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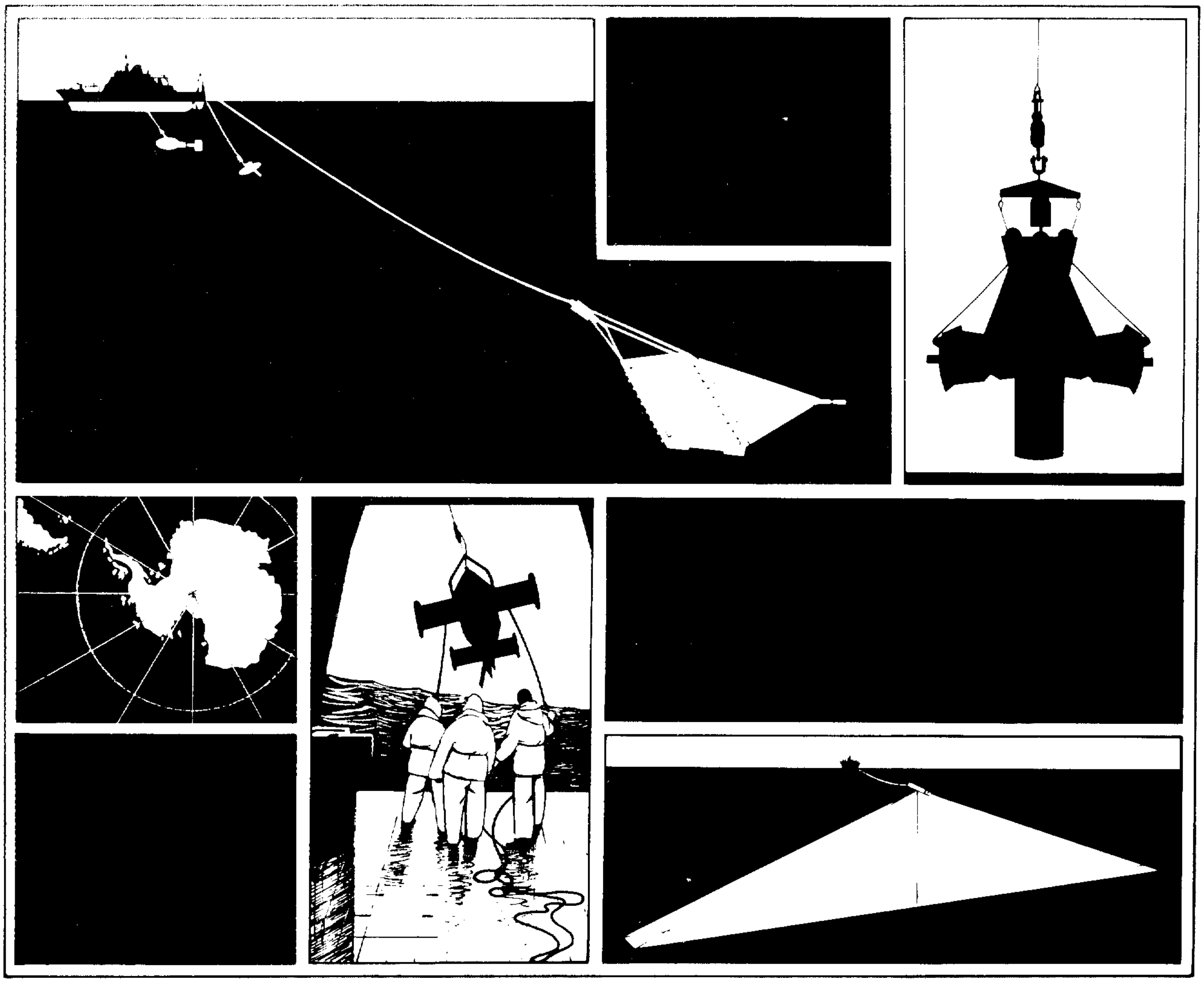
D Smythe-Wright, R Paylor & S E Holley

Report No 303 1993



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The measurement of oxygen in seawater by photometric and amperometric techniques

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ABSTRACT

Staff of the James Rennell Centre for Ocean Circulation (part of the Institute of Oceanographic Sciences Deacon Laboratory), have recently upgraded their oxygen measuring technique for chemical tracer studies. Such use of oxygen requires high quality data sets. With this in mind analytical techniques have been developed using an SIS photometric endpoint detection system and a Metrohm autotitroprocessor amperometric system. The analyses are based on the original Winkler titration method. Details of the design and full analytical methods are given.

