLVED ORTHOPHOSPHATE

Method 1 - SINGLE SOLUTION METHOD (recommended)

Reagents and Apparatus

(a) Molybdate solution. Dissolve 15 g of A.R. ammonium paramolybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, preferably finely crystalline, in 500 ml of distilled water. Keep in a plastic bottle out of direct sunlight. The solution is stable for a few weeks.

(b) Sulphuric acid solution. Add 140 ml A.R. conc.H_2SO_4 to 900 ml of distilled water and mix, taking the usual precautions.

(c) Ascorbic acid solution. Dissolve 5.4 g of high quality ascorbic acid, $C_6H_8O_6$, in 100 ml of distilled water. Prepare this solution on the day it is to be used.

(d) Potassium antimonyl tartrate solution. Dissolve 0.34 g of high grade potassium antimonyl tartrate, $KSbO.C_4H_4O_6$, in 250 ml of water, warming if necessary. Stored in a plastic or glass bottle, the solution is stable for many months.

(e) Mixed reagent. Mix together 100 ml ammonium molybdate, 250 ml sulphuric acid, 100 ml ascorbic acid, and 50 ml potassium antimonyl tartrate solutions. Prepare shortly before use and discard any excess. Keep for no longer than 6 hours.

(f) Standard phosphate solution. Dissolve 0.439 g of desiccated potassium dihydrogen phosphate, KH_2PO_4(A.R.), in distilled water and make up to 1000 ml. Add a few drops of chloroform and store in a dark bottle. This solution contains 100,000 µg PO_4^{3-}-P/litre. Prepare 1 litre of PO_4^{3-} solution containing 1000 µg PO_4^{3-}-P/litre by diluting 10.0 ml of the above stock. The dilute solution should not be kept for longer than 3 months.

(g) Glassware. Clean thoroughly with conc. H_2SO_4 and rinse well. It is advisable to retain the same flasks for this determination with occasional H_2SO_4 cleaning. Apparatus not in use should be kept filled with 0.1% v/v H_2SO_4 in distilled water solution.

(h) Spectrophotometer. 10 cm cuvettes.

Procedure

Carefully make up reference solutions containing 0, 2, 5 and 7 µg PO_4^{3-}-P by taking 0, 2, 5 and 7 ml of the 1000 µg PO_4^{3-}-P/litre standard solution and diluting to 100 ml with distilled water.

Warm all samples to a temperature between 15 and 30°C in a thermostatically controlled water bath and measure the absorbance of samples to obtain a turbidity correction.

To 100 ml of each sample and to the reference solutions add 10± 0.5 ml of mixed reagent from a 25 ml measuring cylinder and mix at once.

After 10 minutes and within 2-3 hours measure the absorbance of the solutions in a 10 cm cuvette against distilled water at a wavelength of 885 nm.

Notes

1. The samples should preferably be analysed within an hour of collection and certainly before 6 hours has elapsed. Otherwise they must either be frozen or kept in iodine-impregnated plastic bottles(3).
   The sample may be frozen by careful immersion in a mixture of solid CO_2 and alcohol and/or stored in a deep-freeze cabinet. To impregnate plastic bottles with iodine, fill them with a solution containing 8% KI and 5% I_2 and allow to stand for one week. Then wash the bottles with distilled water. The iodine preservation technique cannot be used if the stannous chloride method is to be used to determine the phosphate. (Method 2).
2. The effect of temperature on colour development is small, and in many instances there may be no need to warm the samples first.
3. Turbidity measurements, if consistent, need not be performed on every sample.
4. An alternative to preparing a daily requirement of ascorbic acid solution is to make up a larger quantity (27 g dissolved in 500 ml of distilled water) and store, frozen solid in a plastic bottle. Thaw for use and refreeze the remainder at once. With this treatment the reagent is stable for several months.

5. Orthophosphate - phosphorus levels in surface waters are frequently $<10\mu g/litre$, but at depths >100 m may range up to 60 µg/litre or more.

6. "Dissolved orthophosphate" is also variously described as "reactive phosphorus" or "inorganic phosphate". It includes labile organic phosphorus as well as the soluble phosphate ions. Since the particulate matter in seawater is unlikely to contain these forms of phosphorus, it matters little whether the determination is made on unfiltered or filtered water, although for reasons of turbidity and stability the determination on filtered water is becoming favoured.

   The unreactive phosphorus (organic and inorganic) level in seawater is found by difference: it equals the total phosphorus determination minus the dissolved orthophosphate determination.

7. There is no "salt error" in this method. A given quantity of PO_4^{3-}-P develops the same absorbance in both distilled and seawater.

References

1. Murphy, J. and Riley, J.P. (1962).- A modified single solution method for the determination of phosphate in natural waters. Analytica chim. Acta, 27, 31-36.

2. Strickland, J.D.H. and Parsons, T.R. (1968).- A practical handbook of seawater analysis. Bull. Fish. Res. Bd Can., 167, Section II.2.1.

3. Heron, J. (1962).- Determination of phosphate in water after storage in polyethylene. Limnol. Oceanogr. 7, 316-321.

4. DISSOLVED ORTHOPHOSPHATE

Method 2 - STANNOUS CHLORIDE REDUCTION

Reagents and Apparatus

(a) Molybdate solution (10% w/v). Dissolve 25 g A.R. ammonium paramolybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, in distilled water and make up to 250 ml. Store in a plastic bottle out of direct sunlight. Stable for several days.

(b) Sulphuric acid (50% v/v). Add 500 ml A.R. H_2SO_4 (arsenic free) to 500 ml of distilled water with stirring and cooling.

(c) Molybdate reagent. Add, with stirring, 100 ml of 10% ammonium molybdate solution to 300 ml of the 50% H_2SO_4 and store in the dark. Not more than 400 ml of this solution should be made at a time as it is unstable over a long period of time.

(d) Concentrated stannous chloride solution (40% w/v). Dissolve 40 g A.R. $SnCl_2.2H_2O$ in A.R. HCl to a total volume of 100 ml. Keep under liquid paraffin in a burette assembly. Only clear solution should be used.

(e) Dilute stannous chloride solution (1% w/v). Deliver 0.25 ml of the conc. $SnCl_2$ solution from the burette into a 10 ml measuring cylinder and dilute to 10 ml with distilled water. Transfer to a stoppered bottle. This solution is unstable; do not keep for more than half a day.

(f) Standard phosphate solution. Dissolve 0.439 g of desiccated potassium dihydrogen phosphate, KH_2PO_4(A.R.), in distilled water and make up to 1000 ml. Add a few drops of chloroform and store in a dark bottle. This solution contains 100,000 µg PO_4^{3-}-P/litre. Prepare a standard PO_4^{3-} solution containing 1000 µg PO_4^{3-}-P/litre by diluting 10 ml of the above stock to 1 litre. The dilute solution should not be kept longer than 3 months.

(g) Burette assembly comprising a 1 ml capacity, 2-way tap, burette, and a bulb bellows mounted in the stopper of a dark glass bottle. When air is forced into the bottle above the liquid paraffin, $SnCl_2$ solution is displaced up into the burette.

(h) Syringe assemblies: 20 ml capacity for molybdate reagent, 2 ml capacity for dilute $SnCl_2$. Mount with barrel in the horizontal position. A screw mechanism moves the piston forward when dispensing solutions. See note 2.

(i) Spectrophotometer. 4 cm cuvettes.

Procedure

Carefully make up reference solutions containing 0,2,5 and 7 µg PO_4^{3-} - P by taking 0,2,5 and 7 ml of the 1000 µg PO_4^{3-} - P/litre standard solution and diluting to 100 ml with distilled water. To each add 1 ml of molybdate reagent from the syringe dispenser, shaking well after each addition. After 2 minutes (to allow the phosphomolybdate complex formation) add 0.1 ml of the 1% $SnCl_2$ solution and shake again.

Allow to stand - further shaking may flocculate the colloidal colour complex. Not less than 4 or more than 10 minutes later read the absorbance in 4 cm cuvettes at 700 nm. Compare all solutions against distilled water. If the cuvettes are not optically matched make a cell correction.

After obtaining acceptable readings for blank and standards and making sure that samples are not turbid, proceed with the addition of reagents to 100 ml aliquots of samples. These should be at the same temperature as the reference solutions.

Samples showing signs of pollution by bottom sediment or debris, or otherwise discoloured, should be filtered or decanted before processing. To measure the turbidity, add molybdate reagent to 100 ml of filtered sample in a conical flask, shake well and read the absorbance (= "turbidity") against distilled water. Return the contents of the cells to the conical flask, add the $SnCl_2$ reagent and repeat the reading with the blue colour developed (="turbidity + phosphate").

Notes

1. Orthophosphate - phosphorus levels, especially in surface waters, are frequently $< 10 \mu g/litre$, but at depths greater than 100 m may range up to 60 µg/litre or more.

2. The phosphate reagent dispensers used in this laboratory are: one all-glass 20 ml "Summit" side-tip syringe and one 2 ml "Eva" brand syringe, together with a revolution-counting device on each, all horizontally mounted in ring-clamps on a stainless steel base. Before use, air bubbles must be expelled from the barrel or delivery tip by tipping the syringes upside down. Then clamp the syringes in position and set the counter to zero. The volume of reagent is not read from the horizontally positioned syringe-barrel, but counted off in the number of turns made by the knob driving the syringe piston. One revolution of the screw on the end of each syringe delivers 1.0 ml and 0.1 ml of reagent respectively. (However 25 ml and 1 ml burettes may be used.)

3. The samples should preferably be analysed within an hour of collection, and certainly before 6 hours. See Method 1, Note 1.

4. It is convenient to treat samples in batches of 3 to 6 at a time - adding reagents, allowing the colour to develop and reading the absorbance values - unless the room (and sample) temperature is greater than 25°C. At higher temperatures the colour develops (and then fades) faster, and the absorbance must be measured not less than 3 and not more than 9 minutes after addition of the stannous chloride. In this case samples should be treated singly or, at most, 3 at a time.

5. Repeat the calibration procedure daily in the laboratory, and, when at sea, at each station. Draw a calibration graph by plotting colour units as a function of PO_4^{3-}-P concentration. Any big variation in the slope or linearity of the calibration line generally denotes faulty chemicals, incorrect preparation of reagents, or a deterioration of the primary standard. Temperature also affects the slope of the calibration line.

6. See Note 6 appended to Dissolved Orthophosphate - Method 1.
7. Salt error. The colour developed in seawater is less than that developed in distilled water for the same amount of PO_4^{3-}-P. It has been found that results obtained by this method on seawater should be multiplied by 1.15 to give the true phosphate concentration.

5. TOTAL PHOSPHORUS

Method 1 - PERSULPHATE OXIDATION

Reagent and Apparatus

(a) Potassium persulphate, $K_2S_2O_8$, A.R. quality.

(b) Apparatus as in Section 4, Method 1 or 2.

Procedure

Measure 100 ml of each sample, filtered or unfiltered as required, into clean, conical flasks, previously rinsed with distilled water.

Add 1 g (or sufficient to effect satisfactory oxidation) of solid $K_2S_2O_8$ to each flask. Cover with a watchglass or inverted small beaker and either heat in a boiling water bath for an exact period of time - 1 hour is usually sufficient -, or place in an autoclave at 125°C for 30 minutes.

Cool, and replace evaporation losses by carefully topping up with distilled water to 100 ml.

Analyse for dissolved orthophosphate as in Section 4, Method 1 or 2.

Blank test on reagent

The oxidation procedure should be carried through with a new batch of $K_2S_2O_8$ using distilled water, and then followed by the phosphate analysis.

Notes

1. The concentration of total phosphorus in seawater may reach as high as 70-75 µg per litre, but it is usually much less than this in the surface zone (0-100 m).
2. As opposed to losses while heating on a water bath, the water vapour losses during autoclaving may be negligible, although this point should be checked by individual workers for their own conditions.

