FISHERIES RESEARCH BOARD
OF CANADA

MANUSCRIPT REPORT SERIES
(OCEANOGRAPHIC and LIMNOLOGICAL)
No. 19

Standard Methods of Seawater Analyses
Volume II

by
J. D. H. Strickland

Pacific Oceanographic Group
Nanaimo, B.C.

July 15, 1958

Programmed
by
THE CANADIAN JOINT COMMITTEE ON OCEANOGRAPHY

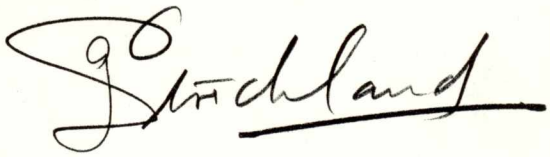
Standard Methods of Seawater Analyses

Volume II

PREFACE

These six methods continue the series published as Volume I of Standard Methods of Seawater Analyses published July 31, 1957.

The general format and policy is unchanged. Methods for the various forms of organic nitrogen and for copper, manganese, zinc and molybdenum are outstanding but it is hoped to complete the series by next year.



J.D.H. Strickland

Chemist (Associate Scientist)

Standard Methods of Seawater Analyses

Volume II

CONTENTS

DETERMINATION OF SALINITY BY CONDUCTIVITY MEASUREMENT

Note. This method is for use with the Mark II Salinometer of the Pacific Oceanographic Group. Changes from method Sal. 3.A. (Vol. I) are minor but important.

Definitions

A. Chlorinity

Halogen ions in sea water are normally estimated by argentimetry. To prevent ambiguity arising from changes in the accepted atomic weights of silver and chlorine the chlorinity, Cl ‰, of sea water is defined as 0.3285234 times the weight of silver precipitated as silver halides from 1 kg. of sea water, all weighings being in vacuo.

B. Salinity

The salt content of sea water is usually expressed as salinity, S‰ , a convention which approximates to the weight in grams, in vacuo, of the solids obtained from 1 kg. of sea water (weighed in vacuo) when the solids have been dried to constant weight at 480° C, the organic matter oxidized and the bromide replaced by an equivalent amount of chloride. (Ocean water contains slightly more salts (halogens, carbonate and bicarbonate) than is expressed by its salinity value.) In practice, the salinity is defined in terms of chlorinity by the Knudsen equation:

$$S‰ = 0.030 + 1.8050\ Cl‰ .$$

A. Capabilities of the Method

Range S ‰ 23 - 38

The following data are based on a statistical treatment of results obtained by skilled operators, using the procedure exactly as described.

1. Precision at the 35 ‰ Salinity Level

The correct value lies in the range: Mean of duplicate determinations ± 0.004 ‰ under sea-going conditions or ± 0.003 ‰ in a shore base laboratory under favourable conditions.

Reject duplicate determinations if the readings of the salinometer differ by more than 0.17 using different cells or by more than 0.12 using the same cell. If duplicates differ by less than these amounts, take a mean value.

Note: The above statistical data apply at the 0.05 probability level, i.e., the odds are 19 to 1 in their favour. By this criterion two samples of sea water cannot be considered to be significantly different in salinity unless the values differ by more than 0.008 ‰ when determined at sea.

B. Outline of Method

The sample is placed in a conductivity cell in one arm of an AC conductivity bridge and its resistance compared with the resistance of a reference cell containing sea water which is placed in another arm of the bridge. The resistance of sea water of known salinity is then similarly compared with the resistance of the reference cell and from these values the ratio of the resistance of the unknown sample to the resistance of sea water of known salinity is obtained. Using this ratio and a calibration curve the salinity of the sample can be evaluated.

C. Special Apparatus and Equipment

The conductivity salinometer used by the Pacific Oceanographic Group is required for this method. The salinometer consists, essentially, of a high precision conductivity bridge and a constant temperature bath containing up to seven specially designed conductivity cells. The procedure for filling cells and taking a measurement with the bridge is fully described in the following method. The constant temperature bath is normally run below room temperature by means of a heating system working against refrigeration. Once adjusted the bath requires little or no attention. Directions for adjusting the bath temperature to any desired value are given in Section K. No other special equipment is required but the temperature of the laboratory in which the salinometer is used must be greater than the bath temperature employed (5° C or 15° C).

D. Special Reagents and Solutions Required

1. Standard Sea Water

The primary standard for this method must be a sample of Eau de Mer Normale with the stated Cl ‰ value (near to 19.38) obtained from the Depot d'Eau Normale at Charlottenlund Slot, Charlottenlund, Denmark. To conserve this primary standard, it is essential that a large volume (20 - 40 liters) of a secondary standard be prepared, as standard sea water is required to the extent of at least 1 1/2 liters a working day. The secondary standard should, if possible, have a salinity exceeding 32 ‰ and should be filtered and stabilized by adding a few small crystals of thymol. It is stored in a carboy under a layer of paraffin oil from which it may be syphoned to reach the cells in the salinometer. The salinity of the secondary standard is determined using the salinometer and Eau de Mer Normale by the procedure described in Section H.

2. Sea Water for the Reference Cell.

The salinity of the sea water used for the reference cell in the salinometer need not be known but it is desirable to use a sample of water with a salinity near to that of the samples being analysed (within 2 to 3 parts per thousand). An extra bottle of water from a cast may be set aside for this purpose or, if the salinities being measured are near to that of the secondary standard, see above, this water may also be used to fill the reference cell.

E. Sampling Procedure and Sample Storage

Use standard flat 8-ounce medicinal bottles for collecting, shipping and storage of samples used for salinity determinations. The bottles manufactured for use with size no. 6 corkd should be used. The corks are soaked for 30-40 seconds in melted paraffin wax and drained and dried by placing them on a wire screen. Extruded excesses of wax are removed. The bottles are rinsed thoroughly three times with the sample and then filled to the shoulder. They are sealed by forcing the waxed corks down to a few mm. below the level of the neck. If analysis is delayed a further seal should be made within a few days by dipping the neck in melted wax. No changes in salinity will then result over a period of many years provided that "seasoned" bottles are used, i.e. bottles that have contained sea water for many months prior to use.

When these bottles, or the containers of Eau de Mer Normale, are opened the salinity determination must be carried out within a few minutes. Repeat determinations on re-corked bottles should not be delayed for more than about one hour.

F. Experimental

Procedure

1. Switch on bridge and bath and allow sufficient time for the bath to reach temperature (Note (a)). Adjust the bath temperature to 15° C (Note (b)) making sure that the temperature of the laboratory housing the salinometer is maintained at a temperature above 15° C (see Note (i)). The bath temperature should be checked and the value noted, by reading the thermometer every hour, as a precaution against a failure in the heating or refrigeration units going undetected for a prolonged period.

2. Zero the meter on the bridge by turning the meter gain to zero (fully counterclockwise) and adjusting the meter zeroing knob. Turn the meter gain one half to three-quarters full up by rotating the knob in a clockwise direction. Adjust the eye by turning the gain to zero (fully counterclockwise) and adjusting the "adjust" knob until the eye opens a few degrees. Turn the gain up one third of a turn clockwise (Note (c)).

3. Change the solution in the reference cell (marked R), if necessary, (Note (d)) with a suitable supply of sea water (see Section D.2).

4. Determine the cell constant for each cell, if necessary, (Note (e)) using the secondary standard sea water (see Section H).

5. Commence a sequence of measurements by rinsing and filling cell No. 1 and progressing thus to cell No. 6 in an anti-clockwise direction (Note (f)). Take the reading on cell No. 1 at least 15 minutes after filling this cell (Note (g)). Empty and rinse the cell, fill it with the next solution to be analysed and then take a reading on cell No. 2. Proceed in this fashion (Note (f)) round the ring of cells in an anticlockwise direction noting the time

that each cell is filled and the time that the measurement is subsequently made and checking that the time interval is never less than 15 minutes. Do all samples in duplicate, using a different cell for the repeat (Note (h)). Full details follow:-

Rinsing and Filling

Remove the cell stopper and fasten the tubing from the suction pump to the cell outlet. Press the pump activator until the cell is emptied. Turn off the pump and fill the cell with the solution to be analysed by using a small polyethylene funnel or by puring the sample from a polyethylene beaker. A jigger (liquor dispensing unit) can also be used, being corked into each sample bottle in turn, but a small length of rubber tubing must be placed on the spout to give a flexible contact with the cells. Rinse the stopper and the mouth of the cell during this operation. Again empty the cell. Fill the cell with sample until it appears above the level of the lucite bath cover. Pinch the pump tubing by the finger and thumb and turn on the pump. Gently release the pressure on the tubing until the liquid in the cell is sucked down just level with the lucite bath cover (Note (i)). Stop the pump and replace the cell stopper. Remove the rubber tubing from the cell outlet. The above procedure, rinsing once, is adequate for most routine work. If, however, two samples, or a sample and standard sea water, are known to differ by more than about 5 ‰ an extra rinsing cycle (empty and fill) should be included. This extra cycle should always be used when cell constants are determined and whenever a cell full of sea water has stood for more than about one hour, such as over a meal break (Note (j)).

Taking a Reading

Move the pointer on the cell selector switch (located in the center of

the bath) until the pointer is opposite the white line directed at the cell containing the sample solution. Dial on Arm Y of the bridge the value of the cell constant for the cell concerned to two decimal places. With the "eye" gain half on and the meter gain 3/4 on (see 2. above) adjust the resistances on Arm X, starting at the 100 ohm dial and moving to the right, until the best balance is obtained on the meter down to the dial marked in 0.01 ohm units. Now check that the capacity switch is in the center position and turn the far left hand side capacity knob (marked "Capacity Balance") until the "eye" is open as far as possible. Readjust the 0.01 ohm dial until the meter is again balanced. Check that the "eye" is fully open and, if not, tune the capacity again and once more zero the needle with the 0.01 ohm dial (Note (k)). If the meter does not zero exactly use the 0.01 ohm setting which gives the lease meter deflection. Note the reading immediately (Note (l)) and write down this reading. In addition write down the cell constant showing on Arm Y in a record sheet opposite the cell number, the sample number, the time of filling the cell and the time of taking the reading.

Notes

(a) The bridge requires only about a minute warming period (when the "eye" should be fully lit) and should be switched off when not in use. The bath requires at least an hour to reach thermal stability and should be left on overnight when in continual use. For shut-down periods exceeding 24 hours, turn off the heating, stirring and refrigeration units. If a prolonged shut-down is envisaged the refrigeration coils should be emptied (see Section K).

(b) For details of the procedure used to bring the bath to temperature see Section K.

(c) The meter should be zeroed each time the bridge is switched on but is generally stable, thereafter, for many hours. The bridge is perfectly balanced when the eye is fully open. This point is most easily detected when the maximum opening is restricted by the "adjust" knob to be only a few degrees, but the exact amount is a matter of personal preference.

(d) The reference cell should be closed at the exit end with a small rubber cap (policeman). It is desirable (see Section D.2.) for the salinity of the water in the reference cell to be within 2 to 3 ‰ of the sample salinity.

(e) The cell constants for all the cells must be determined every time that the water in the reference cell is changed. They must also be determined first thing at the commencement of each day's work and last thing before the apparatus is closed down at the end of each day's work. In addition, the constant of one of the cells, chosen at random, is determined at approximately two-hour intervals throughout the working day. If this constant changes by more than 0.1 from its previous value the cell constants for all the cells must be re-determined.

(f) The circulation of the bath is such that when a cell is filled with sample solution, which may be 10° C or more different in temperature from the bath fluid, this has no effect on the temperature of the adjacent cell in an anticlockwise direction. The adjacent cell in a clockwise direction, however, is affected slightly for a period of a few minutes. When cell No. 1 is filled, therefore, it is best to wait 2 to 3 minutes before taking a reading on any other cell as the temperature of the reference cell (R) may be slightly disturbed.

(g) The main purpose of comparing the conductivity of the sea water sample with that of another sea water sample in the reference cell is that the tempera-

ture sensitivity is greatly reduced. Both water samples change their conductivity in an identical and parallel manner. However, both sample and reference cell must be at an identical temperature (to within 0.002° C or better) and to ensure this each new solution must be allowed to stand in the cell for at least 15 minutes. If the sample temperature is initially more than 10° C from the bath temperature 18 minutes should be allowed for thermal equilibrium.

(h) The main purpose of a duplicate is to ensure that serious errors are not encountered due to an error in rinsing procedure or due to bubbles being trapped in the cell. For the acceptable deviation of duplicates see Section A.

(i) The resistance of the electrolyte in these cells is not entirely independent of the level of the liquid in the filling arm, even when this level is many cm. above the top electrode. The level must, therefore, be reproducible from sample to sample and be the same as the level used when the cell constant was measured. The exact level is unimportant, provided that it can be reproduced to within 0.5 cm. or a little better, but experience indicates that it is not good practice to fill the cell right up to the stopper. The top of the lucite bath cover provides a useful reference level. Once the cells have come to temperature the liquid above the level of the bath should not be disturbed as it is not at bath temperature. An air-tight cell stopper ensures that ship's movement will not disturb the contents of the cell and, provided that the laboratory temperature always exceeds 15° C, there will be no convection of the warmer upper layer in the cell. Having a refrigerated bath also minimizes the formation of nitrogen bubbles on the cell electrodes which may form if the sea water temperature is raised by more than about 10° C from its *in situ* temperature.