KEYWORDS

AMPEROMETRIC
ANALYTICAL TECHNIQUES
DISSOLVED OXYGEN
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TRACERS
WINKLER TITRATION

ISSUING ORGANISATION

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1 INTRODUCTION

Oxygen has been widely used as a characteristic of water masses. Its distribution in the oceans is the net result of the balance between physical processes (advection and mixing) and biological production and consumption. In general surface waters are supersaturated with oxygen due to the action of wind and waves trapping bubbles of air that dissolve in the water. The final concentration at the surface is largely determined by the solubility of oxygen in seawater and hence is temperature and salinity dependent, the cold fresher polar seas carrying more dissolved gas than warm saline tropical waters. In addition oxygen is produced by phytoplankton photosynthesis in surface waters and is utilised by all organism for respiration and for the bacterial decomposition of decaying organisms. Consequently the longer a water mass has been isolated from the atmosphere, the lower, in general, will be its content of dissolved oxygen. The main features of oxygen distribution throughout the world's oceans are the presence of an oxygen minimum region at mid depth and the relatively high oxygen concentration in deep waters. These are the results of a balance between the biological oxygen consumption and the physical transport of oceanic waters.

The concentration of dissolved oxygen found in seawater ranges from 0-8 ml l^{-1} with a large proportion of the values within the more limited range of 1-6 ml l^{-1}. The highest dissolved oxygen concentrations > 7 ml l^{-1} are found in the North Atlantic. As a consequence North Atlantic Deep Water (NADW) which forms in the area, is characterised by high oxygen levels and appears as a mass of oxygen rich water extending from 0-2000 m at 60°N. Its penetration south dominates the oxygen distribution throughout the Atlantic resulting in an oxygen concentration maximum at about 3000 m at 40°S (see Figure 1). NADW retains its high oxygen characterisation during penetration of all the world's oceans.

Antarctic Intermediate Water (AIW) and Antarctic Bottom Water (ABW) also have relatively high oxygen concentrations. AIW is evident as an intrusion of oxygen rich water in all oceans from the surface at latitudes >50°S to about 800 m at 25°S in the Atlantic and Pacific Oceans and 10°S in the Indian Ocean (see Figure 1). The oxygen signal of ABW is clearly evident at depth in the Pacific and Indian Oceans to latitudes north of the Equator but is not well pronounced in the Atlantic Ocean. It is clear from Figures 1b and c that the combined contributions of Antarctic waters and NADW flowing into the Pacific and Indian Oceans result in a distinct North-South oxygen gradient.

In contrast to the above, low oxygenated water is found at intermediate depth in the North Pacific and North Indian Oceans. There is also a significant oxygen minimum at 500 m in all oceans at 10°N. The cause of this minimum is unknown. It has been suggested that the area is either a region of minimal motion with little circulation or mixing to refresh the water and replace the oxygen consumed, or that biological detritus accumulates in the region and uses up the oxygen.

Neither explanation is satisfactory and highlights the fact that whenever oxygen is considered as a water property it must be used with caution since it is non-conservative.

The information contained in this report describes a single method of sample collection and pretreatment, and two methods for the determination of oxygen in seawater. Both techniques have been set up for use within the WOCE Hydrographic Programme and meet the <1% accuracy and 0.1% precision specified by the WHP Office (WHPO, 1991). Section 2 deals with the analytical principle, Section 3 deals with reagent preparation and Section 4 with sample collection, fixing and storage. The photometric determination of oxygen is covered in Section 5 and the amperometric technique in Section 6. Section 7 deals with manual titrations in the case of equipment failure and Section 8 with final calculation and correction factors.

Whilst Sections 5 and 6 are mainly self contained there is information in Section 5 which will be useful for amperometric determinations. The operator is, therefore, advised to read all sections irrespective of which end point detection method is used.