Reference

1. Menzel, D.W. and Corwin, N. (1965).- The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulphate oxidation. Limnol. Oceanogr. 10, 280-1.

5. TOTAL PHOSPHORUS

Method 2 - PERCHLORIC ACID DIGESTION

Reagents and Apparatus

(a) 70-72% A.R. perchloric acid, $HClO_4$.

(b) Ammonia solution. Dilute 60 ml of concentrated ammonia to 1 litre with distilled water.

(c) Sand trays.

(d) Apparatus as in Section 4, Method 1 or 2.

Procedure

Deliver 100 ml aliquots of the well-shaken samples, into 150 ml conical flasks which have been rinsed with distilled water. Add 6 drops (0.3 ml) of $HClO_4$ to each and transfer to a high temperature sand tray (200°C). Digest down without vigorous boiling until crystallization has commenced. Then transfer to a low temperature sand tray (110°C) and digest without spattering to dryness.

After cooling, add 100 ml of distilled water followed by 1 drop of phenolphthalein indicator. If the solution is alkaline, bring back to the acid condition with the dropwise addition of $HClO_4$. Cover the flasks with watch glasses and allow to stand overnight or until the solution of salts is complete; this can be hastened by gentle swirling of the flasks.

Neutralize the samples with ammonia. Then analyse for dissolved orthophosphate as in Section 4, Method 1 or 2.

Blank tests on reagents

Whenever a new batch of perchloric acid or ammonia is to be used, deliver 0.3 ml of the acid into 100 ml of distilled water in one of the digestion flasks, evaporate to dryness, refill with 100 ml of distilled water and neutralize with ammonia. Then treat as for the other samples.

6. REACTIVE SILICATE

Method 1 - FORMATION OF REDUCED BETA SILICO-MOLYBDATE

Reagents and Apparatus

(a) Water free of silica. Collect from a glass or quartz still which has been in use for some years. Alternatively, use distilled water passed through an anion-exchange resin column. Store in high density (HD) polythene containers.

(b) Standard silicate solution. Weigh 0.960 g of sodium silicofluoride, Na_2SiF_6, (Fisher Certified Reagent Cat. No. S-410) in a nickel crucible or plastic beaker. Crush any lumps and dissolve in 100-200 ml of water free of silica by stirring with a nickel spatula. Using water free of silica, transfer the solution and beaker washings to a 1000 ml volumetric flask and make up to the mark. Mix well and transfer quickly to high density polythene bottles. The solution picks up silica rapidly from glass and should not be kept in the flask for more than a few minutes. The solution is stable indefinitely in H.D. polythene bottles.
1 ml contains 5 µ mole of Si (140 µg Si).

(c) Molybdate solution. Place 4.0 g of A.R. ammonium paramolybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, in a 1 litre plastic beaker and use about 300 ml of water free of silica to dissolve the salt. Add 12.0 ml of concentrated hydrochloric acid, mix and make the volume up to 500 ml with water free of silica.

(d) Metol-sulphite solution. Dissolve 6 g of A.R. sodium sulphite, Na_2SO_3, in 500 ml of water free of silica and then add 10 g of "metol" (p-methylaminophenol sulphate, $CH_3NHC_6H_4OH.H_2SO_4$). When the metol has dissolved, filter the solution through filter paper. Store in a clean glass bottle and stopper tightly. This solution keeps for no more than one month.

(e) Saturated oxalic acid solution. Shake 50 g of A.R. oxalic acid crystals, $(COOH)_2.2H_2O$, with 500 ml of water free of silica. Decant the supernatant solution for use. The solution may be stored in a glass bottle and is stable indefinitely.

(f) Sulphuric acid solution, 50% v/v. Carefully pour 250 ml of A.R. concentrated sulphuric acid H_2SO_4 into about 250 ml of water free of silica. Cool and make the volume up to 500 ml with a little more water.

(g) Reducing reagent. Mix 100 ml of metol-sulphite solution with 60 ml of saturated oxalic acid solution. Add slowly with mixing, 60 ml of 50% sulphuric acid solution and make the final volume of the mixture up to 300 ml with water free of silica. This reagent should be prepared immediately before use.

(h) Artificial seawater. Dissolve 25 g of NaCl A.R. and 8 g of magnesium sulphate, $MgSO_4.7H_2O$, A.R. in 1 litre of water free of silica. This solution is also best prepared shortly before use. If kept for any time, it must be stored in a polythene container.

(i) Set of plastic containers. One is required for each silicate determination. H.D. polythene bottles of at least 2 oz (60 ml) capacity are suitable.

(j) Plastic beakers.

(k) Pipettes. One of 25 ml capacity for measuring out seawater samples. Others (e.g. 1, 2, 5, 10, 20 ml) for preparing standard solutions.

(l) Automatic dispensers (optional). 10 ml capacity for dispensing molybdate solution; 15 ml capacity for the reducing agent.

(m) Spectrophotometer. 1 cm, 4 cm and 10 cm cuvettes.

Procedure

Sample solutions should be at a temperature between about 18° and 25°C.

Add 10 ml of molybdate solution to a clean, dry (or drained) polythene bottle. Add 25 ml of the seawater sample, mix by swirling, and allow the mixture to stand for 10 mins (but for no more than 30 mins).

Add 15 ml of the reducing reagent rapidly and mix immediately.

Allow the solution to stand for 2 to 3 hours then read the absorbance at 810 nm. For concentrations of 0-20 μ mole Si/litre

use 4 or 10 cm length cells, and for higher values use 1 cm cells.

The colour is stable for 6 hours.

Standards

A secondary standard is prepared by diluting 4 ml of the standard silicate solution to 200 ml with artificial seawater.

1 ml contains 0.10 μ mole of Si. (2.8 μg Si).

Standard solutions corresponding to the sample values expected are prepared by diluting 5, 10, 25, 50, 75, and 100 ml aliquots of this secondary standard to 100 ml with artificial seawater (see Note 2).

For 125, 150, 200 μ mole/l standards dilute original standard to 100 ml with artificial seawater.

Take 25 ml volumes of these standards and treat as under "Procedure".

These standards should be prepared and treated quickly as the reactive silicate content decreases in a few hours due to polymerization.

Blank determinations should be done on distilled water free of silica for unknowns, and on artificial seawater for standards.

Notes

1. "Reactive silicate" comprises ionized silicate, molecularly dispersed silicon dioxide. SiO_2, and the less highly polymerized part of the colloidal material. It can be utilized by diatoms.
2. Levels of reactive silicate in the sea range from 0-200 μg Si/litre in surface waters up to 6000 μg Si/litre in some deep ocean waters.
3. Samples may be analysed immediately or preserved with mercuric chloride, $HgCl_2$, as for nitrates (see Section 7, Method 2) and analysed later. In the latter case 0.5 ml of 5% w/v $HgCl_2$ should also be added to the distilled water blanks. Alternatively samples frozen at -20°C keep for many months without change in silicate concentration provided the latter is less than about 50 μ mole/litre.
4. Samples of seawater for silicate analysis should not be stored in glass bottles prior to analysis. Waxed glass or polythene containers are suitable. Unpreserved samples may be kept for a day before analysis provided they are kept cool and in darkness.

5. This method for the determination of reactive silicate as described is considered (see Reference 2) to give more reliable results than that given under Method 2.

References

1. Strickland, J.D.H. and Parsons, T.R. (1968).- A practical handbook of seawater analysis. Bull. Fish. Res. Bd Can., 167, Section II. 5.

2. Liss, P.S. and Spencer, C.P. (1969).- An investigation of some methods used for the determination of silicate in sea water. J. mar. Biol. Assoc. U.K. 49: 589-610.

6. REACTIVE SILICATE

Method 2 - FORMATION OF REDUCED ALPHA SILICO-MOLYBDATE

Reagents and Apparatus

(a) Water free of silica. Collect from a glass or quartz still which has been in use for some years. Alternatively use distilled water passed through an anion-exchange resin column. Store in H.D. polythene containers.

(b) Standard silicate solution. Weigh 0.960 g of sodium silicofluoride, Na_2SiF_6 (Fisher Certified Reagent Cat. No. S-410) in a nickel crucible or plastic beaker. Crush any lumps and dissolve in 100-200 ml water free of silica by stirring with a nickel spatula. Using water free of silica, transfer the solution and beaker washings to a 1000 ml volumetric flask, and make up to the mark. Mix well and transfer quickly to H.D. polythene bottles. The solution picks up silica rapidly from glass and should not be kept in the flask for more than a few minutes. The solution is stable indefinitely in H.D. polythene bottles. 1 ml contains 5 µ mole Si or 140 µg Si.

(c) Monochloracetic acid solution. Dissolve 100 g of pure acid, $CH_2ClCOOH$, in water free of silica and make up to 1 litre. Store in polythene bottles. Stable.

(d) Molybdate solution. Dissolve 120 g of A.R. sodium molybdate dihydrate, $Na_2MoO_4.2H_2O$, in 1 litre of water free of silica. This solution may need to be filtered after a few days. The solution is stable indefinitely in H.D. polythene bottles.

(e) Reducing reagent. 20 g p-methylaminophenol sulphate, $CH_3NHC_6H_4OH.H_2SO_4$, (metol), 12 g sodium sulphite heptahydrate, $Na_2SO_3.7H_2O$, and 10 g sodium oxalate, $Na_2C_2O_4$, dissolved in 1 litre of water free of silica. Although it is claimed that this reagent is stable for one month, it is best prepared immediately prior to use.

(f) Artificial seawater. 25 g NaCl and 8 g $MgSO_4.7H_2O$ dissolved in 1 litre of water free of silica. This solution is also best prepared shortly before use. If kept for any time it must be stored in a polythene container.

(g) High density polythene bottles.

(h) Plastic beakers.

(i) Plastic measuring cylinders.

(j) Pipettes : 1 ml, 2 ml, 5 ml, 10 ml, 20 ml.

(k) Automatic dispensers: 1 ml, 5 ml.

(l) Spectrophotometer. 1 cm and 4 cm cuvettes.

Procedure

Dispense 50 ml aliquots of each sample into 100 ml polythene bottles to which 1 ml of $CH_2Cl\ COOH$ solution has been added. Add 1 ml of molybdate solution. Shake the mixture and allow to stand for at least 1 hour. This allows time for the formation of the alpha silico-molybdic acid. If desired the samples may stand for up to 6 hours.

Now add 5 ml of reducing agent, shake the sample, and allow to stand for at least 12 hours.

Read the absorbance of the samples using a Spekker absorptiometer with red filter 608 or a spectrophotometer at 640 nm. For concentrations 0-50 μ mole/litre use 4 cm length cells, and for higher values use 1 cm cells. The colour is stable for up to 60 hours.

Standards

A secondary standard is prepared by diluting 4 ml of the stock silica standard solution to 200 ml with artificial seawater. 1 ml contains 0.10 μ mole of Si.

Standard solutions corresponding to 0, 1, 2, 5, 10, 20, and 50 μ mole/l are prepared by diluting this secondary standard with artificial seawater (see Note 2 and Method 1). Prepare standards of higher concentration, if required, by diluting original standard.

These standards should be prepared and treated quickly as the reactive silicate content decreases in a few hours due to polymerization.

Blank determinations should be done on distilled water free of silica for unknowns, and on artificial seawater for standards.

Notes

1. "Reactive silicate" comprises ionized silicate, molecularly dispersed silicon dioxide, SiO_2, and the less highly polymerized part of the colloidal material. It can be utilized by diatoms.
2. Levels of reactive silicate in the sea range from 0-200 µg Si/litre in surface waters up to 6000 µg Si/litre in some deep ocean waters.
3. Samples may be analysed immediately or preserved with mercuric chloride, $HgCl_2$, as for nitrates and analysed later. In the latter case, 0.5 ml of 5% w/v $HgCl_2$ should be added to the distilled water blanks.