(j) For reasons that are not yet fully understood, cells that have stood for several hours, or overnight, with sea water may require more rinsing than normal before they regain the highest possible reproducibility of resistance.

(k) The capacity of the bridge network should be capable of being balanced by the extreme left-hand knob unless the salinity of the sea water in the reference cell is many ‰ different from that of the sample. The other (decade type) capacitors are used with the switch set to "Arm X" or "Arm Y". Should it be necessary to employ these capacitors with water of the same salinity in reference and sample cells the electrodes have probably deteriorated and should be replatinized (see Section J).

(l) The current passing through the cell whilst the bridge is balanced may cause very slight heating and for this and other reasons the reading on Arm X may change slowly with time (a few units in the 0.01 ohm range). When making a measurement on solutions (or determining the cell constant, see Section H) it is best to note the first resistance balance that is obtained and not attempt to obtain a value that is strictly constant with time.

G. Calculations

Variations in the conductivity of the reference cell and variations in the behaviour of the sample cells are eliminated by frequent determinations of the cell constant (Section H) so that the readings in Arm X are always "Normalized" and are directly usable for the determination of salinity. The readings of Arm X are inversely proportional to the resistance of the sample cell and hence proportional to the conductivity of the sample in this cell.

Over a relatively small range of salinity values the conductivity, expressed as the reading R or Arm X, is related to the salinity of a sample

by an expression of the form:

$$S‰ = \alpha + \beta R + \gamma R^2 + \ldots\ldots$$

where α, β and γ are constants and higher powers of R can be neglected.

The nature of the bridge is such that residual resistance remain in Arms X and Y even when the dial readings are zero. For this reasons, the constant α depends on the bridge being used and cannot be assigned an absolute value. Because of the very high precision attempted in this method the constant β will also incorporate the slight relative inaccuracies of the nominal resistances in Arm X and Arm Y of the bridge and will therefore depend upon the bridge being used. An empirical calibration must, therefore, be made with every bridge. The slope of the salinity/conductivity curve changes with temperature, about 0.015‰ per °C per ‰ difference between measured and reference salinities near S = 35 ‰ and a calibration is therefore required for each bath temperature. The bath temperature must be re-set, each time it is changed, to within about ± 0.2° C of its nominal value. A greater latitude is permissible (0.5 - 1° C) provided that samples are not more than 1 ‰ from 35 ‰ salinity and cell constants are redetermined everytime the temperature changes. For convenience a salinity of 35.000 ‰ has been arbitrarily assigned an R value of exactly 700.00 ohms on Arm X and, using this arbitrary fix, a careful calibration against sea water samples of known salinity has resulted in the following equation relating S ‰ and R at 15° C:

$$S‰\,(15°C) = 3.32435 + 0.0414724R + 5.398 \times 10^{-6}.R^2.$$

This equation applies as from June 1958 but may be changed as a result of subsequent calibrations. Tables for use between R values of 440 and 755 (S ‰ about 22.6 - 37.7) have been computed.

Note. The salinities derived from the above equation or from the accompanying

table are directly comparable with the salinities derived from chloride titration and the use of the Knudsen formula (see Standard Method Sal 1.A). In calibration the assumption was made that the conductivity of solutions is a function of their chlorinity when they are diluted with distilled water. The corresponding salinities were then evaluated from the Knudsen equation.

H. Calibration

1. Determination of Cell Constant

1. Fill cells 1 to 6 with secondary standard sea water rinsing twice (see Section F.5 and Notes e , g, and j).

2. Set Arm X to the correct R value for the salinity of the secondary standard at the given bath temperature.

3. Balance Arm Y in exactly the same manner as described for the balancing of Arm X in Section F.5 (see also Note 1).

4. Note the value of Arm Y and take this as the cell constant applying to all subsequent samples put in this cell until the next cell constant determination is made. If this subsequent determination shows a statistically significant change of constant it is doubtful whether the practice of correcting the sample readings between the two standardizations (by taking a "mean value" cell constant or a linearly interpolated value) has much justification unless the exact form of the cell constant variation is known. With good experimentation the constant should change very little over a period of a few hours and for work of the highest precision there is no substitute for making frequent evaluations.

2. Evaluation of the Secondary Standard Salinity.

1. Undertake this determination only when the bridge and bath are

working in a trouble-free manner.

2. Choose four sample cells, either at random or the four most stable cells if any behaviour differences are suspected. Rinse these cells three times and finally fill them with Eau de Mer Normale (see Section F.5).

3. Allow these cells at least 15-20 minutes to come to thermal equilibrium with the reference cell.

4. Set Arm X to the R value for the salinity of the Eau de Mer Normale which is calculated from the stated chlorinity value and the Knudsen equation (see the Definitions at the beginning of this method). The salinity must be calculated to the fourth decimal place to enable R to be calculated to the nearest 0.01 ohm.

5. Balance the bridge for each cell to determine the cell constant as described in Section G.1 above.

6. Without delay empty the cells and rinse them three times with the secondary standard and finally fill them with this standard (see Section F.5).

7. Set Arm Y to the cell constant value for each cell, just evaluated, and after 15-20 minutes balance Arm X. The four values for the reading R thus obtained should have a spread not exceeding 0.16. If the spread is greater the standardization should be repeated, if not, take a mean R value of the four determinations.

8. Evaluate the salinity of the substandard from the mean R value and the equations or table (see Section G). This value is used for the carboy of secondary standard sea water and must be re-determined for each new batch of this standard.

Note. It is known that a very small number of Eau de Mer Normale ampules contain water the salinity of which is not that calculated from the stated chlori-

nity values. The operator must be constantly on guard against this possibility. More than one bottle of Eau de Mer Normale is required for the standardization of the secondary standard so that a "rogue" bottle should be detected by the apparently poor agreements of two cells when compared with the other two cells.

J. Care and Maintenance of the Conductivity Cells

1. General

The electrodes in the conductivity cells must never be allowed to dry or the cell be empty of water for more than a minute or two. Sea water may be left in the cells overnight or for a similar period of shut-down but for longer intervals fill the cells with distilled water (having first rinsed out salt water with one or two fillings) and close the exit end of the cells with a small rubber cap.

2. Detection of Electrode Failure

Apart from breakage or the severence of electrical contacts, both of which are easily detectable, the only other likely defect that will develop in a cell is the deterioration of the surface of the two platinum electrodes.

If the cell constant of a particular cell changes markedly with respect to the other cells the cell is suspect. The most likely changes, however, will be found to occur in the capacity balance of the bridge which will become steadily larger as the electrodes of a cell deteriorate. Although it may still be practicable to balance the bridge network the resulting resistance is no longer of necessity a correct measure of the resistance of the sea water in the cell. The electrodes themselves contribute a resistance R_p known as the polarization resistance which is normally negligible but which increases to a significant amount as the electrodes deteriorate. Whereas the resistance of

the sea water, R, should be independent of the frequency of the alternating voltage applied to a cell the R_p value depends on the frequency and may be detected by measuring the total resistance $R + R_p$, at two different frequencies. For this purpose the 400 C.P.S. and 1000 C.P.S. (1 k.c.) switches are available on the bridge.

Once every week, or whenever the equipment is used after a shut-down period, or whenever a cell is suspect for any reason the cell constant should be checked using 400 C.P.S. immediately after the usual determination at 1 k.c. If the values so obtained differ by more than 0.2 ohms (the value at 400 C.P.S. will be greater) the cell requires replatinization.

Once every six months or so a full check of polarization should be made, as the check described above only detects cells that have deteriorated relative to the reference cell; all cells may possibly have developed polarization troubles but to approximately the same extent.

Disconnect the lead to the reference cell (Y in Mark II bridge, A in Mark I bridge) and connect the terminals to a 1000 ohm precision resistor, either a wire wound secondary standard resistor or a 1000 ohm resistor in an AC decade resistance box. Set the resistances in Arm X to 400.9 (368.2 in Arm A of the Mark I bridge) and balance the bridge at 1000 C.P.S. using Arms Y or B, with the cell to be tested in circuit. A large capacity may be required to obtain correct capacity balance. Without delay (as temperature fluctuations are now not compensated) turn the frequency control knobs to 400 C.P.S. and quickly rebalance with Y (or B). A small change in capacity balance may be necessary. The difference in resistance found should not exceed 0.2 ohm (the value at 400 C.P.S. being greater). The values of 400.9 (or 368.2) used in the above experiment enable the true resistance of the cell under study to be evaluated. The re-

sistances can have any value near to 500 ohms, however, if we are concerned solely with finding the polarization of the cells.

3. Replatinization of Cells

The cell assembly must be removed from the bath for replatinization.

Clean off the old platinum black coating by immersing the electrodes for a few seconds in a mixture of one part by volume of nitric acid and two parts by volume of hydrochloric acid which has been prepared a few hours before use. Rinse the cell two or three times with distilled water to remove the cleaning fluid and then fill the cells with a solution containing 1 per cent by weight of platinic chloride, 0.03 per cent by weight of lead acetate and 2 to 3 ml. of normal hydrochloric acid per 100 ml. Place a small sheet of platinum foil about 1 cm. long and 2 to 3 mm. wide on a platinum wire down into the cell and through the hole in the back of the top electrode. With this foil in the core of each electrode (but not touching) pass a direct current of about 50 milli-amp. for 1 to 2 minutes making the cell electrode the cathode. The interior of the electrodes and some of the back surface should then attain a dull grey-black coating. Empty the platinizing solution from the cell (retaining it for future use) and rinse once with distilled water, discarding the rinsing. Fill the cell with a 2 per cent by volume solution of sulphuric acid, insert the auxiliary electrode as described above and "clean" each electrode by passing a current of about 50 milli-amp. for 1 to 2 minutes making the cell electrode the cathode. Finally rinse the cell three times with distilled water, replace it in the bath and fill with sea water. Test the cells for polarization again as described in J.2 above and check that the difference of resistance at 400 C.P.S. and 1000 C.P.S. is not greater than 0.2 ohm (the difference should

generally be less than 0.02 ohm).

K. Adjusting the Bath Temperature

Full details of the construction and maintenance of the refrigeration and heating unit of the bath are not in the scope of this method. It will be necessary, however, to check and change the bath temperature from time to time and to pump refrigerant from the bath cooling coils prior to a prolonged shut-down period. Instructions for these operations are as follows:-

1. Shut-down and Start-up Operation

Note. The switch on the voltage stabilizer unit (at the back of the main panel) and the switch on the Electron-o-Therm heater control (above the main panel) should be kept permanently at "on". The main power supply can be kept permanently alive but must have a good earthing.

For a short period shut-down (maximum 2 days, i.e., a week-end) switch off the "Pump", "Stirrer", "Heaters", "Voltage Regulator" and "Refrigerator", switches on the main panel. To start up again switch on the "Refrigerator", "Pump" and "Stirrer" switches. Then turn on the "Voltage Regulator" switch followed one minute later by the "Heaters" switch. Wait until the thermometer in the bath registers the required temperature (this may take several hours) and then allow a full hour after this for the bath to reach thermal equilibrium.

For a long period shut-down the refrigerant must be emptied from the coils in the bath and pumped into the reservoir of the refrigerator unit. To accomplish this, close the liquid line shut off of the refrigeration system by turning the yellow valve fully clockwise. Allow the system to pump until the motor stops. Turn off the oval red needle valve (fully clockwise). Open the blue and cream coloured valves when the readings should be equal and a few

pounds above zero. The sight glass should be empty of refrigerant. Turn off the "Pump", "Stirrer", "Heaters", "Voltage Regulator", "Refrigerator" and "Main Power" switches. Close the blue and cream valves. To start up again switch on the "Main Power", "Refrigerator", "Pump" and "Stirrer" switches. Open the yellow valve of the refrigeration system (fully anticlockwise) and the oval red needle valve half a turn anticlockwise.

Finally adjust the bath temperature by the procedure given in the following:-

2. Bath Temperature Adjustment

General. The electrical and refrigeration system is either already turned full on or it is turned on as described in the last paragraph. Switch on the "Voltage Regulator" switch followed one minute later by the "Heaters" switch. Open the orange valve and check that the pressure is around 90-120 lbs. Close the orange valve. Open the cream valve and check that the pressure is around 40-50 lbs. Close the cream valve. Open the blue valve and keep this open during the temperature adjustment. The reading on the dial should read 20-25 lbs. (always lower than the pressure on the gauge above the cream valve) when the refrigerator motor switches on and should decrease to a few lbs. above zero before the motor switches off. This valve must never read below zero or the refrigeration system will be under vacuum and oil or moist air may leak into the pipes. The pressure switch on the refrigeration panel is never touched.

To lower the bath temperature switch off the "Heaters" switch. Turn the oval red needle valve half a turn or more open and let the refrigeration take the bath temperature down to about 0.5° C lower than the desired temperature. Continue as in the next paragraph. To raise the bath temperature switch

off the "Refrigerator" switch and turn up the Powerstat on the Electron-o- Therm heater control to read 85 and leave it there until the temperature is about 0.5° C lower than the desired temperature. Switch on the "Refrigerator" switch and then continue as in the next paragraph.