Reference

1. Grasshof, K. (1964).- On the determination of silica in sea water. Deep-Sea Res., 11; 597-604.

7. NITRATE

Method 1 - CADMIUM REDUCTION

Reagents and Apparatus

(a) Coarse cadmium powder (Merck's cat. no. 2001). Sieve through a No. 25 sieve (B.S. opening 0.0236") and use the coarse fraction.

(b) Copper sulphate solution. Prepare an approx. 2-5% solution in distilled water.

(c) Hydrochloric acid solution (10% v/v).

(d) Concentrated ammonium chloride (35% w/v) solution. Dissolve 175 g of A.R. NH_4Cl in 500 ml of distilled water and filter it into a plastic or glass storage bottle.

(e) Dilute ammonium chloride (0.85% w/v) solution. Dilute 50 ml of the concentrated solution to 2 litres with distilled water.

(f) Standard nitrate solution. Weigh out 0.202 g of A.R. potassium nitrate, KNO_3, and make up to 1 litre with distilled water. This stock solution is quite stable. Shortly before use, take 10 ml of this stock, add 50 ml of conc. NH_4Cl solution and dilute with distilled water to 2 litres. This working solution contains 10 μ mole/litre of nitrate in 0.85% NH_4Cl solution.

(g) Standard nitrite solution. Make up 2 litres containing 138 mg A.R. sodium nitrite, $NaNO_2$, in distilled water. With a few drops of chloroform it will keep for several weeks in a dark bottle. On the day it is to be used, take 5 ml of the stock solution, add 12.5 ml of conc. NH_4Cl solution and dilute to 500 ml. This standard contains 10 μ mole/litre of nitrite in 0.85% NH_4Cl solution.

(h) Sulphanilamide solution. Prepare a 1% w/v solution of sulphanilamide, $C_6H_4(NH_2)SO_2NH_2$, in 10% HCl solution. This reagent is stable.

(i) N(-1-naphthyl) ethylenediaminodihydrochloride, $C_{12}H_{14}N_2.2HCl$, solution. Make up a 0.1% w/v solution with distilled water and store in a dark bottle. It is stable for up to a month.

(j) Glass wool.

(k) Wash bottle.

(l) Automatic pipettes (optional). For dispensing the colour-developing reagents.

(m) Glass columns and supports (rack or clamps).
One convenient design of a prepared column is shown in Fig.1.

(n) Spectrophotometer. 1 cm cuvettes.

Procedure

(a) Preparation of Column

11 to 12 ml of the coarse cadmium powder is needed for one column. This volume, or a multiple of it depending on how many columns are required, (4 or 5 are a convenient number to handle at one time), is placed into a 150 ml conical flask and covered with 30 ml of distilled water.

Add 30 ml of 10% HCl solution to the flask, and shake gently for about 2 minutes. The cadmium powder turns from dark grey to a bright metallic appearance. Pour the acid off, and rinse the acid-treated cadmium powder with distilled water several times, then leave covered with distilled water.

In a flask prepare a 2-5% solution of copper sulphate (50 ml is sufficient for preparing 4 columns), pour this on to the acid-treated cadmium, and shake gently. The metallic appearance of the powder turns to dull black. After 2 minutes pour the liquid off and wash the copperized cadmium powder with distilled water, avoiding exposing it to the air. With the first 2 or 3 washings quite a lot of fine black copper powder comes off the prepared cadmium, turning the supernatant quite black. Very gentle shaking while washing will produce a clear supernatant after 5 or 6 rinses.

If cadmium powder is needed for more than one column, transfer the treated material with the help of a wash bottle into lots of 11 ml in 25 ml graduated cylinders, letting the washing water overflow over the cylinders; try not to expose the prepared cadmium to the air.

Set up the columns on a rack or in retort stands. With a glass rod, push a loose plug of glass wool to the bottom of each column, then fill with distilled water. Make sure there are no air bubbles trapped. With a jet of water from a wash bottle transfer the copper-cadmium mixture from the cylinders. As this is done, tap the outside of the columns to settle the powder firmly. Cover the powder with glass wool.

At no time should the copperized cadmium powder be exposed to the air.

The newly-packed column has to be conditioned by flushing through with half a litre of dilute NH_4Cl (0.85%) solution followed by a further half litre of standard nitrate solution. Collect 20 ml of the reduced nitrate solution near the end of this procedure. The column must then be checked for over-reduction using the standard nitrite solution. Put about 75 ml of the latter through the column, collecting a 20 ml sample as described under "Analytical Procedure". Develop the colour. Over-reduction is indicated if the absorbance values obtained for the two 20 ml samples are different. Correct by passing through more dilute NH_4Cl solution and standard nitrate solution. Further checks for satisfactory reduction depend on the frequency of column use.

A 10 μ molar standard solution of nitrate should, on a good instrument, give a nett absorbance (standard minus blank) of about 0.490 for a 1 cm cell. Significantly lower values are an indication of over-reduction taking place, or of an inefficient column.

Freshly prepared columns do not give steady results until one or two days after packing.

Between runs keep columns covered with dilute NH_4Cl solution.

(b) Analytical Procedure

Add 2.5 ml of conc. NH_4Cl (35%) to a 100 ml volumetric flask and make up to the mark with sample. Mix. Displace the previous dilute NH_4Cl or sample solution left in the column with two or three lots of 25 ml rinses and discard the effluent. Then pass through the final 25 ml of the sample and collect 20 ml in a 25 ml graduated cylinder. Transfer into a 50 ml test tube.

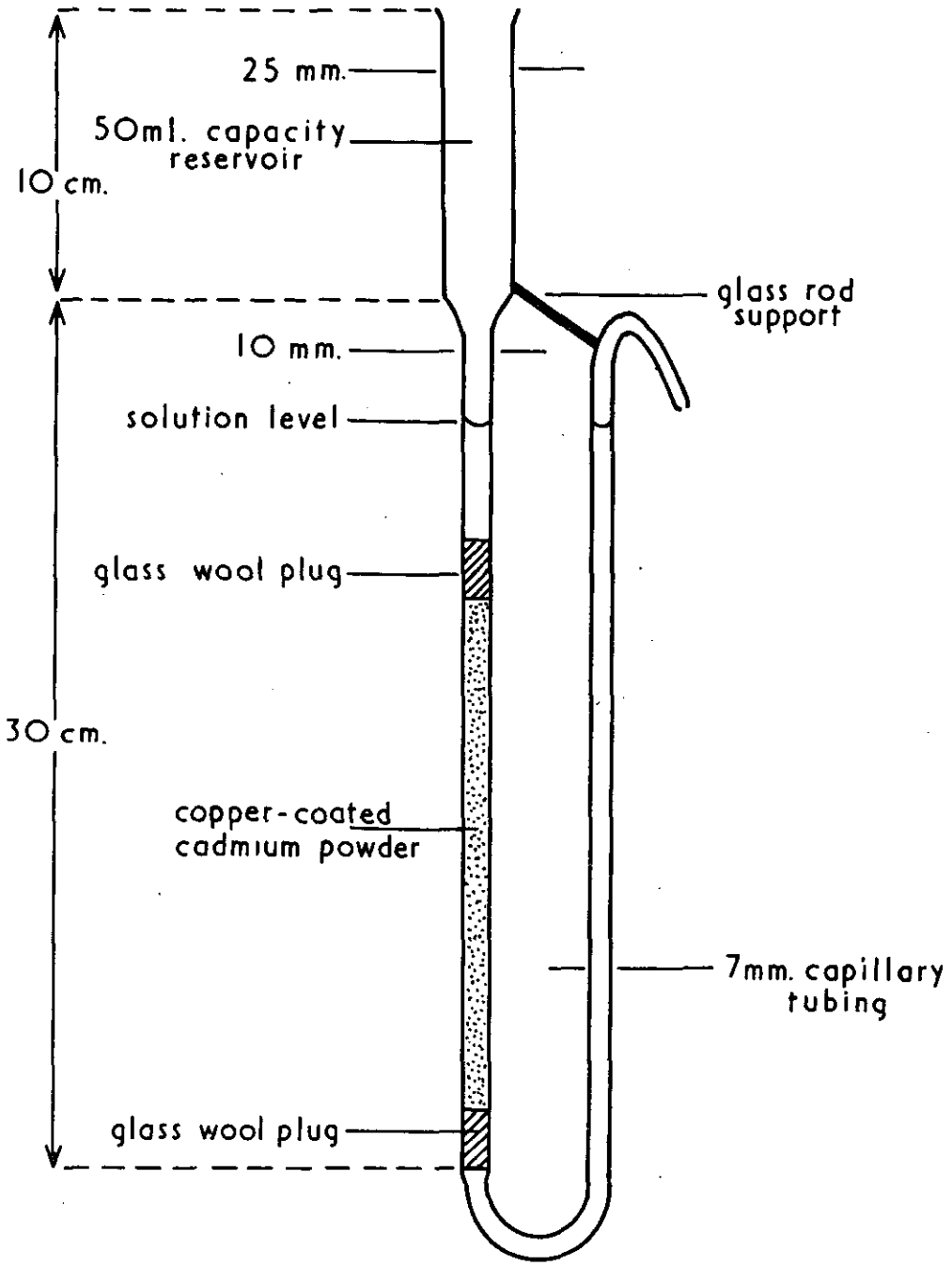

Figure 1.- Nitrate reduction column.

Within 2 hours, add 0.5 ml of 1% sulphanilamide in 10% HCl to each and mix. Not before 2 minutes or after 8, add to each tube 0.5 ml of 0.1% N-1-naphthyl ethylene-diaminohydrochloride and mix.

Allow to stand for 15 minutes, then read the absorbance at 543 nm using 1 cm cells. The colour is stable for at least 2 hours.

(c) Standards

Standard solutions containing say, 1, 5 and 10 μ mole NO_3^-/litre, (equivalent to 14, 70 and 140 μg NO_3^--N/litre), may be prepared by diluting the 10 μ molar nitrate standard with dilute NH_4Cl solution. The absorbance values for the standards of different columns should be within 2% of each other.

Notes

1. Concentrations of nitrite and nitrate in seawater generally lie in the range:-

   NO_2^-: 0.1 to 50 μg NO_2^-- N/litre;

   NO_3^-: 1 to 500 μg NO_3^- - N/litre;

   both increasing with depth. Commonly, nitrate is present at levels 10 times greater than nitrite.
2. The method described above is suitable for nitrate (and also nitrite) determinations on fresh or frozen samples. Analysis of freshly collected water should not be delayed, but the samples are stable for up to 12 hours if they are kept cold and in the dark.
3. The purpose of the HCl treatment is to clean and to increase the surface area of the cadmium powder.
4. Mercuric chloride, $HgCl_2$, interferes with the copper coated cadmium column and therefore precludes the use of this method on preserved samples.
5. The column reduces about 5% of the nitrite, and more if the column is somewhat deactivated. Correction for this is not needed if the column is calibrated by passing through a standard nitrate solution.

6. Column design is flexible. The one described here has a satisfactory throughput, (about 10 ml per minute), and the syphon arrangement minimizes accidental exposure of the copper-cadmium to air and avoids the operator having to manipulate taps.
7. Columns need not be washed with blank solution between consecutive samples of a batch.
8. Slight differences in flow rate do not affect the reducing properties of a column. However when using more than one column it is important to maintain comparable flow rates for convenient working. Having the same volume of copper-cadmium powder in each tube helps to minimize such variations in flow.
9. When the flow rate drops off significantly, columns require reconditioning. During use, the copper-cadmium particles become finer, fill more voids, and restrict the passage of solution as also does clogging by debris in the water. Phosphates and arsenates at the relatively high levels used in some media also react and clog the system. Sieve out the undersized fraction and treat with HCl again, etc.
10. If nitrate levels are over 220 µg N/litre, diluted samples should be used, otherwise the reducing capacity of the column is over-taxed. The absorbance reading for higher concentrations may reach the upper, more crowded part of the scale, and also the azo dye may precipitate.

References

1. Morris, A.W. and Riley, J.P. (1963).- The determination of nitrate in sea-water. Analytica Chim. Acta, 29, 272-9.
2. Grasshof, K. (1964).- Zur Bestimmung von Nitrat in Meer- und Trinkwasser. Kieler Meeresforsch. 20, 5-11.
3. Wood, E.D., Armstrong, F.A.J., and Richards, F.A. (1967).- Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J. mar. Biol. Ass. U.K., 47; 23-31.

7. NITRATE

Method 2 - STRYCHNIDINE METHOD

Reagents and Apparatus

(a) Strychnidine reagent. Dissolve 0.640 g strychnidine (Gallard Schlesinger Corp. N.Y.) in 1 litre of nitrate-free conc. H_2SO_4. This solution should be only a pale pink or straw colour, and should be kept for at least 2 weeks before use.

(b) Nitrate-free H_2SO_4. Prepare by taking 500 ml A.R. H_2SO_4 (arsenic free) in a 1 litre Erlenmeyer flask, adding 1 g A.R. $(NH_4)_2SO_4$, and heating to 250°C until copious fumes of SO_2 are evolved.

(c) Artificial seawater. Dissolve 70 g A.R. NaCl in distilled water, add 20 ml of 5% w/v $HgCl_2$ solution, and make up to 2000 ml.

(d) Mercuric chloride solution. Dissolve 5 g A.R. $HgCl_2$ in 100 ml of cold distilled water. Store in a plastic bottle.

(e) 50% v/v H_2SO_4 in artificial seawater. Carefully mix equal volumes of artificial seawater and conc. sulphuric acid, observing the normal precautions of adequate cooling and ventilation. As this solution is used for dilution only, immediately prior to reading, one may use A.R. H_2SO_4 (arsenic free) instead of nitrate-free H_2SO_4.

(f) Standard nitrate stock solution. Dissolve 0.607 g A.R. $NaNO_3$ (or 0.7214 g A.R. KNO_3) in distilled water. Add 1 ml of 5% $HgCl_2$ solution and adjust to 1 litre in a volumetric flask. This solution contains 100,000 µg of NO_3^-- N/litre.

(g) Dilute standard solution. Using artificial seawater dilute the stock solution to contain 2000 µg of NO_3^- -N/litre.

(h) Erlenmeyer flasks.

(i) PVC compartmented box.

(j) 50 ml polythene bottles.

(k) Spectrophotometer. 5 mm cuvettes.

Procedure

Dispense 5 ml samples into 30 ml Erlenmeyer flasks. Chill to approximately 5°C in a cold room or refrigerator. Samples are usually

dispensed in the morning and stored in the cold room until late afternoon.

After cooling, add 5 ml of strychnidine reagent slowly down the side of the flask to ensure that minimal mixing (and therefore minimal heating) occurs.

Allow samples to stand overnight (18-24 hrs) without shaking. Diffusion ensures slow mixing and uniform colour development. Samples should be contained in a polythene or PVC compartmented box with a neatly fitting lid to exclude light and air.

Read the absorbance in a spectrophotometer at 530 nm using 5 mm cells.

Standards and Blank

Use 5 ml of artificial seawater to determine the reagent blank.

Using artificial seawater make up standards corresponding to the expected sample concentrations. For shallow waters use 20, 40, 60, 80, 100 and 200 µg NO_3^- -N/litre standards. For deeper waters make up 100, 200, 300, 400 and 500 µg NO_3^- -N/litre standards.

Dispense, in triplicate, 5 ml aliquots of the set of standards appropriate to the collected samples and treat as under "Procedure".

Standards and samples having an absorbance of more than 1.0 unit on the spectrophotometer scale should be diluted to ½, 1/3, ¼ or 1/5 strength using 50% v/v H_2SO_4 in artificial seawater just prior to re-reading.

Construct a graph of standard absorbance versus concentration and read off the nitrate concentration of samples from this. (The reaction does not always follow Beer's Law; generally the graph comprises two definite straight lines with an inflexion at about 100 µg NO_3^- - N/litre).

Notes

1. Samples should be kept in 50 ml high-density polythene bottles which have been first rinsed with the sample water. Add 0.5 ml of 5% $HgCl_2$ solution to each sample. Samples so preserved show no change in NO_3^- concentration over several months.
2. The strychnidine method tends to be more inconvenient than Method 1, but may be used on fresh or frozen samples as well as on preserved ones.

Reference

1. Rochford, D.J. (1947).- The preparation and use of Harvey's reduced strychnidine reagent in oceanographic chemistry. Bull. Coun. scient. ind. Res., Melb., 220.

8. AMMONIA

Reagents and Apparatus

(a) Phenol-alcohol solution. Dissolve 10 g of reagent grade phenol, C_6H_5OH, in 100 ml of 95% v/v ethyl alcohol, C_2H_5OH.

(b) Sodium nitroprusside solution (.5% w/v). Dissolve 1 g of sodium nitroprusside, $Na_2Fe(CN)_5NO.2H_2O$, in 200 ml of water. Store in an amber bottle. The solution keeps for no more than a month.

(c) Alkaline citrate solution. Dissolve 100 g of A.R. trisodium citrate, $C_3H_4OH(COONa)_3.2H_2O$, A.R. and 5 g of A.R. sodium hydroxide, NaOH, in 500 ml of distilled water.

(d) Sodium hypochlorite solution. Use a solution of commercial hypochlorite, NaOCl. (12.5% available chlorine.) Store in a cool place; even so it decomposes to about half strength in 3 years. Old stock may be discarded or its strength checked by thiosulphate titration. It should be at least 1.5 N (5.3% available chlorine).

(e) Oxidizing solution. Mix 4 volumes of alkaline citrate solution and 1 volume of hypochlorite. This mixture is unstable and should be used the same day.

(f) Standard ammonia solution. Dissolve 45.8 mg of ammonium chloride, NH_4Cl, (best grade available) in distilled water and make up to 1 litre in a volumetric flask. A ten times dilution of this stock gives a working solution containing 1.2 µg of ammonia nitrogen per ml. It is quite stable.

(g) Reagent bottles or stoppered flasks, 100-150 ml capacity.

(h) Dilute HCl solution (about 10% v/v) for washing of glassware.

(i) Spectrophotometer. 10 cm cuvettes.

Procedure

Using a graduated cylinder measure 50 ml of each sample into 100 ml reagent bottles or stoppered flasks. Also take 3 additional 50 ml aliquots from one of the samples to prepare standards - preferably from water collected from a zone of minimal biological activity (80 metres or deeper).

Spike the standard aliquots with the standard ammonia solution; 0.25 ml, 0.50 ml and 1.00 ml (≡ 6, 12 and 24 µg NH_3-N per litre).

Add 2 ml of phenol alcohol solution, 2 ml of sodium nitroprusside solution and 5 ml of oxidizing solution in succession to each 50 ml of sample or standard, mixing thoroughly after each addition.

Allow the blue colour of indophenol to develop at room temperature, 22-27°C, for at least 1 hour. Since samples drawn from different depths may have markedly different initial temperatures, it may be preferable to use a water bath, especially for short developing times. The blue colour is stable for at least 18 hours.

Read the absorbance at 640 nm in a 10 cm cuvette, with distilled water in the reference cell.

The absorbance due to the turbidity of each seawater sample should be measured first on duplicate 50 ml samples without reagents. Treat 50 ml of de-ionized distilled water with the colour-developing reagents to get a reagent blank absorbance. This value, together with the respective turbidity absorbance, should be subtracted from the final absorbance of each sample or standard with the colour developed.

Notes

1. The concentration range of ammonia in seawater is normally about 0.1 to 2 µ mole per litre (1 µ mole = 1 µg-atom = 14 µg NH_3-N/litre).
2. Analysis should begin within 2 hours of collecting the samples. Since filtering usually introduces contamination, unfiltered samples must be used, and hence turbidity blanks must be run, as described above.
3. All glassware used must be cleaned by washing initially with warm dilute hydrochloric acid. Alternatively the HCl may be left standing in the glassware between sets of determinations. Rinse thoroughly with distilled water and then with the sample seawater. Allow to drain; do not dry. The HCl may be used repeatedly.
4. Care must be taken at all times to avoid extraneous contamination by NH_3.
   (a) Distilled water used for washing and for making up reagents should be freshly removed from a cation exchange resin column.

(b) Materials containing NH_3 should be kept and used elsewhere. This includes floor and bench cleaning agents.

(c) Samples should be handled in stoppered bottles or flasks and not be unnecessarily exposed to the atmosphere or to rubber.

(d) No smoking should be allowed in the laboratory.

5. The reagent blank absorbance should be about 0.060 in a 10 cm cuvette. The yellow colouration in the blank does not absorb at 640 nm. This 0.060 absorbance corresponds to about 0.45 µ mole NH_3-N/litre. Samples of zero ammonia content often occur and may be used as a check of the reagent blank, i.e. if the latter is higher than the lowest sample absorbance the distilled water supply is probably contaminated.

6. Sensitivity varies from day to day. The extreme range encountered by us has been 0.098 to 0.140 absorbance for 1 µ mole NH_3-N/litre.

7. Turbidity absorbance values range from 0.001 to 0.005 in relatively "clean" waters.

Reference

1. Solorzano, L. (1969).- Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14, 799-801.

9. UREA

Reagents and Apparatus

(a) Reagent (A). Dissolve 85 g of A.R. sodium dihydrogen phosphate, $NaH_2PO_4.2H_2O$, in one litre of concentrated sulphuric acid, H_2SO_4, A.R. quality.

(b) Reagent (B). Warm 100 ml of distilled water to about 60°C, and dissolve in it 5 g of diacetyl monoxime, $CH_3COC(NOH)CH_3$. Cool, and add 0.06 g of semicarbazide hydrochloride, $NH_2CONHNH_2.HCl$.

(c) Reagent (C). Dissolve 200 g of manganese II chloride, $MnCl_2.4H_2O$, and 4 g of potassium nitrate, KNO_3, in 500 ml of distilled water.

(d) Sodium chloride, NaCl. A.R. quality.

(e) Standard urea solution. Using distilled water prepare a stock solution of urea which contains 50 µg N/ml (107.26 g urea, $CO(NH_2)_2$, per litre). This is stable for several months. Dilute 100 times with distilled water to obtain a working standard containing 0.5 µg urea-N/ml. Keep only for a few weeks.

(f) Reagent bottles. Approximately 100 ml capacity (see Note 3).

(g) Thermostatically controlled water bath.

(h) Fast-running burette.

(i) Pipettes.

(j) Glass measuring spoon or ladle. (Optional).

(k) Spectrophotometer. 10-cm cuvettes.

Procedure

Place (ladle out) approximately 7½ g (the same mass to each sample) of solid NaCl in a clean 100 ml capacity reagent bottle. Add 45 ml of the seawater sample and dissolve the salt completely by swirling. (The salt takes several minutes to dissolve.)

Mix equal volumes of reagents (B) and (C).

From a burette, add 6 ml of reagent (A) to the sample. Mix by swirling. Then dispense 1½ ml of mixed reagent (B) + (C) into the sample and mix.

Stopper the reagent bottle and immerse in a stirred water bath maintained at 70°C. After 90 minutes remove the bottle and cool rapidly in running tap water.

Measure the absorbance in a 10 cm cuvette at 520 nm. The colour is stable for at least three days.

Discard any unused portion of mixed reagent (B) + (C).