Adjustment. When the bath temperature is 0.5° C or a little less below the required temperature (5° C or 15° C) have the refrigerator units and heaters on, put the Powerstat to read about 50 and turn the oval red needle valve about one-eighth to one-quarter turn on (anticlockwise). Watch the thermometer carefully until the temperature is exactly correct and then immediately turn the Powerstat to zero (where it must always remain whilst the bath operates) and adjust the "Temperature Range" dial on the Electron-o-Therm until the needle of the milliammeter reads anywhere between 40 and 60. If necessary use the "Fine Temperature Adjust" to set the meter reading. It is essential that this adjustment be made when the bath temperature is exactly at the desired value as the heater control will attempt to control to the temperature of the bath when the needle is first adjusted to "float" in the 40-60 range. Now watch the meter needle. If it continually points to values over 50-60 the refrigeration is too strong and the oval red needle valve should be turned down. The adjustment on this valve is quite fine and the valve will probably not be needed more than an eighth or quarter turn open. Adjust this valve (do not touch the "Temperature Range" dial again) until the meter stays most of the time between 40 and 50. Do not touch the "Thermostat Expansion Valve" unit at the bottom left of the refrigeration panel. On the Mark II Salinometer this valve is set at the correct value and should not require adjustment. Turn off the blue valve. The bath is now stabilized at the desired temperature and should not

require adjustment for many weeks. The refrigeration cycle (time between the motor switching off and on again) will vary with external conditions but should not be more frequent than about once every 1 to 1 1/2 minutes.

Approved December 1957 Standard Method No. P. 3.A

DETERMINATION OF TOTAL PARTICULATE PHOSPHORUS IN SEA WATER

Note. Reference is made to Standard Method No. P. 2.A and Addendum No. 1 (Vol. I)

A. Capabilities of the Method

If a volume of V ml. is filtered for this determination, so as to give some 0.15 - 0.25 μg.at.P., the correct value for the particulate phosphorus content may be assumed to line in the range:

$$\text{Mean of n determinations} \pm \frac{7}{Vxn^{1/2}} \mu\text{g.at.P/l.}$$

and will approximate to $\pm \frac{3\text{-}4\%}{n^{1/2}}$ of the amount of phosphorus being determined. The limit of detection cannot be specified as, ideally, there need be no upper limit to the volume filtered for this determination. In practice the method will rarely be used to estimate less than 0.05 μg.at.P/l.

The procedure should be reserved for occasions when high precision values are required of amounts of total particulate phosphorus less than about 0.4 - 0.5 μg.at.P/l, as the method requires rather a large volume of sample and has other drawbacks for routine shipboard use. The method determines the sum of particulate and adsorbed phosphorus (see Standard Method No. P.4. A).

B. Outline of Method

Sufficient sea water sample is taken for the amount of particulate phosphorus therein to lie between about 0.15 and 0.25 μg.at.P. The sample is filtered through a membrane filter that retains particles in excess of 0.4 micron diameter. The filter is digested in a mixture of perchloric and

nitric acids until all forms of phosphorus are converted to orthophosphate and the element is then determined as described in Standard Method No. P. 2.A.

C. Special Apparatus and Equipment

H.A. White Plain Filters, 47 mm. diameter , manufactured by the Millipore Filter Corporation of Watertown 72, Mass., U.S.A.

Pyrex filter holder and clamp supplied by the Millipore Filter Corporation for use with their membrane filters.

500 or 1000 ml. glass Pyrex Buchner flask and source of moderate vacuum (water aspirator pump).

A Beckman DU Spectrophotometer or any other absorptiometer capable of measuring the extinction of light of wavelength near to 7000 A.

Spectrophotometer or absorptiometer cells having a path length of at least 10 cm. and holding less than 40 ml. of solution.

One hundred and twenty-five ml. pyrex Erlenmeyer flaska. Three small dents, 1 to 2 mm. deep are made in the lip of each flask symmetrically placed round the rim. The flasks are covered with 2-inch diameter coverglasses.

7 cm. $\pm$ 0.5 cm. lengths of melting-point capillary closed at one end, one for each flask.

Full details for the preliminary cleaning and conditioning of this glass are given in Section G below.

D. Special Reagents Required

The same special reagents are required as in Standard Method No. P. 2.A. In addition there is a requirement for analytical reagent quality nitric acid, s.g. 1.42, stored in a clean glass bottle.

E. Sampling Procedure and Sample Storage

Samples for total particulate phosphorus must be filtered (see Section F below) within 1 to 2 hours of sampling. As it will rarely be possible to carry out the present method on board ship the filters holding the particulate phosphorus must be stored. They should be placed in the Erlenmeyer flasks used for the subsequent analysis (see Section F below) and the mouth of the flask sealed with Parafilm. One drop of concentrated hydrochloric acid should be placed in each flask before they are sealed. After use flasks should be rinsed thoroughly with distilled water, dried by gentle heat and, when cool, sealed immediately with parafilm until required or ship for a further sample.

F. Experimental

Procedure

1. Before and during filtration the sample should be vigorously shaken to ensure the even distribution of particulate matter. The best volume to be filtered can only be judged from an estimate of the quantity of particulate phosphorus present. This amount will rarely lie outside the range 0.1 - 1 μg.at.P/l. in the N.E. Pacific oceanic and coastal waters. The volume should be varied so that the phosphorus held by the filter is not less than about 0.15 μg.at.P (the blank from the filter itself may become a significant fraction of the total) and does not exceed about 0.3 μg.at.P (the maximum amount that can conveniently be determined by the spectrophotometric procedure). As a rough guide the following table may be consulted:

Anticipated Particulate P (µg.at.P/l.)	Volume to be filtered
0.1 or less	1500 ml.
0.1 - 0.2	1000 ml.
0.2 - 0.3	750 ml.
0.3 - 0.5	500 ml.
0.5 - 0.8	250 ml.
0.8 - 1.0	200 ml.
1.0 or more	50 - 100 ml.

If the true content falls in a range on either side of the anticipated range the consequences will not be too serious.

2. Assemble the Millipore filtration equipment and Buckner flask and fit a filter in place using clean forceps. Wash the filter by pouring through at least 100 ml., preferably more, of distilled water (note a). Filter a suitable volume of sample (see above) and suck the filter dry but do not wash it. Remove the filter membrane with forceps and place it into the special 125 ml. Erlenmeyer flask. Add 8 ml. of perchloric acid solution (Reagent No. 1) and 2 ml. of concentrated nitric acid. The filter should rest on the bottom of the flask, beneath the acids.

3. Evaporate the contents of the flask with vigorous boiling on a hot plate until the nitric acid is dispelled and fumes of perchloric acid appear (note b).

4. Remove the flask from the hot plate, allow the contents to cool and add 50 ml. of synthetic sea water.

5. Continue the determination exactly as described in Standard Method No. P. 2.A, Section F, paragraphs 2 to 7 inclusive, notes c to n inclusive.

6. Correct the measured extinction by subtracting a reagent blank (see Section G). Calculate the total particulate phosphorus concentration in microgram atoms of phosphorus per liter (μg.at.P/l.) from the expression

$$\mu\text{g.at.P/l.} = \text{Corrected extinction} \times F \times \frac{50}{V}$$

where V is the volume of sample filtered and F is the factor for each batch of samples evaporated (determined as in Section H below). Report results to two significant figures. If the volume filtered was misjudged and extinction values exceed 1.6 remeasure the extinction value after dilution as described in Addendum No. 1 to Standard Method No. P. 2.A.

Notes

(a) The 'soluble phosphorus' in HA Millipore filters appears to be very small (of the order of 0.005μg.at.P/filter) and is completely removed by passing about 100 ml. of water through the filter before use. In no circumstances should the filters be touched with the fingers before or after filtration.

(b) Provided that a little nitric acid is present there is no hazard in the destruction of the small amount of cellulose compounds comprising a single millipore filter. Complete volatilization of the nitric acid is indicated by the appearance of a heavy oily ring of perchloric acid refluxing down the sides of the flask and characteristic dense white fumes that escape only slowly from the mouth of the Erlenmeyer. Fuming at this stage should not be prolonged for more than a few minutes to minimize the loss of perchloric acid. The determination is continued as in Standard Method No. P. 2.A to ensure a more reproducible standardization and to enable determinations by the present method to be run simultaneously with a batch of total phosphorus determinations.

(c) Notes c to n inclusive are the same as those found in Standard Method No. P. 2.A.

G. Determination of Blanks

1. Cell to Cell Blanks

When both sample and distilled water cells are filled with distilled water the extinction of one against the other should be 0.000. Due to slight optical defects a slight positive or negative value may be found. This is allowed for when turbidity blanks are subtracted (see below) but the value should be found when determining the reagent blank. The water in the distilled water cell should be changed every day as marked turbidities can result even in distilled water if it remains in the cell for too long.

2. Reagent Blank

The phosphorus content of a single, water-washed, Millipore filter has been found to be small (about 0.02 μg.at.P/filter or less) but it cannot be neglected and may vary from batch to batch of filters. For safety, therefore, a blank should be determined, as the mean of duplicates, with each set of samples. A blank on the filter, nitric acid and other reagents is carried out as follows:

Place a washed Millipore filter into a special Erlenmeyer flask, using forceps, and add 8 ml. of Reagent No. 1, 2 ml. of concentrated nitric acid and 2 to 3 drops of Reagent No. 2. Add 1 to 2 ml. of synthetic sea water (see Section H) to provide a little solid in the flask to minimize any volatilization of phosphorus. Evaporate, without coverglass, until solid separates and only a few tenths of a ml. of perchloric acid remains. Cool somewhat, add 5 ml. of dilute ammonia solution, Reagent No. 3, and evaporate

to dryness but do not bake. Continue exactly as described in Standard Method No. P. 2.A, Section F, paragraphs 5 to 7 and correct the resulting extinction for any cell to cell blank. The final blank should not exceed an extinction of 0.1 - 0.25.

3. Preliminary Cleaning of Glassware

The Millipore filtration equipment and Buchner flask should be kept spotlessly clean and rinsed very thoroughly with distilled water immediately before use.

Clean pyrex Erlenmeyer flasks have been found to give no phosphate contamination. Flasks should be retained especially for the present method and not put into general circulation. Before their initial use, they should be boiled to fumes after adding about 30 ml. of water, 20 ml. of concentrated hydrochloric acid and 10 ml. of Reagent No. 1. The perchloric acid is then refluxed vigorously in each flask for about one hour. A blank determination (G.2. above, omitting the filter and nitric acid) should then be carried out on each flask before it is finally put into use. The flasks are rinsed thoroughly with distilled water after each determination and stored dry. The capillary antibump devices and the 2-inch coverglasses should be boiled for a few minutes in 50 per cent V/V hydrochloric acid solution before use or after a prolonged shut-down period.

H. Calibration

The calibration procedure is identical with that described in Standard Method No. P. 2.A, Section H.

Approved December 1957 Standard Method No. P. 4.A

DETERMINATION OF PHOSPHORUS IN ITS VARIOUS COMBINED FORMS IN SEA WATER

Note. Reference is made to Standard Methods Nos. P. 1.A, P. 2.A with Addendum No. 1, (Vol. I) and P. 3.A.

This method stipulates the data and equations required by the analyst to calculate the distribution of total phosphorus amongst the various forms of combination in which it may occur in a sea water sample. No new chemical procedures are involved.

Definitions

The following forms of phosphorus are considered. Their differentiation is based on their reactivity with acid molybdate and their particle size. 'Insoluble' material is defined as that retained by a 0.4 micron membrane sieve and it is unlikely that any substantial fraction of particulate organic or inorganic phosphate will escape this classification. It is assumed that fuming perchloric acid will mineralize phosphorus from whatever form it may occur in sea water.

Symbol	Description
IM	Inorganic phosphorus held in true solution which reacts in 3 minutes with acidified molybdate. Condensed polyphosphates, if present, may not react but no evidence has yet been obtained as to the occurrence of such material in sea water.
OM	Phosphorus held in organic combination in true solution which nevertheless hydrolyzes and reacts in 3 minutes

with acidified molybdate. Such material may well exist in sea water although this has not been proven. Phosphorus methods using reductants that necessitate heating or prolonged periods to complete the reduction (e.g. metol or ascorbic acid) can bring about considerable hydrolysis of soluble organic phosphorus compounds. Stannous chloride which reacts in a few minutes in the cold, is superior in this respect to any other reagent described.

1. <u>(IM+OM) or "Soluble Reactive"</u>

No known method will differentiate the above two forms of soluble phosphorus and only their sum can be estimated. Enzymatic attack may eventually lead to some separation.

IP — Particulate phosphorus, inorganically combined, that is non-reactive to acidified molybdate for a period of 3 minutes.

OP — Particulate phosphorus, organically combined either in living material or detritus, that is non-reactive to acidified molybdate for a period of 3 minutes.

2. <u>(IP+OP) or "Particulate"</u>

No known method will differentiate the above two forms of particulate phosphorus and only their sum can be estimated. Enzymatic attack may eventually lead to some separation.

3. AD

Inorganic or organic phosphorus that is adsorbed onto particles or is otherwise out of solution in sea water but which becomes soluble and reacts with acid molybdate in 3 minutes. Such material, which may include "colloidal" phosphates, etc., has been detected in some inshore waters.

4. SO or "Soluble Unreactive"

Phosphorus held in organic combination in true solution but which is non-reactive to acidified molybdate for a period of 3 minutes. Any condensed inorganic phosphate may be included in this classification.

Data Required

All results should be expressed as μg.at.P/l.

Determination 1. Total phosphorus on an unfiltered sample. Ref. Standard Method No. P. 2.A and addendum No. 1.