Standards and Blank

Add approximately 9 g, (the same mass to each), of solid NaCl to each of four reagent bottles. Add 45, 44.5, 44, and 43 ml of distilled water, and 0, 0.5, 1, and 2 ml of the working standard to each respectively. These standards contain 0, 0.25, 0.5 and 1 µg of urea-N each.

Add reagents, develop and read the colour (faint pink) as for the samples.

From the calibration curve calculate the amount of urea-N in each sample, and multiply by $\frac{1000}{45}$ to obtain µg urea-N per litre of seawater.

Notes

1. The concentration of urea-nitrogen in seawater varies from 1 to 20 µg N/litre.
2. Reagent (B) is stable for up to a fortnight. The appearance of a slight turbidity does not seriously impair its function. Reagents (A) and (C) are quite stable. Any slight black precipitate of manganese dioxide, MnO_2, in reagent (C) may be disregarded, and avoided by using the supernatant liquid only.
3. The use of relatively thick-walled reagent bottles dissipates the heat generated after adding reagent (A). If less heat-absorbing stoppered flasks are used, the samples must be allowed to stand for a few minutes and cool to 50°C or so before adding the final mixed (B) + (C) reagent.
4. The effect of heating is important. Irregular heating or cooling produces erratic results. Above 70°C destruction of the pink colour is accelerated and low yields are obtained : below 70°C colour formation is slow.

5. The sensitivity of the method increases with the salt content. $7\frac{1}{2}$ g additions in the samples and 9 g additions to the standards are specified to produce approximately 20% NaCl solutions.

6. The suggested volume of seawater sample, 45 ml, is chosen because it is the volume required to fill one particular design of 10 cm absorption cell.

Reference

1. Newell B.S., Morgan, B. and Cundy, Jane (1967).- The determination of urea in seawater. J. mar. Res. 25, 201-2.

10. METHOD OF FILTRATION TO COLLECT PARTICULATE MATERIAL IN SEAWATER

The amount of particulate material which is collected from a sample of seawater depends on the type of filter used and the conditions employed. It is therefore important to specify these data along with the analytical results. Material which is retained by the filter is arbitrarily considered to be "particulate"; that which passes through "soluble".(1)

Whatman GF/C glass fibre filters with a disc diameter of 25 mm are convenient to handle, and produce low blank values. Their effective pore diameter seems to be about 1 µm.

If particulate COD is to be one of the parameters estimated then these filters should be heated at 500°C for 30 mins before use.

The vacuum applied should not exceed 1/4 to 1/3 of an atmosphere (8"-10" mercury gauge).

A seawater sample of about 6 litres is big enough to provide sub-samples to determine several materials on the unfiltered seawater, e.g. NH_3, urea, NO_3^-, salinity, PO_4^{3-}, SiO_2, as well as leaving enough water to filter and provide sufficient particulate material for analysis.

After drawing off any unfiltered water sub-samples as may be required, connect the 6 litre sample container to a conventional 25 mm diameter glass or plastic filter holder, (e.g. Millipore, Gelman). A sieve of nylon netting of about 200-300 µm mesh should be placed in the line to prevent gross particles and zooplankton from passing to the filter pads.

Every precaution should be taken to ensure that the particulate matter is collected evenly over the filter pad. Fill the holder above the filter with sample water before applying any vacuum. Damaged filters should not be used. Handle filters with tweezers at all times. A second fibreglass filter should be placed between a cellulose support pad on the filter holder and the top collecting filter to prevent the latter from being contaminated with material from the support pad when it is being lifted.

When filtration is complete, or when sufficient particulate material has been collected, the filter should be sucked as dry as

possible, and the slightly damp top disc lifted on to a jig where it can be precisely cut into as many as eight equal sectors, the razor blade used for the cutting being guided by slots on either side of the disc. Once again the filter disc should be protected from contamination during the sectoring by a second GF/C underneath.

Different sectors of the same filter can be analysed for different elements or used for duplicate determinations. This procedure has been shown to be statistically more reliable than using separate filters (and therefore separate water samples) for each analysis.[2] A suitable distribution could be to use 5/8 of the filter to determine plant pigments and 1/8 each for particulate nitrogen, phosphorus and oxidizable matter.

See figures 2 to 5 in the appendix (Section 17) for photographs of apparatus described in this section.

References

1. Sheldon P.W. and Sutcliffe, W.M. (1969).- Retention of marine particles by screens and filters. Limnol. Oceanogr., 14, 131, 441-4.

2. Newell B.S. and Kerr, J.D. (1968).- Suspended organic matter in the south eastern Indian Ocean. Aust. J. mar. Freshwat. Res., 19, 129-38.

11. PARTICULATE TOTAL NITROGEN (KJELDAHL DIGESTION)

Reagents and Apparatus

(a) Digestion mixture. Dissolve 50 g of A.R. potassium sulphate, K_2SO_4, in 500 ml of 10% v/v H_2SO_4 - use analytical grade materials.

(b) $HgCl_2$ (1% w/v) solution. Dissolve 1 g of A.R. mercuric chloride, $HgCl_2$, in 100 ml of distilled water.

(c) NaOH (10% w/v) solution. Dissolve 10 g of A.R. sodium hydroxide, NaOH, in 100 ml of distilled water.

(d) 0.4% (0.1N) NaOH solution. Dilute 1 vol. of (c) to 25 vols with distilled water.

(e) Thymol blue indicator solution (B.D.H brand or equivalent).

(f) Bromocresol purple indicator solution (B.D.H brand or equivalent).

(g) Test tubes, thick walled, hard glass, 20 ml capacity.

(h) Aluminium heating block drilled with holes to accept the tubes.

(i) Hot plate.

(j) Test tube shaker or mixer.

(k) Pipettes. Also one Pasteur pipette.

(l) 150 ml conical flasks.

(m) 50 ml glass stoppered graduated cylinders.

(n) Reagents and apparatus for NH_3 analysis (Section 8).

Procedure

Using tweezers, place the filter sectors (1/8 of a GF/C per sample) with adhering particulate matter into open test tubes. Add 2 ml of digestion mixture plus 1 drop of $HgCl_2$ solution to each, and heat in the aluminium block, raising the temperature slowly from 110°C to 350°C as the water evaporates. Reflux at 350°C until clear. Expose about $\frac{3}{4}$ the length of the tubes to allow refluxing.

When all the organic matter has been digested (3-5 hours), allow to cool, then carefully neutralize with concentrated and dilute NaOH. Add 1 drop of thymol blue indicator first, and after this colour change add 1 drop of bromocresol purple and neutralize further

to the faint purple endpoint. A test tube buzzer is a convenient means of mixing the tube contents during the neutralization step.

Transfer carefully, with washings, to glass-stoppered graduated cylinders and make up to 50 ml with de-ionized distilled water. Mix and then pour into clean 150 ml conical flasks. (With care the amount of solution left behind will be constant between samples). The conical flasks must be cleaned prior to use with warm dilute HCl and distilled water rinses, as in the preparation for NH_3 analysis.

The rest of the method follows that for ammonia analysis in Section 8; all reagents are the same except for the composition of the oxidizing solution. Make up the latter by mixing 8 volumes of the alkaline citrate solution, 1 volume of sodium hypochlorite solution and 1 volume of distilled water. (These proportions minimize the blank, but a longer time is required - 3 hours - to develop the indophenol colour).

It is not necessary to incubate the flasks in a water bath; leave them undisturbed on a bench, preferably covered with a cloth to exclude dust and bright light.

The glass filter fibres will sink to the bottom of the flasks during the time the colour is developing, leaving a clear supernatant from which, with care, samples free from fibres can be withdrawn with a Pasteur pipette to the absorption cell.

Read in 1 or 2 cm cuvettes at 640 nm.

Standards and Blank

Digest GF/C filters with the digestion mixture in tubes the same as for the samples. Neutralize and transfer carefully to graduated cylinders.

Make up to 50.0, 47.5, 45.0 and 40.0 ml total volume with de-ionized distilled water; mix and pour into conical flasks into which have been dispensed 0, 2.5, 5.0 and 10.0 ml of standard ammonia solution (1.2 µg NH_3-N per ml) respectively. Develop the colour and read the absorbance.

This set of standards contains 0,3,6 and 12 µg NH_3-N per flask. Only 2 standards as well as the blank need be prepared if they straddle the sample concentration values.

Notes

1. The level of particulate total nitrogen in seawater varies from 5 to 50 µg/litre depending on the fertility of the water; even more for very rich (or polluted) water.

2. Calculate the NH_3-N present in each sample flask and multiply by $\frac{8}{V.n}$, where V = volume (litres) of seawater filtered, and n = number of sectors used (usually one).

12. PARTICULATE TOTAL PHOSPHORUS

Reagents and Apparatus

(a) Potassium persulphate, $K_2S_2O_8$, solution (5% w/v). Make up fresh stock weekly from A.R. quality reagent.

(b) Sulphuric acid solution. 6N. Dilute 100 ml of A.R. conc. H_2SO_4 to 600 ml with distilled water.

(c) Molybdate solution. (2.5% w/v). Dissolve 10 g of A.R. ammonium paramolybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, in 250 ml of distilled water. Store in a plastic bottle away from direct sunlight. The solution is quite stable.

(d) Ascorbic acid solution. Prepare a 10% w/v solution of ascorbic acid, $C_6H_8O_6$, in distilled water. This solution is unstable. It may be prepared shortly before use. Alternatively it will keep for up to 10 weeks if kept frozen in a plastic bottle. Thaw for use and refreeze.

(e) Standard phosphate solution. 10 µg PO_4^{3-}-P/ml. Dissolve 439 mg of A.R. potassium dihydrogen phosphate, KH_2PO_4, in distilled water, make up to 1 litre, and then dilute the solution 10 times. To prepare a working standard, take 5 ml of the above and dilute to 50 ml in a standard flask with 5% $K_2S_2O_8$ solution. The working standard contains 1 µg PO_4^{3-}-P/ml.

(f) Colour developing reagent. Mix 1 volume each of the sulphuric acid, ammonium molybdate and ascorbic acid solutions plus 2 volumes of distilled water. Prepare freshly for each set of determinations.

(g) Screw-capped tubes. 20 ml capacity.

(h) Aluminium block drilled with holes to accept the tubes.

(i) Hot plate; water bath.

(j) Pipettes.

(k) Spectrophotometer. 1 cm cuvettes.

Procedure

Place the GF/C filter sectors into screw-capped tubes.

Add 4.0 ml of 5% $K_2S_2O_8$ solution to each, and screw the caps on.

Heat the aluminium block to 100°C. Place the tubes in and digest for 1 hour exactly. Remove and allow to cool. Add 4.0 ml of the colour developing reagent and incubate for 2 hours in a water bath at 37°C.

Read in 1 cm cuvettes at 820 nm, or, if off-scale, at 620 nm (for 1/3 sensitivity.) The colour is stable for at least 24 hours.

Standards and Blank

The level of particulate phosphate-phosphorus in seawater may vary from 0.1 to 10 µg/litre.

Prepare standards containing 0, 1 and 2 µg of PO_4^{3-}-P as follows:

To GF/C sectors in 3 tubes add 4.0 ml, 3.0 ml and 2.0 ml of 5% $K_2S_2O_8$, plus 0 ml, 1.0 ml and 2.0 ml of the 1 µg/ml PO_4^{3-}-P working standard solution respectively.

Carry through the same digestion and colour development procedure as for the samples.

Calculate the phosphorus present in each unknown sample and multiply by $\frac{8}{V.n}$ to obtain µg PO_4^{3-} -P/litre seawater, where V = volume (litres) of seawater filtered, and n = number of sectors used (usually one).