Determination 2. Total phosphorus on filtrate passing through a millipore HA filter. Ref. Standard Method No. P. 2.A and ref. Method No. P. 3.A for filtration technique.

Determination 3. Inorganic phosphorus on an unfiltered sample. Ref. Standard Method No. P. 1.A.

Determination 4. Inorganic phosphorus on filtrate passing through a millipore HA filter. Ref. Standard Method No. P. 1.A and ref. Method No. P. 3.A for filtration technique.

Calculations

"Soluble Reactive" μg.at.P/l. = Result of Determination 4.

"Particulate" μg.at.P/l. = Detm'n 4 + Detm'n 1 - Detm'n 2 - Detm'n 3.

AD μg.at.P/l. = Determination 3 - Determination 4.

SO μg.at.P/l. ("Soluble Unreactive") = Determination 2 - Determination 4.

Note.

The result for total particulate phosphorus determined as described in Standard Method No. P. 3.A is a precise figure for "Particulate" plus "AD" combined phosphorus and is probably a close enough approximation to "Particulate" for most purposes. "Soluble Reactive" phosphorus, as defined above, is generally reported as the ionic phosphate in sea water. Determination 2 - Determination 3 is generally equated to soluble organic phosphorus (the "SO" phosphorus) and this is a good enough approximation for most purposes but the result will be low by the amount of any "AD" phosphate present.

Approved December 1957. Standard Method No. N. 2.A

DETERMINATION OF NITRATE IN SEA WATER

Note. Reference is made to Standard Method No. N. 1.A (Vol. I).

A. Capabilities of the Method

Range 0.3 - 45 μg.atoms N/liter

The method is designed for use with sea water of salinities between 20 and 35 ‰ (See Section H 3).

The following data are based on a statistical treatment of the results obtained in a shore base laboratory using the procedure exactly as described.

1. Precision at the 30 μg.at. N/l level (1 cm. cell)

The correct value lies in the range: Mean of n determinations $\pm \frac{1.3}{n^{1/2}}$ μg.at. N/l.

2. Precision at the 4.5 μg.at. N/l level (10 cm. cell)

The correct value lies in the range: Mean of n determination $\pm \frac{0.3}{n^{1/2}}$ μg.at. N/l.

3. Limit of Detection

The smallest amount of nitrate nitrogen that can be detected with certainty is about 0.3 μg.at. N/l. using a 10 cm. cell.

Reject duplicate determinations if the extinction* values differ by

---

The extinction, E, is defined as $\log_{10} \frac{I_o}{I}$ where I_o and I are incident and transmitted light intensities, respectively. The Beckman DU Spectrophotometer is calibrated directly in extinction units. If the adsorptiometer employed reads per cent transmission, T, calculate E as $\log_{10} \left(\frac{100}{T}\right)$.

more than 0.06 in the extinction range 0.5 - 1.0 on a 1 cm. cell,

or more than 0.03 in the extinction range 0.1 - 0.5 on a 1 cm. cell,

or more than 0.1 in the extinction range 0.5 - 1.0 on a 10 cm. cell,

or more than 0.06 in the extinction range 0.1 - 0.5 on a 10 cm. cell.

If the duplicate extinction values differ by less than the above limits, take a mean value.

Note. The above statistical data apply at the 0.05 probability level, i.e., the odds are 19 to 1 in their favour.

B. Outline of Method

The nitrate in the sea water is reduced with hydrazine (catalysed by copper) in a solution buffered at pH 9.6. The nitrite thus produced is determined by diazotizing with sulphanilamide and coupling with N-(1-napthyl) ethylenediamine to form a highly coloured azo dye the extinction of which is measured using 1 or 10 cm. cells and light of wavelenght near to 5400 A.
If much nitrite is present in a sample of low nitrate concentration the nitrite content of the sample must be determined and a correction made to the nitrate result.

C. Special Apparatus and Equipment

A Beckman DU Spectrophotometer or any other seaworthy spectrophotometer capable of measuring the extinction of light of wavelength near to 5400 A.

Spectrophotometer or absorptiometer cells having path lengths of 1 cm. and 10 cm. The 10 cm. cells should hold less than 40 ml. of solution.

A thermostatically controlled water bath set for 23° C ± 1° C. At sea it is a great advantage to have this bath suspended by gimbals.

12.5 cm. No. 42 Whatman filter papers, filter funnels and 100 ml. beakers for collecting filtrates.

D. Special Reagents Required

1. Phenol Solution

Dissolve 46.0 g. of analytical reagent quality phenol in 1000 ml. of distilled water. Filter the solution rapidly through a fine porosity glass filter and store in a dark glass bottle with a glass stopper in a cool place. If the bottle is tightly stoppered this reagent is stable indefinitely.

2. Sodium Hydroxide Solution

Dissolve 29 ± 0.5 g. analytical reagent quality pellet-form sodium hydroxide in distilled water. Cool and dilute to 2000 ml. with distilled water. Store in a well-stoppered polyethylene bottle. The solution should be renewed every few months to ensure that carbonation is not excessive.

3. Buffer Reagent (Reagent No. 1)

Pipette 25 ml. of phenol solution (not by mouth) into a dry beaker and add from a pipette 25 ml. of sodium hydroxide solution. Mix and use at once. The solution should be prepared for use. Do not store the solution for more than 60 minutes.

4. Copper Sulphate Solution

Dissolve 0.10 g. of analytical reagent quality copper sulphate

pentahydrate ($CuSO_4.5H_2O$) in 1000 ml. of distilled water. The solution may be stored in a glass bottle and is stable indefinitely.

5. Hydrazine Sulphate Solution

Dissolve 14.5 g. of analytical reagent quality hydrazine sulphate $\left[(NH_3)_2SO_4\right]$ in 2000 ml. of distilled water. Filter the solution through a fine porosity glass filter and store in a well-stoppered dark glass bottle. The solution should be renewed once a month.

6. Reducing Reagent (Reagent No. 2)

Pipette 25.0 ml. of copper solution and 25.0 ml. of hydrazine sulphate solution into a dry beaker. The reducing agent should be prepared immediately before use and not stored for more than 60 minutes.

7. Acetone (Reagent No. 3)

Reagent grade acetone should be used.

8. Sulphanilamide Solution (Reagent No. 4)

Dissolve 5 g. of sulphanilamide in a mixture of 50 ml. of concentrated hydrochloric acid (s.g. 1.18) and about 300 ml. of distilled water. Dilute to 500 ml. with water. The solution is stable for many months.

9. N (1-napthyl) Ethylenediamine Dihydrochloride Solution (Reagent No. 5)

Dissolve 0.50 g. of the dihydrochloride in 500 ml. of distilled water. Store the solution in a dark bottle. The solution should be renewed once a month.

E. Sampling Procedure and Sample Storage

50 ml. of sample are required for this determination but several ml. are consumed in filtration and rinsing so that 70 to 80 ml. of sample should be taken and stored in the 125 ml. Erlenmeyer flask used in the subsequent analysis. Samples are stable for several hours at room temperature in the dark but the analysis should not be delayed for more than about 24 hours. If greater delays are unavoidable the samples should be frozen.

The flask used in this method must be pre-treated by the cleaning procedure given in Section G, below.

F. Experimental

Procedure

1. Filter about 10 ml. of sample through a dry 12.5 cm. No. 42 Whatman filter paper into a 100 ml. beaker, both funnel and beaker having been rinsed thoroughly with distilled water and drained, by inverting and shaking, immediately prior to use. Rinse the beaker with the filtrate and discard this filtrate. Pour the remainder of the sample through the paper and collect all the filtrate. Use about 10 ml. of the filtrate to rinse, vigorously, the 125 ml. Erlenmeyer flask that contained the sample. Discard this rinsing and drain the flask by inverting and shaking it. Measure 50 ml. of the filtered sample by a 50 ml. measuring cylinder and pour it into the Erlenmeyer flask (note a).

2. Place the samples in a thermostatically controlled water bath at 23° C $\pm$ 1° C. Prepare the buffer and reductant, Reagents Nos. 1 and 2, as described in Section D above and place these solutions in the water bath (note b).

3. When the samples have reached bath temperature (this should take less than 30 minutes) remove them one at a time from the bath, add 2.0 ml. of buffer (Reagent No. 1) accurately from a clean automatic pipette (note c) mix by swirling and return each flask to the bath.

4. After the buffer has been added to all the samples remove each sample, again one at a time, add with rapid mixing 1.0 ml. of reducing solution (Reagent No. 2) accurately from a clean automatic pipette (note c) and return each flask immediately to the bath (note d). Shield the samples from direct light (daylight or artificial light) (note e) and leave them at 23° C in the bath for at least 8 hours. After about 8 hrs., temperature control is no longer critical and the flasks may be removed from the bath, if desired. They are then stored, lightly corked, in the dark for at least a further 12 hours at a temperature that should be between 18° C and 25° C, making a minimum standing time of 20 hours from the addition of the reductant (note f).

5. Add 2 ml. of acetone (Reagent No. 3) to each sample from an automatic pipette (note g).

6. At least 2 minutes after adding the acetone add 1.0 ml. of sulphanilamide solution (Reagent No. 4) from an automatic pipette (note h). Allow the reagent to react for a period greater than 2 minutes but not exceeding 8 minutes (note i).

7. Add 1.0 ml. of napthylethylenediamine solution (Reagent No. 5) and mix immediately. Between 10 minutes and 2 hours afterwards measure the extinction of the solution in a 1 cm. cell against distilled water (note j). A wavelength of 5430 A and slit width of 0.03-0.04 mm. should be

used with the Beckman DU Spectrophotometer (blue sensitive cell). If the extinction is less than 0.15 on a 1 cm. cell repeat the reading on a 10 cm. cell and use the latter value for calculation purposes. Unless adjacent samples are known to have extinction values within about 25 per cent of each other the absorptiometer cell should be rinsed with each new solution before filling.

8. If a filter type absorptiometer is used choose a filter having a peak transmission as near to 5400 A as possible and use only an absorptiometer giving meaningful readings for an extinction as high as 1.5. If this is not possible the 1 cm. cell readings should be repeated on a 10 cm. cell only if the 1 cm. cell extinction is less than 0.10. Record extinction values measured by a 1 cm. cell to the nearest 0.002 units in the range 0.150 - 0.250, to the nearest 0.005 for extinction values exceeding 0.250. If a 10 cm. cell is used, values need only be recorded to the nearest 0.01 at any level of reading.

9. Correct the observed extinction by that of a reagent blank (1 or 10 cm. cell) obtained as described is Section G. Calculate the nitrate present as described in Section J.

Note: Although some 18 to 20 hours is required for complete reduction about 85 per cent of the reduction takes place in the first 6 hours and a rough estimate of the nitrate in a sample can be obtained by completing the method after 6 hours and using a factor of 1.2 on the extinction so obtained.

Notes

(a) Reduction of nitrate to nitrite is said to be inhibited by

particulate matter, phytoplankton, etc. This effect probably depends on the nature of the particulate matter but for safety all samples should be filtered through a fine filter paper before the analysis commences.

(b) Although the reduction of nitrate to nitrite is not dependent on the temperature at which the reduction takes place, between 20° C and 25° C, it is very dependent on slight temperature changes and it is essential that the temperature of the sample does not change by more than ± 1° C during the first 4 hours following the addition of reductant. After this period the reduction can be carried out at any temperature in the range 18° C - 25° C, and a thermostatically controlled bath is unnecessary. The final extinction value can drop by 10 to 20 per cent if the initial temperature when reductant is added is a few °C higher or lower than the temperature at which the reduction takes place during the first 4-8 hours. Failure to realize this fact is probably responsible for much of the trouble that has been experienced with this method. Any bath temperature in the range 20° C - 25° C can be used without affecting the final extinction to a significant extent, but 23° C is chosen so that the same water bath may be used for this determination as is used for methods P. 1.A, P. 2.A, etc. The effect of salinity and the very important effect of movement on the method is discussed fully in Section H.

(c) The amounts of buffer and reductant, especially the former, are quite critical and should be measured to better than 10 per cent. This precision is quite feasible with rubber bulb automatic pipettes provided that care is taken when the pipettes are adjusted and the pipettes are kept clean and rinsed with water after use. The reduction to nitrite must take place in the pH range 9.50 to 9.65. Below a pH of 9.5 the amount of nitrite formed decreases to some 75 per cent of the maximum at pH 9.0. The amount

formed decreases as the pH exceeds 9.65 and is only a few per cent of the maximum by pH 9.8, probably due to the inactivation of the copper catalyst by the formation of colloidal magnesium hydroxide.

(d) Hydrazine will not reduce nitrate to nitrite without a catalyst. Copper is suitable provided that at least a minimum amount is added. The amount of copper is not very critical once it exceeds this minimum quantity (about 50 per cent of the amount used in the present method).

(e) Somewhat erratic values may have arisen as a result of the exposure of samples to bright light during the reduction stage and for safety, therefore, it is recommended that the reduction take place in the dark or in subdued light. Absolutely light-tight conditions are not required.

(f) The maximum nitrite formation occurs in about 18 hours at 20 - 25° C but for safety a period of at least 20 hours should dlapse after adding reductant and measuring the nitrite produced. No change in nitrite concentration occurs then for at least a further 24 hours.

(g) Acetone is added to destroy excess hydrazine.