Reference

1. Chen, P.S., Toriba, T.Y., and Warner, H. (1956).- Determination of lipid phosphorus. Analyt. Chem. 28: 1756.

13. PARTICULATE CARBON (CHEMICAL OXYGEN DEMAND)

Reagents and Apparatus

(a) Standard potassium dichromate solution. Weigh out about 4.90 g of A.R. quality potassium dichromate, $K_2Cr_2O_7$, dissolve in distilled water and make up to 1 litre. The solution is perfectly stable; 4.9035 g of the salt per litre has a concentration of 0.100 N.

(b) Ferrous ammonium sulphate solution. Weigh out 9.80 g of A.R. ferrous ammonium sulphate, $(NH_4)_2SO_4.FeSO_4.6H_2O$, and dissolve in 300-400 ml of distilled water to which 20 ml of conc. H_2SO_4 has been added. Make up to 1 litre with distilled water. Its strength is nominally 0.025 N, but it must be standardized before each set of determinations, by titration against the dichromate solution. Keep for no more than 1 month.

(c) Ferroin (tri-ortho-phenanthroline ferrous sulphate) indicator solution. (B.D.H. brand indicator or equivalent.)

(d) 4.5% w/v sodium sulphate, Na_2SO_4, solution.

(e) Conc. H_2SO_4. A.R. quality.

(f) Thermostatically controlled hot plate (or aluminium block).

(g) 5 ml capacity burette, or alternatively an automatic titrimeter e.g. "Dosimat".

(h) Test tubes or beakers.

Procedure

The GF/C filters used should be precombusted at 500°C for 30 minutes to eliminate all organic contaminants.

After filtering the seawater sample, disconnect and wipe the inside surfaces of the upper part of the filter holder free of salt water. Reassemble, add 2 ml of Na_2SO_4 solution, suck gently dry, and repeat with a further 2 ml of Na_2SO_4 solution.

Dismantle. Cut up the GF/C's.

Transfer the sample (1/8 of the glass filter disc) to a tube or small beaker.

Carefully dispense 1 ml of $K_2Cr_2O_7$ solution into each sample, add 2 ml of conc. H_2SO_4, and mix.

Place the tubes/beakers on the aluminium block/hot plate at 105°C for 1 hour exactly.

Cool, and titrate the residual dichromate against the ferrous solution in the presence of one drop of ferroin indicator.

Calculation

1 ml of 1 N $K_2Cr_2O_7$ = 8 mg oxygen
= 8000 µg oxygen

Hence; Chemical Oxygen Demand
= $(N_1V_1 - N_2V_2)$ 8000 µg O_2 per sector

where N_1 = concentration of $Cr_2O_7^{2-}$ solution (normality)

N_2 = concentration of Fe^{2+} solution (normality)

V_1 = volume of $Cr_2O_7^{2-}$ solution (mls)

V_2 = volume of Fe^{2+} solution (mls)

Multiply by $\frac{8}{V}$ to obtain µg C.O.D/litre of seawater

where V = volume (litres) of seawater filtered.

Notes

1. The reason for washing the particulate matter on the filters with Na_2SO_4 solution is to displace all the chloride present without causing osmotic losses at the same time.
2. Sufficient $K_2Cr_2O_7$ should be present to oxidize the organic material present with an excess of at least 75%.
3. Clean the test tubes (or beakers) in hot chromic acid mixture and keep in a dust free container (dessicator).
4. The range of Chemical Oxygen Demand values usually lies within 50-300 µg oxygen per litre of seawater. In the case of seawater suspended matter, C.O.D values may be expressed as carbon equivalent from the approximate ($\pm$ 5%) relation 2.9 µg oxygen = 1 µg carbon.

Reference

1. Maciolek, J.A. (1962).- Limnological organic analyses by Dichromate Oxidation. Res. Rep. U.S. Fish. Wildl. Serv., 60.

14. PLANT PIGMENTS (CHLOROPHYLLS)

Reagents and Apparatus

(a) 100% acetone. CH_3COCH_3.
(b) Screw-cap tubes (20 ml capacity).
(c) Tube "buzzer". e.g. Vortex Genie.
(d) Spectrophotometer and 2 cm cuvettes.
(e) Filtration equipment, etc (see section 10).
(f) Centrifuge.
(g) Pasteur pipette.

Procedure

Using tweezers, place the damp filter sectors with adhering particulate materials, into screw-cap tubes, and add 6 ml of 100% acetone.

Screw the caps on. Expose the tubes to as little light as possible. Mix the contents several times on a tube buzzer and leave the tubes standing overnight in the dark in a cold room or cupboard to allow complete extraction of pigments. The extract is stable for 3 or 4 days if kept cool and away from light.

Mix again and centrifuge for 10 minutes at 2000 r.p.m. Transfer the supernatant with a Pasteur pipette into 2 cm cuvettes, taking care not to suck up any glass fibres.

Read each sample at 750, 665, 645 and 630 nm. Match the cells when filled with acetone and if necessary apply corrections. Use 100% acetone for the blank. There are no convenient standards.

Calculation

Calculate the concentration of the chlorophylls in the seawater (expressed as µg/l) from the following equations:

$$\text{chlorophyll } p = \frac{8}{n}\left[\frac{p}{d} \times \frac{\text{vol of acetone (mls)}}{\text{vol of seawater filtered (litres)}}\right]$$

where d = light path of cuvette, (cm)

n = number of filter sectors used,

and p = a, b or c

and where $a = 15.6 \times (abs_{665}-abs_{750})-2 \times(abs_{645}-abs_{750})-0.8\times(abs_{630}-abs_{750})$

$$b = -4.4 \times (abs_{665}-abs_{750})+25.4x(abs_{645}-abs_{750})-10.3x(abs_{630}-abs_{750})$$

$$c = -12.5x(abs_{665}-abs_{750})-28.7x(abs_{645}-abs_{750})+109.0x(abs_{630}-abs_{750})$$

abs_x = absorbance at x nm.

15. PARTICULATE PROTEIN

Reagents and Apparatus

(a) Conc. HCl A.R. quality.

(b) 50% v/v HCl solution. Dilute one volume conc. HCl with one volume of distilled water.

(c) 10% v/v HCl solution. Dilute 1 vol. 50% HCl to 5 volumes with distilled water.

(d) 4% w/v NaOH solution. Dissolve 4 g A.R. sodium hydroxide in 100 ml distilled water.

(e) 0.4% w/v NaOH solution. Dilute 1 vol of (d) to 10 volumes with distilled water.

(f) "2M sodium citrate" solution, pH = 5.20. Dissolve 180 g of A.R. citric acid, $C_3H_4O_6(COOH)_3$, in 500 ml of distilled water, and 80 g of A.R. sodium hydroxide, NaOH, in 500 ml of distilled water. Cool both solutions and combine.

(g) 0.4M sodium citrate solution. Dilute 1 volume of the 2M solution with 4 volumes of distilled water.

(h) Thymol blue indicator solution. B.D.H. brand or equivalent.

(i) Bromocresol purple indicator solution. B.D.H. brand or equivalent.

(j) 2% w/v ninhydrin solution. Dissolve 2 g triketohydrindene hydrate in 100 ml of a 70:30 v/v mixture of glycerol and isobutanol. Keeps for 2-3 weeks.

(k) Bovine serum albumin (B.S.A.) standard. Dissolve 31.63 mg of high grade B.S.A. in 50 ml of faintly alkaline (1 drop of 0.4% NaOH) distilled water. This solution contains 100 µg of nitrogen per ml. Make up fresh standard for each analysis.

(l) Screw-capped tubes and test tubes.

(m) Aluminium heating block with holes drilled to accommodate the screw-capped tubes.

(n) Water bath.

(o) Hot plate.

(p) Test tube buzzer (optional).

(q) Pipettes.

(r) Spectrophotometer. 1 cm cuvettes.

Procedure

The same sectors as were used to determine chlorophylls may, with little error, be used to estimate proteins, in which case any acetone remaining in the tubes must be poured out and discarded, and the tubes warmed in the aluminium block (about 80°C) for an hour or so. But due to the risk of failing to remove all traces of acetone it may be preferable to use other unused sectors.

To the dry filter and residue add 2 ml of 50% HCl solution. Screw the caps on, place the tubes in the aluminium block, tighten the caps after a few minutes' warming, and heat at 112°-115°C. Let the acid reflux for 24 hours with about half the length of each tube exposed above the block. Remove and allow to cool.

After hydrolysis, neutralize the acid, initially to a thymol blue end point then to the bromocresol purple end point, using the 40% and 0.4% NaOH solutions. The use of a test tube buzzer during this operation is helpful. 10% HCl solution may be used to compensate for any over-neutralization.

Add 2 ml of 2M sodium citrate and make the volume up to 10 ml (giving a final concentration of 0.4 M sodium citrate).

Centrifuge and dispense 1 ml of the supernatant into test tubes used for the colour development. To this 1 ml of supernatant add 1 ml of 0.4 M sodium citrate and 2 ml of ninhydrin reagent.

Mix and place in a boiling water bath for 30 minutes. Cool quickly and make the volume up to 10 ml with distilled water. Mix.

Read at 570 nm in 1 cm cuvettes.

Because the colour is unstable, the absorbance should be read within about half an hour of the tubes being removed from the water bath.

Calibration

Because hydrolysis does not convert all peptide nitrogen, and the colour response of individual amino-acids to ninhydrin varies, it is best to calibrate this method directly with standards made up of bovine serum albumin or egg albumin. The total nitrogen of these standards may be checked by a Kjeldahl digestion (use method of Section11).

Dispense 0, 0.1, 0.2 and 0.3 ml (containing 0 (blank), 10, 20 and 30 µg N respectively) of the B.S.A. standard into 4 screw-capped tubes. Make up to 1 ml with distilled water.

Add 1 ml of conc. HCl (A.R.) and put all 4 tubes through the hydrolysis, neutralization and colour development procedure.

Construct a calibration curve. From this curve calculate the amount of protein nitrogen present in each unknown and then multiply by $\frac{8}{V.n}$ to obtain the amount of particulate protein per litre in the original water, where V = the total volume (litres) of sea water filtered, and n = the number of sectors used.

Notes

1. The level of particulate protein nitrogen in seawater is usually of the order of 5-30 µg/litre.
2. The main source of interference contributing to the blank is probably ammonia from reagents and the working environment.
3. The effect of clean GF/C filters on the blank is usually undetectable. If one is confident that this is so, then the blank and standard solutions may be prepared (as herein described) without the filters.
4. A salt error is incurred unless all solutions are buffered at the same salt concentrations. Ensure that samples are at pH 5.2 before the ninhydrin reagent is added. (A check may readily be made on an extra blank prepared for the purpose).

16. ZOOPLANKTON TOTAL NITROGEN (KJELDAHL DIGESTION)

Reagents and Apparatus

(a) Digestion mixture (10% w/v K_2SO_4 in 10% v/v H_2SO_4 as in Section 11).

(b) $HgCl_2$ solution (1% w/v as in Section 11).

(c) NaOH solutions : (10% w/v and 0.4% w/v as in Section 11).

(d) Thymol blue indicator solution. B.D.H. brand or equivalent.

(e) Bromocresol purple indicator solution. B.D.H. brand or equivalent.

(f) Glass cloth filters.

(g) Büchner funnel.

(h) Kjeldahl flasks. 200 ml capacity.

(i) Kjeldahl flask heating rack.

(j) Reagents and apparatus for NH_3 analysis. (Section 8).

Procedure

Transfer the zooplankton sample to a glass cloth disc on a Büchner funnel and wash free of seawater with a few ml of distilled water.

Transfer the cloth with sample to a Kjeldahl flask and digest as in Section 11 but with 50 ml of digestion mixture plus 10 drops of $HgCl_2$ solution. Treat another glass fibre disc similarly for the blank.

Transfer with washings and make up the sample and blank digests to 100 ml in volumetric flasks.

(For large samples not fully digested by the original volume of acid, add, after cooling, a further 50 ml of digestion mixture plus 10 drops of $HgCl_2$ to the sample. In this case, make the final digest up to 200 ml in a volumetric flask.)