(h) Reagents Nos. 4 and 5 are identical with reagents Nos. 1 and 2, respectively, specified in Standard Method No. N. 1.A. The determination of nitrite is identical with that described in N. 1.A. but is repeated here for convenience.

(i) Temperature is not critical at this stage provided that it falls in the range 15° C to 25° C. The diazotising reaction requires 2 minutes for completion but undesirable side reactions and decomposition become significant after about 10 minutes.

(j) Ten minutes are required for complete colour development. The colour is then stable for at least two hours.

G. Determination of Blank

1. Cell to Cell Blanks

When both sample and distilled water cells are filled with distilled water the extinction of one against the other should be 0.000. Due to slight optical defects, a small positive or negative value may be found and should be noted when determining reagent blanks. The water in the distilled water cell should be changed every day as marked turbidities can result, even in distilled water, if it remains in the cell for too long.

2. Reagent Blanks

The blank from reagents is barely significant when working with 1 cm. cells but should be checked occasionally. It assumes considerable importance when a 10 cm. cell is used. Ordinary distilled water may contain an appreciable quantity of nitrate and for use in the determination of reagent blanks distilled water should be redistilled from a little alkaline permanganate, rejecting the first few ml. distilling. Such water is assumed to contain no nitrate.

The blank should be determined when any batch of new reagents is used and checked about once a week on a cruise. If the amount of nitrate is small and 10 cm. cells are used, the blank should be determined once for each batch of samples.

Carry out the method exactly as described in Section F paragraphs 2 to 8, inclusive, using 50 ml. of redistilled water in a clean 125 ml. Erlenmeyer flask. Multiply the resulting extinction by the factor 0.85 to allow for the absence of sea salts (see Section H) and subtract this corrected extinction from the sample extinction values (F. 9 above). The

corrected blank extinction on a 10 cm. cell should not exceed about 0.15.

3. Preliminary Cleaning of Glassware

There is evidence that the condition of the surface of the 125 ml. Erlenmeyer flasks used in this method may affect results to some extent. Flasks should not be cleaned in chromic acid-sulphuric acid cleaning mixture. Before initial use the flasks should be half filled with concentrated sulphuric acid and allowed to stand overnight. The acid is then drained out and the flasks rinsed thoroughly with distilled water and then once with about 10 ml. of 1 per cent W/V sodium hydroxide. Finally the flasks are washed well with distilled water and drained.

The flasks should be rinsed with distilled water after use and stored with corks lightly in place. They should be re-cleaned before each analysis.

H. Calibration

1. Standard Nitrate Solution

Dissolve 1.27 g. of analytical reagent quality potassium nitrate, KNO_3, in 1000 ml. of distilled water. The solution is stable indefinitely in the absence of evaporation.

$$1 \text{ ml.} \equiv 12.5 \ \mu\text{g.at. } NO_3^{1-}\text{.N.}$$

Dilute 5 ml. of this solution to 250 ml. with synthetic sea water (see below) for use. This solution should be stored in a dark bottle and prepared fresh once a week.

$$1 \text{ ml.} \equiv 0.25 \ \mu\text{g.at. } NO_3^{1-}\text{.N.}$$

$$1 \text{ ml.} \equiv 5 \ \mu\text{g.at. } NO_3^{1-}\text{.N/l in 50 ml. of sea water sample.}$$

2. Synthetic Sea Water (Salinity approximately 31 ‰)

Use only analytical reagent quality reagent chemicals. In particular

the calcium chloride and magnesium chloride should be taken from well-stoppered bottles to ensure that the salts have not absorbed appreciable amounts of water. A more exact sea water formula is used in this method than in methods such as Standard Method No. P. 1.A as the magnesium content and buffering power must closely resemble natural sea water.

Dissolve 220 g. of sodium chloride, NaCl, 97 g. of magnesium chloride hexahydrate, $MgCl_2.6H_2O$, 37 g. of anhydrous sodium sulphate, Na_2SO_4, 10 g. of anhydrous calcium chloride, $CaCl_2$, 6.5 g. of potassium chloride, KCl, 1.7 g. of sodium bicarbonate, $NaHCO_3$ and 0.23 g. of boric acid, H_3BO_3, in about 2 liters of good quality distilled water. Filter the solution through an 18 cm. No. 1 Whatman filter paper and make up the filtrate to a volume of 10 liters with distilled water. This solution should be made up in 10 to 20 liter quantities and may be stored indefinitely in a clean glass vessel. The nitrate blank on this water should not exceed about 0.03 on a 1 cm. cell.

3. Reasons for Check Calibrations

The effect of salinity on this method is not very great provided that salinities are in the range 20 ‰ to 35 ‰ and a calibration made with a water of salinity 31 ‰ may generally be used for any sample with a salinity in this range. In pure water the extinction from a given amount of nitrate is about 15 per cent greater than that obtained in normal sea water of the same nitrate concentration. If the salinity exceeds 35 ‰ the extinction decreases quite rapidly and at S = 45 ‰ is only some 90 per cent of the value at S = 31 ‰. The calibration described here should only be used for samples of salinity between about 25 ‰ and 35 ‰ if the highest precision is required. With samples of salinity less than 20 ‰ a calibration is made using the above synthetic sea water diluted with distilled water to be within about ± 5 ‰ of the sample salinity.

The extinction for a given amount of nitrate depends upon the size and shape of the glass vessel in which nitrate reduction takes place. The use of a 125 ml. Erlenmeyer flask, as described in the present method, has much to recommend it. These flasks are more convenient and easier to handle at sea than are 50 ml. volumetric flasks and, for reasons not yet understood, the extinction for 50 ml. of a given sample is nearly double that obtained when using a 50 ml. volumetric flask. 125 ml. Erlenmeyer flasks have one drawback, however, in that the extinction value obtained is reduced when the flasks are rocked during the commencement of the reduction, as is the case on board ship. The sensitivity may decrease some 30-40 per cent in a rough sea. The sensitivity in volumetric flasks, although initially much lower, is not affected by movement. However the reproducibility in rocked Erlenmeyer flasks is still good, the non-linearity correction (see Section J) still applies and satisfactory results are easily obtained at sea, provided that each batch of samples is standardized by standards carried through the method simultaneously. The present method should not require repeated standardization when used is a shore-base laboratory, as the factor stays sensibly constant. At sea, the use of a small constant temperature serological bath in gimbals prevents most of the reduction in sensitivity brought about by ship movement.

4. Procedure

Prepare three standard solutions consisting of 6.0 ml. of the dilute nitrate standard (equivalent to 30.0 μg.at. N/l) made to a volume of exactly 50 ml. with synthetic sea water in a 50 ml. graduated measuring cylinder. Transfer the solutions to three dry 125 ml. Erlenmeyer flasks and add 50 ml. of synthetic sea water to a fourth flask to act as a blank. Carry out the nitrate determination exactly as described in Section F above (paragraphs 2

to 8 inclusive). Correct the mean of the three extinctions thus obtained by the blank extinction. Use this value to calculate the amount of nitrate present in samples as described in Section J below.

5. Preparation of a Calibration Curve

For reasons of chemistry, rather than absorptiometry, a strait line relationship is not obtained in this method between the concentration of nitrate in sea water and the resulting extinction. A calibration curve is needed for amounts of nitrate exceeding 10 μg.at. N/l. Once obtained this curve should not require re-determining and it can be adjusted by means of the experimentally determined extinction value at a 30 μg.at. N/l level if this extinction value varies from batch to batch of samples. This adjustment is described in the next section.

The calibration curve has the form:

$$E = a.\mu - b.\mu^2$$

where μ is the nicrogram atoms of NO_3^- nitrogen per liter and the constants a and b have values near to 2.3×10^{-2} and 3.4×10^{-5} respectively. Constant a may vary significantly from batch to batch but changes in the value of $b.\mu^2$ can be neglected (see Section J).

The calibration curve is <u>not</u> determined as a routine procedure but details are given here in case a calibration is made for training purposes.

To construct a calibration curve prepare a series of standards containing 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml. respectively of dilute nitrate standard, each made to 50 ml. with standard sea water. Prepare two standard sea water blanks and carry all sample and blank solutions through the method described in Section F (paragraphs 2 - 8 inclusive). Correct each extinction by the mean of the duplicate blanks and plot a graph showing corrected ex-

tinctions (1 cm. cell) on the ordinate against the number of μg.at. N/l on the abscissa.

J. Calculations

1. Correction for Nitrite

Some nitrite is destroyed by the hydrazine-copper reagent that reduces nitrate to nitrite. This probably explains why the main reduction does not exceed some 50 to 60 per cent of the theoretical. The fraction of nitrite destroyed increases somewhat as the quantity of nitrite decreases below 1 μg.at. NO_2^{1-}.N/l. As the nitrite content of sea water will not exceed 1 - 1.5 μg.at. N/l, and will only exceed 0.5 μg.at.N/l in exceptional circumstances it is unnecessary to correct for the presence of nitrite if the nitrate content of a sample exceeds 10 μg.at. NO_3^{1-}.N/l. For precise values of quantities of nitrate that are less than 10 μg.at. NO_3^{1-}.N/l the initial nitrite in the sample should be determined (Standard Method No. N. 1.A) and the nitrate volue corrected as follows:

Correct NO_3^- (μg.at. N/l) = Found NO_3^- (μg.at. N/l) - 0.45 (μg.at. NO_2^-.N/l)

A mean factor of 0.45 is sufficiently precise for amounts of nitrite between about 0.2 and 1.5 μg.at. N/l. Although this factor decreases to 0.2 or less as the NO_2^{1-} content decreases below 0.2 μg.at. N/l the correction itself then becomes so small that errors in assuming a constant factor of 0.45 may generally be neglected.

2. Calculation of Factor

Although the concentration-extinction relationship for this method is non-linear it is unnecessary to use a curve for calibration purposes or to re-determine the curve should the calibration change. The deviation from

a straight line is so small that the term $b\mu^2$ (see Section H. 4) may be assumed to be a function of E.

A linear factor is used for all E values (1 or 10 cm. cells) after correcting 1 cm. cell extinction values, if necessary, as shown in the accompanying table. This correction is not made for 1 cm. cell extinction values below 0.2 or for any 10 cm. cell extinction value.

$$\text{1 cm. cell factor, } F_{1\text{ cm.}} = \frac{30}{E + e}$$

where E is the mean corrected extinction for 30 μg.at. N/l (see Section H), and e the correction shown in the Table below.

$$\text{10 cm. cell factor, } F_{10\text{ cm.}} = 0.10 \times F_{1\text{ cm.}}$$

$$\mu\text{g. at. } NO_3^-\text{.N/l} = \left[E \text{ (corrected for blank)} + e\right] \times F$$

where the correction, e, is read from the following table according to the found E value. The value for $F_{1\text{ cm.}}$ should be near to 44 for stationary flasks. The maximum error incurred by neglecting the correction, e, does not exceed 5 per cent for most nitrate values but the precision of the present method is sufficiently high to warrant the refinement of a correction for non-linearity.

Table of Corrections

Found E	Add e	Found E	Add e
0.000 - 0.200	Nil	0.685 - 0.730	0.035
0.200 - 0.225	0.002	0.735 - 0.780	0.040
0.227 - 0.250	0.004	0.785 - 0.820	0.045
0.255 - 0.360	0.005	0.825 - 0.860	0.050
0.365 - 0.450	0.010	0.865 - 0.900	0.055
0.450 - 0.520	0.015	0.905 - 0.930	0.060
0.520 - 0.580	0.020	0.935 - 0.950	0.065
0.580 - 0.640	0.025	0.955 - 0.975	0.070
0.645 - 0.680	0.030	0.980 - 1.000	0.080

Approved March 1958 Standard Method No. N. 3.A.

DETERMINATION OF AMMONIA IN SEA WATER

A. Capabilities of the Method

The method, as written, is intended primarily for sea water of salinity greater than 20 ‰ .

Range 0.2 - 10 μg.atoms N/liter

The following data are based on a statistical treatment of the results obtained by trained operators in a shore-base laboratory. Statistics under sea-going conditions are awaited but the precision is not expected to be seriously affected.

1. Precision at 5 μg.at.N/l level

The correct value lies in the range: Mean of n determinations $\pm \frac{0.45}{n^{1/2}}$ μg.at.N/l.

2. Precision at 0.7 μg.at.N/l level

The correct value lies in the range: Mean of n determinations $\pm \frac{0.2}{n^{1/2}}$ μg.at.N/l.

3. Limit of Detection

The smallest quantity of ammonia that can be detected with certainty by a single determination is about 0.2 μg.at.N/l.

Reject duplicate determinations if the extinction* values differ by

---

* The extinction, E, is defined as $\log_{10} \frac{I_o}{I}$ where I_o and I are the incident and transmitted light intensities, respectively. The Beckman DU Spectrophotometer is calibrated directly in extinction units. If the absorptiometer employed reads per cent transmission, T, calculate E as $\log_{10} \left(\frac{100}{T}\right)$.

more than 0.02 in the extinction range 0.1 - 0.2

or more than 0.04 in the extinction range 0.2 - 0.5

or more than 0.06 in the extinction range 0.5 - 1.0.

If duplicate extinction values differ by less than the above limits, take a mean value.

Note. The above statistical data apply at the 0.05 probability level, i.e., the odds are 19 to 1 in their favour. The results apply only to filtered sea water where the ammonia is in true solution. In raw sea water some ammonia may be adsorbed onto particulate matter and more erratic figures are to be expected.