Neutralize 10 ml aliquots of each with the NaOH solutions using thymol blue indicator followed by bromocresol purple, and make up to 100 ml. (see Section 11).

Transfer 2 ml aliquots of the above neutralized solutions to conical flasks, add 48 ml of distilled water and determine the ammonia as in Section 11.

If the zooplankton catch was large, only 1 ml may be needed in 50 ml for the ammonia estimation.

Depending on the amount of dilution undertaken, the total nitrogen in the zooplankton sample will be 500, 1000, or 2000 times the NH_3-N content in the final 50 ml, as may be verified from the procedure.

Calibrate with NH_4Cl standards as in Section 11.

Notes

1. The density of zooplankton in the sea naturally varies tremendously, but material containing 0.3 µg N/litre would indicate a poor to average concentration. A catch which contained 3 µg N/litre would be considered to show a fairly high zooplankton density.

2. If the zooplankton catch is preserved in formalin, some tissue nitrogen will leach out into the preserving fluid. Analyses should be made on fresh material where possible.

17. APPENDIX

SALINITY-DISSOLVED OXYGEN TABLE

Oxygen solubility (ml/l) in seawater from a water-saturated atmosphere of which oxygen is 0.2094 mole fraction, excluding water vapor.

Temp. °C	Salinity ‰																
	33.00	33.25	33.50	33.75	34.00	34.25	34.50	34.75	35.00	35.25	35.50	35.75	36.00	36.25	36.50	36.75	37.00
0	8.15	8.14	8.12	8.11	8.10	8.08	8.07	8.05	8.04	8.02	8.01	8.00	7.98	7.97	7.95	7.94	7.93
1	7.95	7.94	7.93	7.91	7.90	7.88	7.87	7.86	7.84	7.83	7.82	7.80	7.79	7.77	7.76	7.75	7.73
2	7.76	7.75	7.73	7.72	7.71	7.69	7.68	7.67	7.65	7.64	7.63	7.62	7.60	7.59	7.58	7.56	7.55
3	7.58	7.56	7.55	7.54	7.52	7.51	7.50	7.49	7.47	7.46	7.45	7.44	7.42	7.41	7.40	7.38	7.37
4	7.40	7.39	7.37	7.36	7.35	7.34	7.32	7.31	7.30	7.29	7.27	7.26	7.25	7.24	7.23	7.21	7.20
5	7.23	7.21	7.20	7.19	7.18	7.17	7.15	7.14	7.13	7.12	7.11	7.09	7.08	7.07	7.06	7.05	7.04
6	7.06	7.05	7.04	7.03	7.01	7.00	6.99	6.98	6.97	6.96	6.95	6.93	6.92	6.91	6.90	6.89	6.88
7	6.90	6.89	6.88	6.87	6.86	6.84	6.83	6.82	6.81	6.80	6.79	6.78	6.77	6.76	6.75	6.73	6.72
8	6.75	6.74	6.73	6.71	6.70	6.69	6.68	6.67	6.66	6.65	6.64	6.63	6.62	6.61	6.60	6.59	6.57
9	6.60	6.59	6.58	6.57	6.56	6.55	6.54	6.52	6.51	6.50	5.49	6.48	6.47	6.46	6.45	6.44	6.43
10	6.45	6.44	6.43	6.42	6.41	6.40	6.39	6.38	6.37	6.36	6.35	6.34	6.33	6.32	6.31	6.30	6.29
11	6.32	6.31	6.30	6.29	6.28	6.27	6.26	6.25	6.24	6.23	6.22	6.21	6.20	6.19	6.18	6.17	6.16
12	6.18	6.17	6.16	6.15	6.14	6.13	6.12	6.11	6.11	6.10	6.09	6.08	6.07	6.06	6.05	6.04	6.03
13	6.05	6.04	6.03	6.02	6.02	6.01	6.00	5.99	5.98	5.97	5.96	5.95	5.94	5.93	5.92	5.91	5.90
14	5.93	5.92	5.91	5.90	5.89	5.88	5.87	5.86	5.85	5.85	5.84	5.83	5.82	5.81	5.80	5.79	5.78
15	5.81	5.80	5.79	5.78	5.77	5.76	5.75	5.74	5.74	5.73	5.72	5.71	5.70	5.69	5.68	5.68	5.67
16	5.69	5.68	5.67	5.66	5.65	5.65	5.64	5.63	5.62	5.61	5.60	5.60	5.59	5.58	5.57	5.56	5.55
17	5.57	5.57	5.56	5.55	5.54	5.53	5.53	5.52	5.51	5.50	5.49	5.48	5.48	5.47	5.46	5.45	5.44
18	5.46	5.46	5.45	5.44	5.43	5.42	5.42	5.41	5.40	5.39	5.38	5.38	5.37	5.36	5.35	5.34	5.34
19	5.36	5.35	5.34	5.33	5.33	5.32	5.31	5.30	5.30	5.29	5.28	5.27	5.26	5.26	5.25	5.24	5.23
20	5.26	5.25	5.24	5.23	5.22	5.22	5.21	5.20	5.19	5.19	5.18	5.17	5.16	5.16	5.15	5.14	5.13
21	5.15	5.15	5.14	5.13	5.12	5.12	5.11	5.10	5.10	5.09	5.08	5.07	5.07	5.06	5.05	5.04	5.04
22	5.06	5.05	5.04	5.04	5.03	5.02	5.01	5.01	5.00	4.99	4.98	4.98	4.97	4.96	4.96	4.95	4.94
23	4.96	4.96	4.95	4.94	4.93	4.93	4.92	4.91	4.91	4.90	4.89	4.89	4.88	4.87	4.86	4.86	4.85
24	4.87	4.86	4.86	4.85	4.84	4.84	4.83	4.82	4.82	4.81	4.80	4.80	4.79	4.78	4.77	4.77	4.76
25	4.78	4.78	4.77	4.76	4.76	4.75	4.74	4.73	4.73	4.72	4.71	4.71	4.70	4.69	4.69	4.68	4.67
26	4.70	4.69	4.68	4.68	4.67	4.66	4.66	4.65	4.64	4.64	4.63	4.62	4.61	4.60	4.60	4.60	4.59
27	4.61	4.61	4.60	4.59	4.59	4.58	4.57	4.57	4.56	4.55	4.55	4.54	4.53	4.53	4.52	4.51	4.51
28	4.53	4.52	4.52	4.51	4.50	4.50	4.49	4.48	4.48	4.47	4.47	4.46	4.45	4.45	4.44	4.43	4.43
29	4.45	4.44	4.44	4.43	4.42	4.42	4.41	4.41	4.40	4.39	4.39	4.38	4.37	4.37	4.36	4.36	4.35
30	4.37	4.37	4.36	4.35	4.35	4.34	4.34	4.33	4.32	4.32	4.31	4.30	4.30	4.29	4.29	4.28	4.27
31	4.30	4.29	4.28	4.28	4.27	4.27	4.26	4.25	4.25	4.24	4.24	4.23	4.22	4.22	4.21	4.21	4.20
32	4.22	4.22	4.21	4.20	4.20	4.19	4.19	4.18	4.18	4.17	4.16	4.16	4.15	4.15	4.14	4.13	4.13
33	4.15	4.14	4.14	4.13	4.13	4.12	4.12	4.11	4.10	4.10	4.09	4.09	4.08	4.08	4.07	4.06	4.06
34	4.08	4.07	4.07	4.06	4.06	4.05	4.05	4.04	4.03	4.03	4.02	4.02	4.01	4.01	4.00	3.99	3.99
35	4.01	4.01	4.00	3.99	3.99	3.98	3.98	3.97	3.97	3.96	3.96	3.95	3.94	3.94	3.93	3.93	3.92

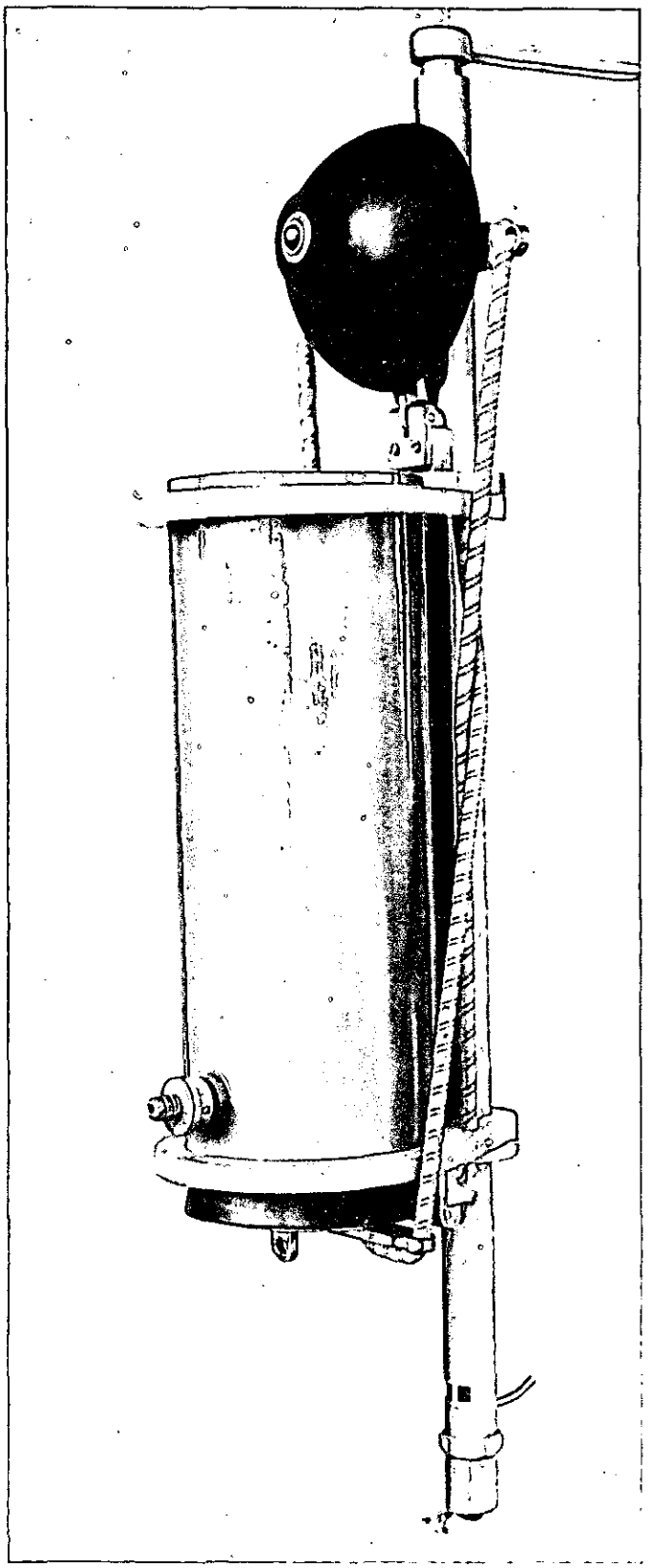

Figure 2.- 2-litre water sampler.

Designed and built at C.S.I.R.O. Cronulla, mainly for use in shallow waters. It is operated in a similar manner to the Jitts sampler (Figure 3). One difference from the latter is that the elastic "springs" connecting the stoppers at either end are external to the sample chamber. The lower plug on this unit is shown in the closed position.