B. Outline of Method

The sea water is treated with chloramine-T and a "pyrazolone" reagent in the presence of pyridine. The complex thus formed with ammonia is extracted into carbon tetrachloride and the extinction of this extract (a yellow solution) is measured using a 10 cm. cell and light of wavelength near to 4500 A.

C. Special Apparatus and Equipment

A Beckman DU Spectrophotometer or any other seaworthy spectrophotometer capable of measuring light of wavelength near to 4500 A.

Short-stem separatory funnels, pear shaped or cylindrical, having a capacity of at least 200 ml. and, preferably, not more than 500 ml.

D. Special Reagents Required

1. Deionized Water

As the limit of ammonia that can be tolerated in the water used in this method is less than about 2 μg. per liter, ordinary distilled water cannot

always be trusted and should be passed through a small column of cation exchange resin just before use and stored in tightly-stoppered glass flask.

2. Carbon Tetrachloride

Use only analytical reagent grade material.

3. Pyridine

Analytical reagent grade pyridine has been found to be satisfactory, although this may not necessarily always be the case. Other sources of pyridine are generally unusable directly, giving a strong blue-green colour in the aqueous phase (see later) but no colour on extraction. To prepare pyridine suitable for use in this method, add a few drops of bromine and distill the pyridine through a fractionating column, collecting the fraction with a 2 to 3° C boiling range near to 113° C (uncorrected reading). About 10 to 20 per cent of the pyridine may have to be rejected as a low boiling fraction.

4. bis-(3-methyl-1-phenyl-5-pyrazolone)

This compound is available commercially under the name 3.3' Dimethyl-1-1' diphenyl-(4-4'-bi-2-pyrazoline)-5-5'dione (E.K. 6969). It is only needed in small amounts and can conveniently be prepared by refluxing 17 g. of 3-methyl-1-phenyl-5-pyrazolone with 25 g. good quality phenyl hydrazine in 100 ml. of ethyl alcohol. The bis compound is separated every few hours for about 24 hours as it precipitates out, washed with hot alcohol and dried in a dessicator. The amount thus produced is sufficient for well over one hundred batches of determinations.

5. Chloramine-T Solution

Dissolve 5 g. of good quality chloramine-T (trihydrate) in 100 ml. of deionized water. Store in a well-stoppered glass bottle. The solution is stable for several days but should be renewed at least once every two weeks.

For long cruises one or two extra 5 g. lots of chloramine-T should be taken, weighed out in small tubes.

6. 3-methyl-1-phenyl-5-pyrazolone solution (Mono Reagent)

Dissolve 5.0 g. of recrystallized (once from hot water) 3-methyl-1-phenyl-5-pyrazolone in 2000 ml. of deionized water. Store the solution in a well-stoppered glass or polyethylene bottle. The solution is stable for many months but should be prepared at least once a cruise to minimize the possibilities of contamination.

7. "Pyrazolone" Reagent

This reagent must be prepared immediately before use and should not be kept for longer than 2 hours. For work at sea 0.08 g. quantities of bis-(3-methyl-1-phenyl-5-pyrazolone) should be taken, already weighed into small tubes.

Dissolve 0.08 g. of bis-(3-methyl-1-phenyl-5-pyrazolone) in 80 ml. of pyridine and add 400 ml. of Mono Reagent. Store in a stoppered 500 ml. glass flask for a maximum of 1 to 2 hours.

8. Acetate Buffer

Dissolve 11 g. of analytical reagent quality sodium acetate trihydrate crystals ($NaC_2H_3O_2.3H_2O$) in a little water. Add 65 ml. of analytical reagent quality glacial acetic acid and make the volume to 500 ml. with deionized water. Store in a tightly-stoppered glass or polyethylene bottle and renew every few months, or before each cruise, to minimize the possibilities of contamination.

E. Sampling Proceudre and Sample Storage

Temporary storage of sea water prior to analysis appears to be satisfactory in either glass or polyethylene but the analysis should not be

delayed for more than 1 to 2 hours at the most. If longer storage periods are necessary freeze the samples solid in a deep-freeze. There are indications that, even with refrigeration, losses may be significant after more than a few days, but more evidence is required.

F. Experimental

Note. About 6 to 8 samples is the maxiumum number that are conveniently analysed at one time.

Procedure

1. Adjust the temperature of the sea water to be between 18° C and 23° C and measure 80 ± 2 ml. of sample into a separatory funnel. Add 1.0 ml. of acetate buffer from a 1 ml. automatic pipette (note a).

2. Add 1.0 ml. chloramine-T from an automatic pipette and wait for at least 90 seconds (note b) before adding 40 ml. of "Pyrazolone" Reagent (note c).

3. After at least a further 1 minute (note b) add 35 ± 0.5 ml. of carbon tetrachloride from a dry 50 ml. measuring cylinder. Extract the ammonia complex by shaking the funnel vigorously for 1 minute and allow the layers to separate (note d).

4. Run off the carbon tetrachloride layer through a dry 9 cm. No. 1 Whatman filter paper into a dry 10 cm. absorption cell and measure the extinction against distilled water (notes d and e). A wavelength of 4500 A and a slit width of about 0.04 mm. should be used with the Beckman DU Spectrophotometer (blue sensitive photocell). If a filter-type absorptiometer is used, choose a filter having a maximum transmission near to 4500 A. Record extinction values (and corrected extinction values, see 5 below) to the nearest 0.005 in the range 0.10 - 0.40 and to the nearest 0.01 for extinction values exceeding 0.40. There is little extract to spare for rinsing cells and, with

this method, it is sufficiently precise simply to drain cells before refilling them. If a very low extinction is to be measured immediately following a high value it would be better to rinse the cell in between with a few ml. of pure carbon tetrachloride.

5. Correct the measured extinction by subtracting a reagent blank obtained as described in Section G. Calculate the ammonia concentration in microgram atoms of ammonia nitrogen per liter (μg.at.N/l) from the expression

$$\mu\text{g.at.N/l} = \frac{\text{Corrected extinction x F}}{(1 - 7.6 \times 10^{-3} \times S)}$$

where S is the salinity of the sample and F is a factor obtained as described in Section H below. Report results to two significant figures. There is no interference in this method from soluble proteins, amino acids, nucleic acids, nucleotides, choline or urea. Cyanide, cyanate and thiocyanate interfere and are partially counted as ammonia, but their occurrence in sea water is unlikely.

Notes

(a) An 80 ml. sample of sea water gives about the same resulting extinction with a given concentration of ammonia as is obtained by taking 50 ml. of fresh water (see later). The reaction takes place only in the pH range 3.2 - 3.8. The acetate buffer should give a final pH near to 3.6. No temperature effect has been noted in the range 18° C to about 23° C but outside this range extinctions decrease a little.

(b) The chloramine-T reaction must be allowed to proceed for at least 70 to 80 seconds and 90 seconds gives a reasonable safety margin. No harm is done if this period is extended to as much as 5 minutes. Similarly, the final complex requires at least 30 to 40 seconds to form after adding the "phrazolone" reagent but solutions can be left for at least 5 minutes, and probably longer, before the extraction with carbon tetrachloride is commenced.

(c) With more than 3 to 4 μg.at.N/l present the aqueous phase will assume a faint purple tint after adding the "pyrazolone" reagent. Normally no colour is noticed. If the solution becomes reddish in colour, quickly changing to blue-green, the pyridine is suspect. Such solutions do not give a coloured extract with carbon tetrachloride.

(d) Low and erratic figures have been obtained with less than about 60 seconds vigorous shaking. After extraction the colour in the carbon tetrachloride layer is stable for a least an hour whilst still in the separatory funnel and for at least 24 hours if stored in a stoppered flask, after removal from the funnel.

(e) Filtration clears the solution of any fine droplets of occluded sea water.

G. Determination of Blanks

1. Reagent Blanks

Carry out the method exactly as described in Section F, paragraphs 1 to 4 inclusive, using 80 ml. of freshly deionized water. The assumption is made that no ammonia is introduced by this water and that all the colour in the extract results from ammonia present in reagents. Multiply the resulting extinction by 0.62 to allow for the presence of salts in sea water (see Section H) and use this value to correct the extinction obtained from samples (ref. Section F. 5). At least one blank determination should be carried out for each batch of samples extracted.

2. Precautions to Reduce Contamination

The greatest care is necessary to prevent the contamination of reagents and samples by ammonia (carried as gas or the vapours of ammonium

salts) in the laboratory. Solutions should be kept in tightly-stoppered bottles, except when in use, and samples should be stored in well-stoppered containers until the analysis commences. In no circumstances must a bottle of ammonium hydroxide be opened in the laboratory, for however brief a period, whilst analyses for ammonia are being carried out. Glass ware should be rinsed very thoroughly with distilled water immediately prior to use.

H. Calibration

1. Standard Ammonia Solution

Dissolve 0.427 g. of analytical reagent quality ammonium chloride, NH_4Cl, in 1000 ml. of distilled water. Add a ml. of chloroform and store in a dark bottle. The solution is stable for many months.

1 ml. ≡ 8.0 µg.at.N

Dilute 10.0 ml. of this solution to 1000 ml. with sea water (see below). Use this solution within one hour.

1 ml. ≡ 0.080 µg.at.N

1 ml. ≡ 1.0 µg.at.N/l on 80 ml. of sea water

2. Sea Water

Sea water taken from about 5 m. depth, especially in the summer, and filtered through a Millipore A.A. filter rarely contains more than 0.5 to 1 µg.at.N/l and has a markedly lower ammonia content than synthetic sea water made from even the purest salts. A supply of several liters of such water is required for standardization. The salinity should be in the range 25 ‰ to 35 ‰ and known to about the nearest 0.5 ‰.

3. Procedure

Add 70 ml. of sea water and 10.0 ml. of dilute ammonia standard to each of six separatory funnels. Add 80 ml. of sea water to two more funnels

to act as blanks. Carry out the method exactly as described in Section F, paragraphs 1 to 4 inclusive on all six standards and on the two blanks.

Evaluate the factor F from the expression:

$$F = \frac{10.0\ (1 - 7.6 \times 10^{-3} \times S)}{(E_s - E_b)}$$

where E_s is the mean extinction of the six standards, E_b is the mean extinction of the two blanks (not corrected for a reagent blank), and S is the salinity in ‰ of the sea water used as a base for standardization.

The value for F should be near to 8.5 and need only be determined once. Subsequent evaluations are only necessary for training purposes or if the technique is suspect.

The corrections made here and in Section F.5, for salinity arise from the fact that the salts in sea water depress the extinction obtained from a given quantity of ammonia. The value at S = 30 ‰ is only some 0.62 times the value obtained in pure water. This factor is sufficiently precise to correct the blank value obtained in distilled water (see Section G) for use with samples having a salinity between about 25 ‰ and 35 ‰. Although the extinction-salinity relationship is curved, the curve flattens appreciably at higher salinities and may be approximated to linear over the range 20 ‰ to 35 ‰. This is the basis of the corrections given in the formulae in this method. For samples of salinity below 20 ‰ the standardization should be repeated, in triplicate at least, making a large addition (say 10 μg.at.N/l) to the sea water concerned.

The maximum error that can be introduced by neglecting the salinity effect in the range 20 ‰ to 35 ‰ is only about 15 per cent so that the use of the correction term is scarcely justified in the more usual ranges of sea water salinity (say 29 ‰ to 34.5 ‰) encountered in the Northeast Pacific. A

factor obtained using sea water of salinity about 30 ‰, from the straightforward relation:

$$\text{factor} = \frac{10.0}{E_s - E_b} = \text{ca. } 11$$

can be used directly with the observed extinction on a sample (see Section F, paragraph 5).

Approved March 1958 Standard Method No. Fe. 1.A.

DETERMINATION OF BIOLOGICALLY REACTIVE IRON IN SEA WATER

DEFINITIONS

1. Particulate and soluble iron

The iron compounds passing through a millipore membrane filter of pore size 0.4 micron will be arbitrarily designated as "soluble". Such compounds will consist of truly soluble complexes of ferric iron and of colloidal ferric hydroxide and phosphate particles. The uncomplexed ferric ion cannot exist at the pH of sea water in measurable amounts and ferrous iron will not normally occur unless exceptional biological conditions have produced an anaerobic environment. As the amounts of both colloidal and complexed ferric iron are largely governed by the presence of organic matter the 'soluble' fraction of the total iron is probably a crude measure of the soluble organic material in sea water. Often not more than about a half of the total iron is found to be 'soluble' and the fraction may often be less than one tenth.

The iron compounds retained on a millipore membrane filter consist either of iron in mineral combination (sand,clay, volcanic ash, etc.) or in organic combination (in plankton and detritus), or as 'flocks' of ferric hydroxide and phosphate, either free or absorbed onto other particles.

2. Biologically active iron

There is evidence that much of the soluble iron that is combined in the form of organic complexes may not be available for plant growth.

The particulate iron that is found in sand or clay particles, or as volcanic ash, etc., will clearly be unreactive, biologically, for rapidly

growing plants. These plants can, however, make direct use of "colloidal" and "particulate" forms of precipitate ferric hydroxide and phosphate.

The treatment with hydrochloric acid described in the present method ensures the true solution of all such precipitated and colloidal forms of phosphate or hydroxide. Ferric hydroxide, dried at 100°C, is also dissolved but all ignited ferric compounds and much of the iron present in plant tissue remain unattacked. Thus a result for iron by this technique is much less of an overestimate of the immediately available biologically active iron than would be a "total" iron figure, obtained by some process of high temperature wet digestion.

Part I. Determination of Particulate Reactive Iron

A. Capabilities of the Method

Range ca. 0.008 - 0.8 μg.atoms Fe/liter using a 1 liter sample

The distribution of particulate iron in sea water is so erratic that a statistical estimate of the precision of this method has little meaning. Most of the variation is introduced from the sampling technique and the *in situ* distribution of the iron. Duplicate samples taken from the same depth in different bottles may each differ by as much as 30 percent from the mean figure.

The precision (0.05 probability level) of the purely chemical part of the procedure is relatively high, being better than:

$$\pm \frac{0.012}{n^{1/2}} \mu g.at.Fe/l$$

on a liter sample, where n is the number of determinations. The limit of detection of particulate iron with a single determination is about

$$\frac{8}{V} \mu g.at.Fe/l$$

where V is the volume of sample taken in ml.

B. Outline of Method

The volume of sea water used in this method is such that the iron contained therein does not exceed 0.8 μg.at.Fe (generally 500 ml. - 2000 ml.). This sample is filtered through a millipore HA filter, retaining particles of diameter exceeding about 0.45 microns, and the filter is treated with hot dilute hydrochloric acid for a few minutes. The iron thus brought into solution is reacted with α-α'-dipyridyl in an acetate buffer in the presence of hydroxylamine to give an orange ferrous complex. The extinction of this complex is measured using 10 cm. cells and light of wavelength near to 5220 A.

C. Special Apparatus and Equipment

HA White Plain Filters, 47 mm. diameter , manufactured by the Millipore Filter Corporation of Watertown 72, Massachusetts, U.S.A.

Pyrex filter holder and clamp supplied by the Millipore Filter Corporation for use with their membrane filters.

Plastic 'bone' tipped forceps for handling filters.

1000 ml. glass Pyrex Buchner flask and source of moderate vacuum (water aspirator pump).

A Beckman DU Spectrophotometer or any other absorptiometer capable of measuring the extinction of light of wavelength near to 5000 A.

Spectrophotometer or absorptiometer cells having a path length of at least 10 cm. and holding less than 40 ml. of solution.

Fifty ml. capacity stoppered graduated glass measuring cylinders,

one for each iron determination.

Full details for preliminary cleaning, etc., are given in Section G below.

D. Special Reagents Required

1. Bathophenanthroline solution (Reagent No. 1).

Dissolve 0.070 g. of 4-7-diphenyl-1-10 phenanthroline (bathophenanthroline) in 100 ml. of ethyl alcohol and then add 100 ml. of distilled water. Keep in a well-stoppered polyethylene bottle.

2. Iso-amyl alcohol

Use the reagent grade alcohol. If this is not available it is safest to distill other grades, rejecting the first 10 per cent and the last 5 per cent of the material during distillation.

3. Hydroxylamine Hydrochloride (Reagent No. 2)

The reagent grade salt should be recrystallized twice, by warming and then chilling in ice water, from firstly, 0.4 times its weight of 10 per cent V/V hydrochloric acid and, secondly, 0.4 times the remaining weight of distilled water. Wash the final product with a few ml. only of cold water and dry it by heating in an air oven at 110° C for one or two hours. The product thus obtained still contains too much iron for the best work and is purified by extraction.

Dissolve 10 g. of the salt in 100 ml. of distilled water in a clean separatory funnel. Add about 5 ml. of Reagent No. 1 and extract with about 10 ml. of iso-amyl alcohol. Run off the lower aqueous layer into a second separating funnel and add a further few ml. of Reagent No. 1 and re-extract with 10 ml. portions of the alcohol until the extracts are colourless. Allow the final extract to separate for 5 to 10 minutes before running the hydro-

xylamine solution into a well-stoppered 130 ml. polyethylene bottle which is kept well stoppered.

4. Sodium Acetate Buffer (Reagent No. 3)

Dissolve 75 g. of reagent grade sodium acetate trihydrate ($NaC_2H_3O_2.3H_2O$) in 100 ml. of distilled water. Add 2 ml. of Reagent No. 2 and 5 ml. of Reagent No. 1 and allow the mixture to stand for about 15 minutes. Extract the solution with about 10 ml. portions of iso-amyl alcohol until the alcohol layers are colourless. Add a further few ml. of Reagent No. 1 and re-extract to confirm that all iron and copper (a yellow extract) have been removed. Allow the final extract to separate for 5 to 10 minutes before running the sodium acetate buffer into a well-stoppered polyethylene bottle.

5. α-α-Dipyridyl Reagent (Reagent No. 4)

Dissolve 0.40 g. of the reagent in 2.0 ml. of concentrated hydrochloric acid (s.g. 1.19) and a little water. Dilute the solution to 100 ml. with distilled water.

6. Iron Extraction Reagent (Reagent No. 5)

Dilute 20.0 ml. of concentrated hydrochloric acid (s.g. 1.19) to 500 ml. with distilled water (giving a solution approximately 0.48 N). Store the reagent in polyethylene.

Sampling Procedure and Sample Storage

Note Very great care is necessary with this method to ensure that all glassware and storage vessels are iron free. For full details see Section G.

Except near the surface in some coastal areas, where the iron content may exceed 1 μg.at.Fe/l, 1000 ml. of sample are generally satisfactory. Only

if precise values are necessary, in low particulate iron areas, need the sample be increased to 2 liters. The sample must be taken in a non-metallic sampling bottle fastened to a stainless steel wire. For the best work, a duplicate, or preferable a triplicate, sample should be taken at each depth, using a separate bottle for each sample. Surface samples open to suspicion and a 5 m. sample is probably equally informative.

When sea water samples are stored in either glass or polyethylene there is a slow deposition of iron on the sides of the containers by processes of adsorption and settling. Prolonged storage of sea water in glass may lead to attack of the surface, with liberation of iron into the sample or subsequent samples. For these reasons, the samples for iron should be filtered within a few hours of removing from the sampling bottles, during which time they can be stored in clean glass or polyethylene. Before transferring iron samples from one container to another, or to a filter, the samples should be vigorously shaken. The filters containing particulate iron may be folded, clipped between paper and stored more or less indefinitely at room temperature, if suitable precautions are taken to prevent contamination. The filtrate for "soluble" iron (see later) should be analysed without delay.

F. Experimental

Procedure

1. Assemble the Millipore filtration equipment and 1000 ml. Buchner flask, having ensured the glassware is iron-free (see Section G). Fit a filter in place using clean plastic ended forceps and commence at once the filtration of a well-shaken sample (generally 1 liter) (note a).

2. Having filtered a suitable volume of sample suck the filter dry (do not wash it), dismantle the filtration unit and rest the glassware on a

clean paper towel until required for the next sample. Remove the filter with the forceps and place it immediately into a suitable storage container or into a 50 ml. measuring cylinder, as described below.

3. Pour 10 ml. ± 0.05 ml. of iron extraction reagent (Reagent No. 5) into a clean well-drained 50 ml. stoppered measuring cylinder. Introduce the filter into this solution by placing it centrally over the mouth of the cylinder and pushing it down into the cylinder with a blunt-ended glass rod of 3 to 4 mm. diameter. The very small fraction of iron from the filter that may adhere to the rod can be neglected. The collapsed filter should be completely submerged below the surface of the extraction reagent.

4. Place the cylinder into a pan of boiling water and allow it to stand in the water near the boiling point for between 10 and 15 minutes (note b).

5. Cool the cylinders containing the extracted iron until they are at room temperature again (note f) and complete the iron determination without delay (note b).

6. Add 1.0 ml. of hydroxylamine solution (Reagent No. 2) from an automatic pipette, followed by 2.0 ml. of sodium acetate buffer (Reagent No. 3) and mix the contents of the cylinder by shaking (notes c and d).

7. Add 1.0 ml. of dipyridyl reagent (Reagent No. 4) from an automatic pipette and then make the volume in the cylinder to exactly 50 ml. with distilled water (note e). Mix thoroughly.

8. Allow the colour to develop for at least 20 minutes (note f) and then measure the extinction of the solution in a 10 cm. cell against distilled water. A wavelength of 5220 A and slit width of about 0.03 mm. should be used with the Beckman DU Spectrophotometer (blue sensitive photocell). If a filter-type absorptiometer is used choose a filter having a maximum trans-

mission in the region of 5000 A. Record extinction values (and corrected values, see 9 below) to the nearest 0.01 unit at all levels.

9. Correct the measured extinction by subtracting that of a blank (see Section G). Calculate the "particulate reactive iron" content in microgram atoms of particulate iron per liter (μg.at.Fe(P)/l) from the expression:

$$\mu\text{g.at.Fe(P)/l} = \text{Corrected extinction} \times \frac{F}{V}$$

where F is a factor obtained as described in Section H, below, and V is the number of ml. of sample initially filtered in this determination. Report results to two significant figures. Should the extinction exceed about 1.5 it may be determined, with some loss of precision, by measuring the value against water in a 1 cm. cell and multiplying the result by ten. The determination is best repeated using a smaller sample of water.

Notes

(a) The Millipore filter should not be touched by hand or metallic forceps. The "bone-ended" forceps used for balance weights are suitable. After assembly, any iron containing dirt or dust that enters the filtration unit may be recorded as particulate reactive iron so that filtration should be started and completed without delay.

(b) As mentioned in the "Definitions" section of the method this treatment is designed to leach out any ferric iron that can reasonably be assumed to be available for growing marine phytoplankton. The treatment should not be prolonged beyond about 15 minutes as the "reactivity" of iron is purely relative and some comparatively inert forms may commence to be extracted. The heating process can be carried out batchwise and filters may remain in cold extraction reagent until a sufficient number of cylinders have accumulated.

However, filters should not soak in cold Reagent No. 5 for more than about 1 to 2 hours before they are heated. The very slight adsorption of dipyridyl complex seen on the filter when determining large amounts of iron results in an error of 1 to 2 per cent at the most, and may be neglected.

(d) Hydroxylamine is used to reduce ferric iron to the ferrous condition, which is the form reacting with the α-α'-dipyridyl. Provided that the volume of Reagent No. 5 is kept in the range 9.5 to 10.5 ml., the pH of the buffered solution should fall in the range 4 - 4.5, which ensures the rapid reduction and complexing of any iron initially present in true solution.

(e) Good quality distilled water should normally be sufficiently iron-free and any iron introduced is allowed for in a blank determination (Section G). However certain sources (old stills, ship's condenser water, etc.) may be suspect. This possibility should always be borne in mind and the water re-distilled from a Pyrex Still if necessary.

(f) Full colour development should occur in about 10 minutes, provided that the temperature of the solution exceeds about 20° C, but for safety a period of 20 - 30 minutes is allowed. The colour is completely stable, thereafter, for at least 24 hours. Solutions strictly obey the Beer-Lambert Law. The presence of sea salts has little or no effect on the extinction and it is therefore not necessary to make corrections for salinity. Calibration (see Section H) can be carried out in distilled water.

G. Determination of Blanks

1. Reagent Blanks

With good quality distilled water the blank extinction from reagents should not exceed about 0.02. The iron extracted from Millipore filters by the present procedure is very small but just about significant, contributing

an additional extinction of about 0.01, if the filters are handled with care. To allow for this, and possible changes from batch to batch of filters, a duplicate blank determination should be carried out with each batch of samples being analysed.

Carry out the method exactly as described in Section F, paragraphs 3 - 9, using a fresh unwashed Millipore filter taken from the packet with forceps. Measure the extinction against water in a 10 cm. cell and use the mean of duplicate values (which should not differ by more than about 0.015) to correct the sample extinction values as described in Section F, paragraph 10.

2. General precaustions and the cleaning of polyethylene and glassware

The necessity for cleanliness whilst carrying out this method cannot be overstressed, especially in a location such as the laboratory on a steel ship.

All solutions, vessels, and filtration equipment should be kept covered when temporarily not in use and the equipment must be thoroughly rinsed before using after even a short shut-down. Before each cruise the apparatus is re-cleaned by the following method:

All bottles used to contain reagents, the 50 ml. measuring cylinders and Millipore filtration unit and flask are freed from iron by rinsing thoroughly with hot 70 per cent V/V hydrochloric acid, followed by liberal quantities of distilled water. When decontaminating apparatus of an unknown history for the first time the cleaning solution should remain in contact with all surfaces for several minutes. When cleaning polyethylene surfaces the solution should be shaken frequently and the addition of a little acid stable cationic wetting agent (of the aryl-trimethyl ammonium type) is advantageous.

H. Calibration

1. Standard Iron Solution

Dissolve 0.392 g. of analytical reagent quality ferrous ammonium sulphate, $FeSO_4(NH_4)_2SO_4.6H_2O$, in a little water. Add 2 ml. of concentrated hydrochloric acid and dilute the solution to 100 ml. in a volumetric flask.

1 ml. ≡ 10 µg.at.Fe

For use dilute 5.0 ml. of the above solution to 500 ml. with distilled water. Do not keep this solution for longer than 1 - 2 days.

1 ml. ≡ 0.1 µg.at.Fe

2. Procedure

Add 10 ml. of extraction reagent (Reagent No. 5) to each of six 50 ml. stoppered measuring cylinders. Reserve two as blanks and to each of the remaining four add 5.00 ml. of dilute standard iron solution. Carry out the determination exactly as described in Section F, paragraph 7 - 9 inclusive.