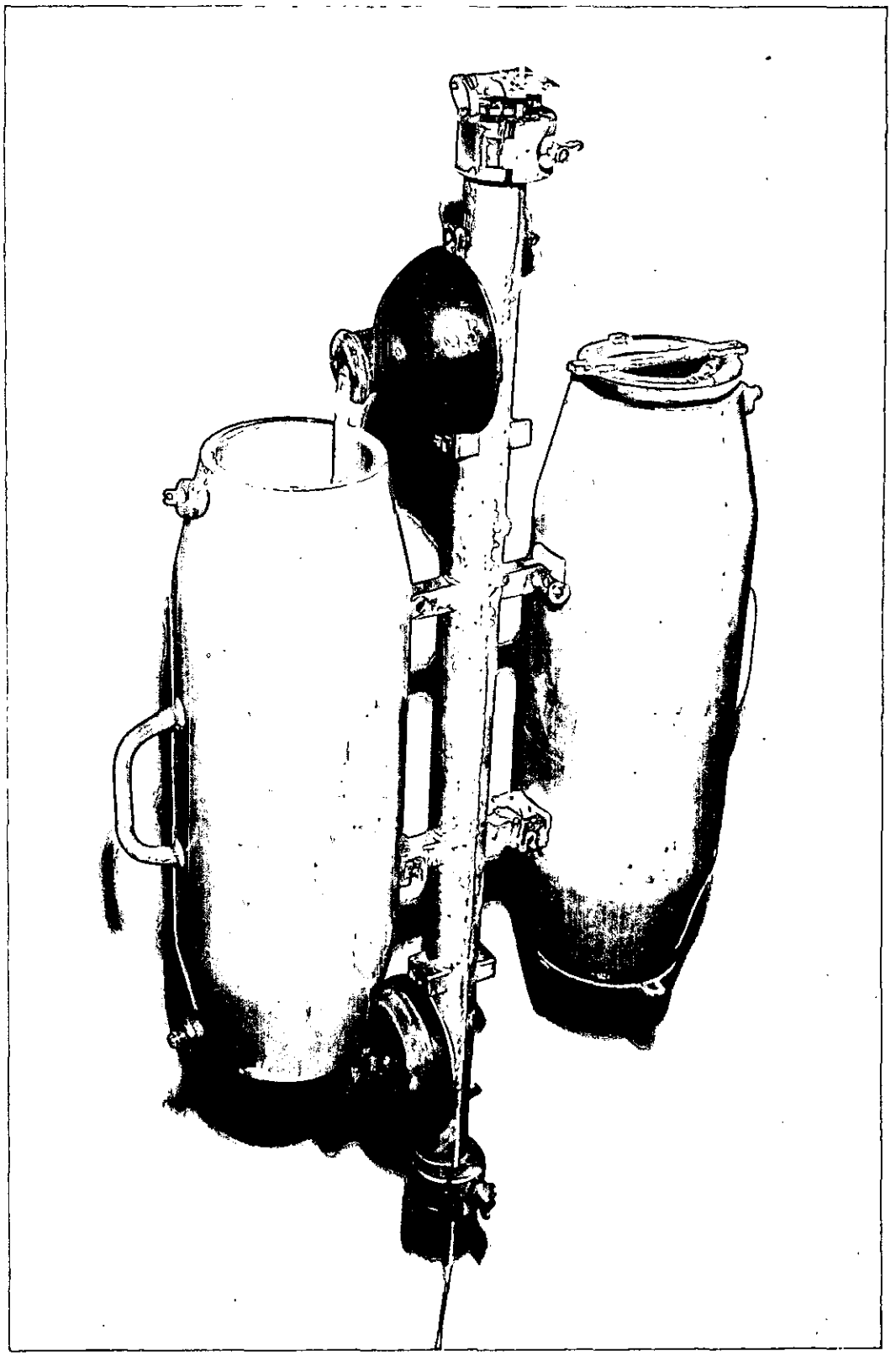

Figure 3.- 6-litre (Jitts) water sampler.

The sampler on the left is open. A taut, rubber "spring" joins the two hemispherical plugs, which are clipped to the frame attached to the wire. At the required depth, a weight ("messenger") is slid down the wire. This releases the plugs by depressing the metal plate at the top of the frame.

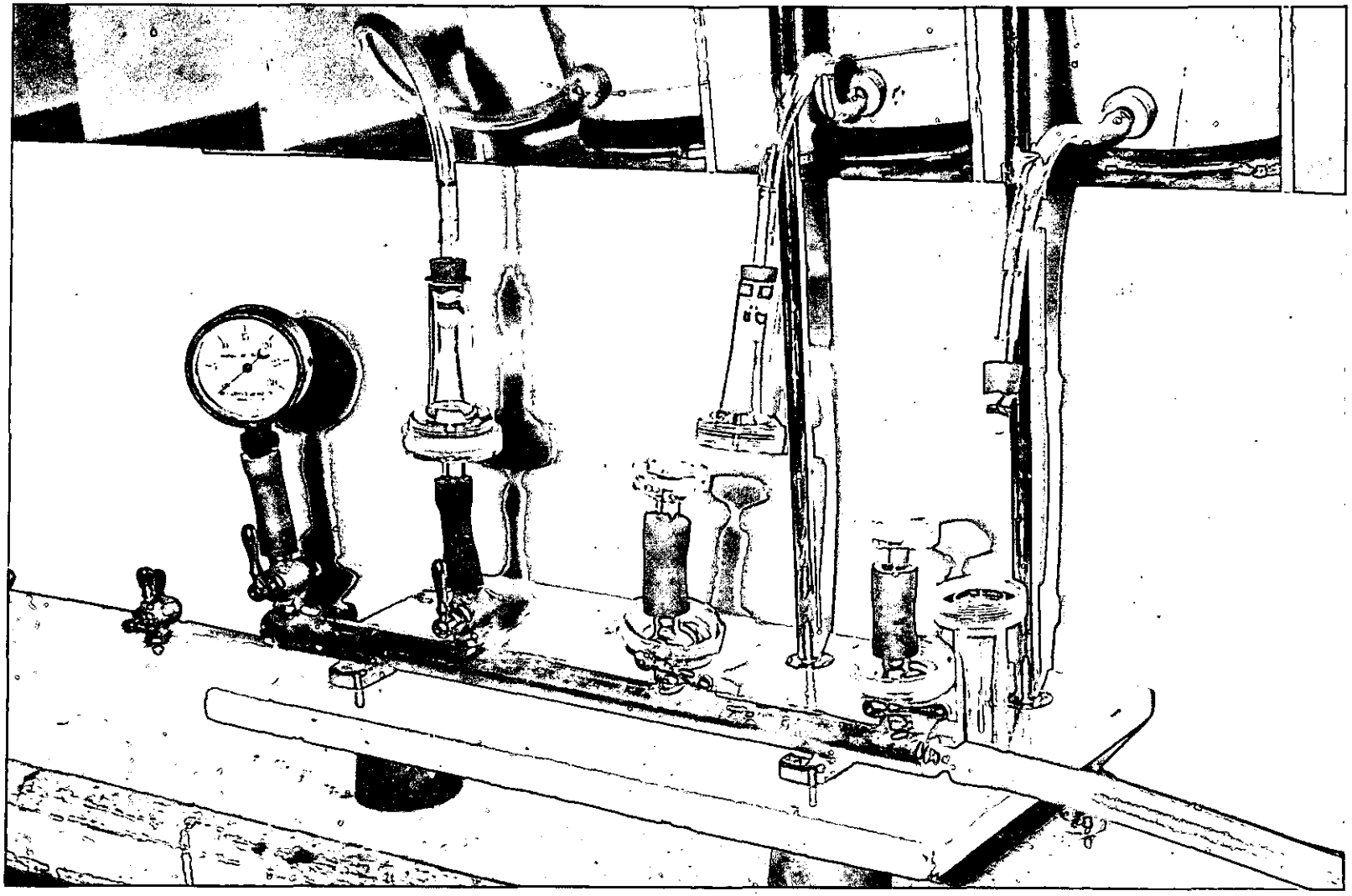

Figure 4.- Filter assembly.
Water passes from the tap on the Jitts sampler through a plastic tube and a glass rod with a nylon sieve strapped across the end. It is then drawn through two glass fibre filters and the cellulose support pad, and finally runs to waste via the water pump vacuum line.
If filtrates were to be collected, modification of the above assembley would be necessary.

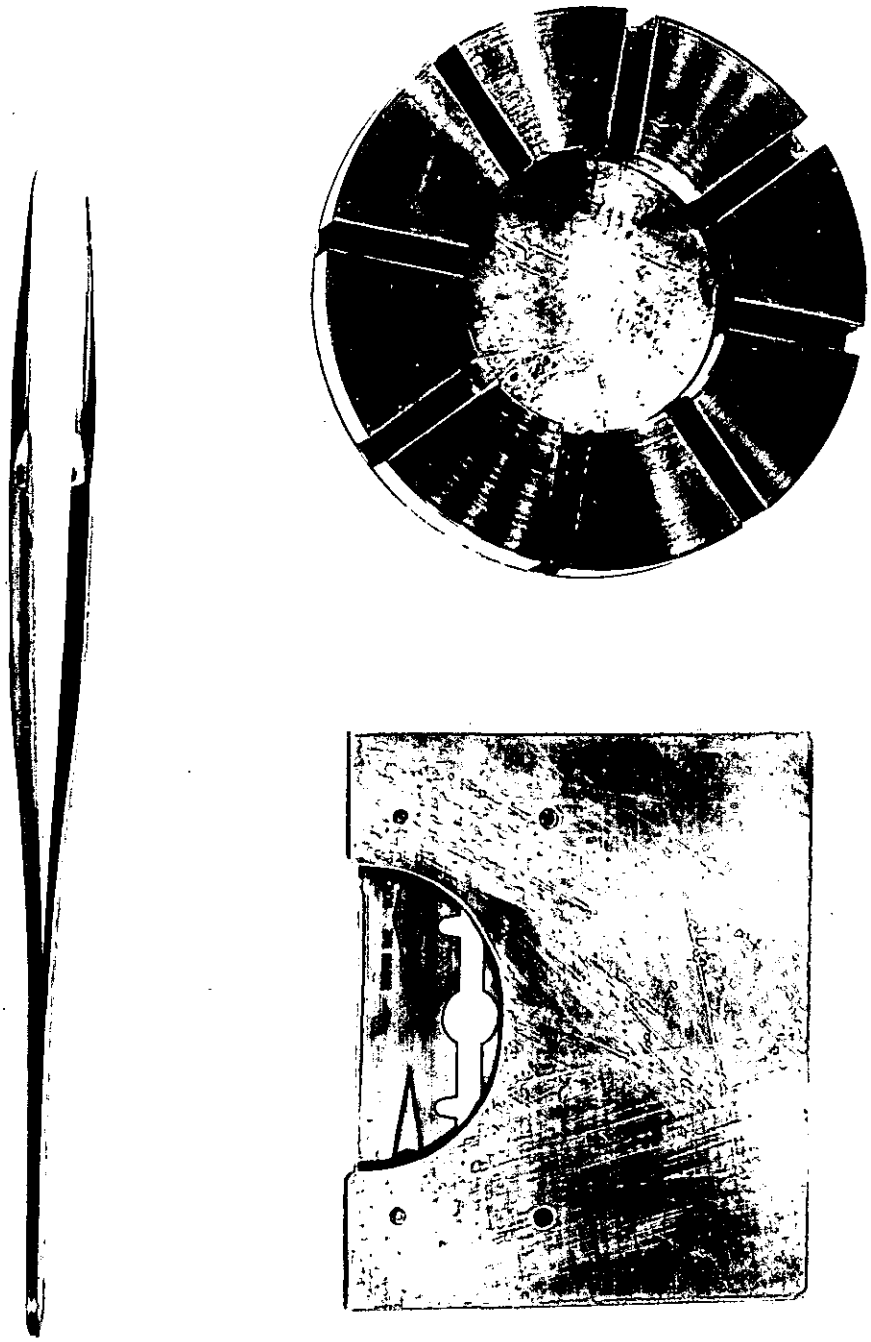

Figure 5.- Filter cutter.
See Section 10 for explanatory details.

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