Calculate the factor F from the expression:

$$F = \frac{500}{E_s - E_b}$$

where E_s is the mean extinction of the four standards and E_b the mean extinction of the two blanks. The value for F should be near to 580 and should not require re-determination, except for training purposes or when there is reason to suspect an error of technique (see note f).

Part II. Determination of Soluble Reactive Iron

A. Capabilities of the Method

Range ca. 0.04 - 1.5 µg.atoms Fe/liter

The following data are based on a statistical treatment of the results

obtained by trained operators in a shore-base laboratory. Statistics under sea-going conditions are awaited.

1. Precision at 0.75 µg.at.Fe/l level

The correct value lies in the range:

Mean of n determinations $\pm \frac{0.03}{n^{1/2}}$ µg.at.Fe/l.

2. Precision at 0.1 µg.at.Fe/l level

The correct value lies in the range:

Mean of n determinations $\pm \frac{0.025}{n^{1/2}}$ µg.at.Fe/l.

3. Limit of Detection

The smallest quantity of soluble reactive iron that can be detected with certainty by a single determination is about 0.025 µg.at.Fe/l.

Reject duplicate determinations if the extinction values differ by more than:

. 0.02 in the extinction range 0.1 - 0.25

or more than: 0.03 in the extinction range 0.25 - 0.50

or more than: 0.04 in the extinction range 0.5 - 1.0

If duplicate extinction values differ by less than the above limits, take a mean value.

B. Outline of Method

One hundred ml. of filtered sea water is treated with hydrochloric acid and then reacted with bathophenanthroline in an acetate buffer in the presence of hydroxylamine. The coloured ferrous complex thus formed is extracted into iso-amyl alcohol and the extinction of the coloured extract is measured using 10 cm. cells and light of wavelength near to 5330 A.

C. Special Apparatus and Equipment

The present determination is carried out on the filtrate from the particulate iron determination and thus the equipment needed for that method is required for the present procedure. In addition there is required, for each determination, a pear-shaped separatory funnel of at least 200 ml. capacity, with the stem shortened to a few cm. length and drawn to a small orifice. Fifty-ml. stoppered measuring cylinders are also required for this method and may constitute an additional requirement if the soluble iron determination is carried out before the particulate determination is concluded.

D. Special Reagents Required.

The same reagents are required as for the particulate iron method Part I, Section D, except for the α - α^1-dipyridyl solution, which is not used.

E. Sampling Procedure and Sample Storage

See Part I, Section E.

F. Experimental

Procedure

1. Transfer 100 ml. of filtrate from the particulate iron determination (note a) into a clean separatory funnel.

2. Add 10 ml. of iron extraction reagent (Reagent No. 5) followed by 2 ml. of phydroxylamine hydrochloride solution (Reagent No. 2) from an automatic pipette. Mix the solutions in the separatory funnel and allow the mixture to stand for 5 minutes (note b).

3. Add 2.0 ml. of acetate buffer (Reagent No. 3) from an automatic pipette followed by 5 ml. of bathophenanthroline solution (Reagent No. 1). Mix and allow the solution to stand for a further 10 minutes (note c).

4. Measure from a dry 50 ml. stoppered measuring cylinder 30 ± 1 ml. of iso-amyl alcohol and drain the alcohol into the separating funnel. Do not wash the cylinder but re-stopper it. Extract the contents of the separatory funnel vigorously for 1 minute and then allow the layers to separate for at least 5 minutes. Discard the lower aqueous layer. Swirl the funnel to dislodge any water droplets, allow the water to collect and again separate the aqueous layer.

5. Run the alcohol layer, without contamination from more than a drop or two of aqueous solution, back into the 50 ml. measuring cylinder. Make the volume to exactly 35 ml. with acetone and mix (note d).

6. Measure the extinction of the solution in a 10 cm. cell against distilled water. A wavelength of 5330 A and slit width of about 0.03 mm. should be used with the Beckman DU Spectrophotometer (blue sensitive photocell). If a filter-type absorptiometer is used, choose a filter having a maximum transmission in the region of 5500 A (note d). Record extinction values (and corrected extinction values, see 7 below) to the nearest 0.001 unit in the range 0.000 - 0.1, to the nearest 0.002 unit in the range 0.1 - 0.40 and to the nearest 0.005 unit for extinction values exceeding 0.40. Rinse the sample cell with acetone after taking each reading.

7. Correct the measured extinction by subtracting that of a blank (see Section G). Calculate the "soluble reactive iron" content in microgram atoms of soluble iron per liter (μg.at.Fe(S)/l) from the expression:

$$\mu\text{g.at.Fe(S)/l} = \text{Corrected extinction} \times F$$

where F is a factor described as in Section H below.

Notes

(a) Careful use of a clean 100 ml. measuring cylinder for this measurement is adequate.

(b) All colloidal forms of ferric hydroxide, etc., are brought into solution. The more stable organic complexes may not be attacked although it is impossible to generalize on this point and the method may well overestimate the soluble iron capable of being utilized by phytoplankton.

(c) At room temperature, all dissolved ferric iron that is not already strongly complexed will form the ferrous-bathophenanthroline complex which can be extracted into amyl alcohol. Much of the increased sensitivity that results from the use of bathophenanthroline, rather than 1:10 phenanthroline or α-α' dipyridyl, is brought about by the fact that the coloured complex can be concentrated by extraction.

(d) Very slight losses of iron owing to incomplete return of the alcohol from the separating funnel into the cylinder and incomplete extraction of the ferrous-bathophenanthroline complex are allowed for in the calibration procedure. The use of acetone ensures that a constant volume of extract is used (35 ml.) and lessens any clouding from droplets of occluded sea water. Extinction values are not affected by the presence of sea water and a calibration may be carried out using distilled water. The slight clouding from entrained water is largely allowed for in the blank determination (Section G) but for the best work, with very small amounts of iron, the extinction at 6500 A should be subtracted from the extinction at 5330 A to allow for turbidity.

G. Determination of Blanks

Note. The general precautions mentioned in Part I apply equally to the pre-

sent method, in fact even greater care in the cleaning of glassware, etc. is called for. For safety it is best to use doubly distilled water for the determination of blanks by the bathophenanthroline extraction method.

Reagent Blanks

Add 100 ml. of doubly distilled water to a 200 ml. extraction funnel and proceed exactly as described in Section F, paragraphs 2 - 6 inclusive. Measure the extinction against water in a 10 cm. cell and use the value to correct sample extinctions as described in Section F, paragraph 7. For safety at least one blank determination should be carried out with each batch of samples. The value should not exceed about 0.1 - 0.15 of an extinction unit.

H. Calibration

The same dilute iron solution is used as is employed in Part I.

1 ml. 1.0 μg.at.Fe/l on 100 ml. of sample.

Procedure

Add 100 ml. of distilled water and 10 ml. of Reagent No. 5 to a series of six separatory funnels. Treat two as blanks and to each of the remaining four add 1.00 ml. of dilute standard iron solution.

Carry out the determination exactly as described in Section F, paragraphs 3 - 6 inclusive.

Calculate the factor F from the expression:

$$F = \frac{1.00}{E_s - E_b}$$

where E_s is the mean extinction of the four standards and E_b the mean extinction of the two blanks. The value for F should be near to 1.5 and should not require re-determination, except for training purposes or if there is reason to suspect the technique.

The following pages contain lists

of the chemicals and equipment required for the

two methods N. 3.A. and Fe. 1.A. described in this volume.

STANDARD METHOD NO. N. 3.A

Title: Determination of Ammonia in Sea Water

CHEMICALS REQUIRED

A. Chemicals per batch of samples analysed

1. Mono reagent n x 400 ml. (if 6 - 10 samples a batch, half this if 1 - 5 samples).

2. Pyridine n x 80 ml. (if 6 - 10 samples a batch, half this if 1 - 5 samples)

3. Bispyrazolone reagent (solid) n capsules (0.04 g.) (assuming 1 - 5 samples), or n capsules (0.08 g.) (assuming 6 - 10 samples).

4. Chloramine T (solid) 5 g. in stoppered tubes, one for every 2 weeks of cruise after the first 2 weeks.

B. Chemicals per sample analysed

1. Acetate buffer n x 1.2 ml. (minimum 100 ml.)

2. Chloramine T solution n x 1.2 ml. (minimum 100 ml.)

3. Carbon tetrachloride n x 40 ml.

C. Other chemicals

To check the method in an emergency each cruise should carry 100 ml. of concentrated ammonium standard.

A liberal supply of distilled water for rinsing glassware is required for this method.

EQUIPMENT REQUIRED

A. Basic Equipment

1. Beckman DU Spectrophotometer and kit (10 cells are required).
2. Small ion exchange column containing well-cycled Dowex 50 resin in the H^+ form for scavenging NH_4^+ from distilled water.
3. One 500 ml. measuring cylinder for measuring mono reagent.
4. 2 x 100 ml. measuring cylinders for initial sample and for pyridine.
5. 3 x 50 ml. measuring cylinders, one for pyrazolone reagent, one for carbon tetrachloride and one spare.
6. Two small glass funnels to take 9 cm. filter papers (one is a spare).
7. 2 x 2 ml. automatic bulb pipettes and one spare, all set at 1.0 ± 0.1 ml. for dispensing reagents 5 and 8 = 3 x 2 ml. automatic pipettes.
8. One ground glass stoppered flask, 250 ml. capacity for temporary storage of ammonia-free water.
9. One 500 ml. stoppered Erlenmeyer flask for holding pyrazolone reagent.
10. One 250 ml. stoppered glass bottle for temporary storage of pyridine.
11. One 250 ml. stoppered glass bottle for temporary storage of carbon tetrachloride.

B. Equipment for the analysis of a batch of samples

1. Short stem separating funnels, capacity at least 200 ml. up to ten (according to anticipated number of samples per batch).
2. 130 ml. screw-capped polyethylene bottles for holding initial sea water sample prior to analysis.

C. Equipment per sample analysed

1 - 9 cm. No. 1 Whatman filter paper per sample.

STANDARD METHOD NO. Fe. 1.A.

Title: Determination of Biologically Reactive Iron in Sea Water

Note: It is assummed below that an equal number of particulate (P) and soluble (S) determinations are to be made. If only one or the other analysis is contemplated the list should be shortened accordingly.

CHEMICALS REQUIRED

A. Chemicals per batch of samples analysed

A little re-distilled water is required for blank determinations with the soluble iron method. Allow 200 ml. a batch. The re-distilled water used in the nitrate method is suitable.

B. Chemicals per sample analysed

1. Iron extraction reagent n x 12 ml. (P) n x 12 ml. (S)
2. Sodium acetate buffer n x 2.2 ml. (P) n x 2.2 ml. (S) (minimum of 100 ml.)
3. Hydroxylamine hydrochloride solution n x 1.1 ml. (P) n x 2.2 ml. (S) (minimum of 100 ml.)
4. α-α' dipyridyl reagent n x 1.1 ml. (P)
5. Bathophenanthroline reagent n x 5.5 ml. (S)
6. Reagent isoamyl alcohol n x 40 ml. (S)
7. Acetone. For rinsing cells and cylinders and as a reagent allow at least n x 25 ml. (S)

C. Other chemicals

To check the method in an emergency each cruise should carry 100 ml. of concentrated iron standard.

A liberal supply of distilled water for rinsing glassware is required for this method.

One Winchester quart of acid cleaning solution should be available on each cruise in case contamination of glassware is suspected.

EQUIPMENT REQUIRED

A. Basic Equipment

1. Beckman DU Spectrophotometer and kit (10 cm. cells are required).

2. One to three pyrex millipore filter holders and clamps (according to the number of samples and pressure of other work. Similarly one to three 1-liter glass Buchner flasks to hold filtrates.

3. Plastic "bone" tipped forceps to handle filters.

4. 1 + 1 = 2 glass rods for pushing filters into measuring cylinders.

5. One large pan to hold up to six 50 ml. cylinders submerged in hot water.

6. One 300 ml. polyethylene wash bottle to contain acetone.

7. 2 x 10 ml. measuring cylinders to measure bath reagent and iron extraction reagent.

8. One 100 ml. measuring cylinder to measure out sample for soluble iron.

9. 3 x 2 ml. automatic bulb pipettes set at 1.0 ml. ± 0.1 ml. One for hydroxylamine, one for α-α' dipyridyl (P) and one spare.

10. 3 x 2 ml. automatic bulb pipettes set at 2.0 ml. ± 0.1 ml. One for buffer (P and S), one for hydroxylamine (S) and one spare.

11. One 250 ml. polyethylene bottle for temporary storage of iron extraction reagent.

12. One 250 ml. glass bottle for temporary storage of amyl alcohol.

B. Equipment for the analysis of a batch of samples

1. Short stem separating funnels of at least 200 ml. capacity, one for each sample (maximum 8 - 10).

2. Up to 10 x 50 ml. stoppered graduated glass measuring cylinders, one for each particulate iron determination plus a further 10 if soluble iron is being determined, as both lots may be in use at the same time.

3. 1-liter screw-capped polyethylene bottles for temporary storage of raw sea water samples prior to analysis. One for every sample in a batch.

4. 130 ml. screw-capped polyethylene bottles for temporary storage of filtered sea water for soluble iron determinations. One for every sample in each batch.

C. Equipment per sample analysed

One HA 47 mm. plain white millipore filter membrane per sample.