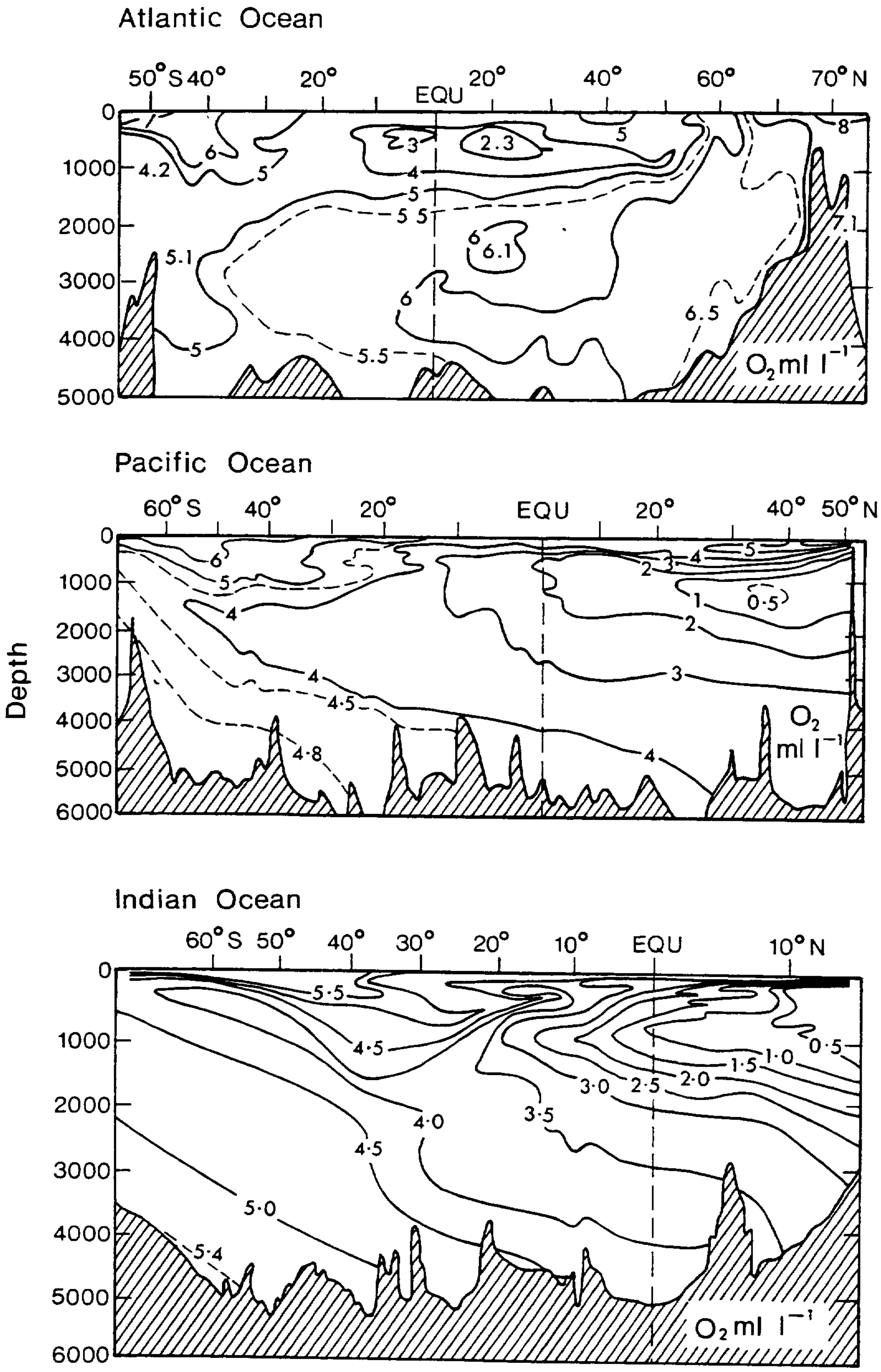

Figure 1. Distribution of oxygen in the Atlantic, Pacific and Indian Oceans (data from Bainbridge et al., 1980; Craig et al., 1981; Spencer et al., 1982).

2 ANALYTICAL PRINCIPLE

The 1888 method of Winkler is still the most precise and accurate procedure for the determination of dissolved oxygen in sea water. It was first applied to seawater analysis by Natterer (1892) and since that date many minor modifications have been described (see eg Kalle, 1939; Thompson and Robinson, 1939; Jacobsen et al., 1950; Carrit and Carpenter, 1966; Strickland and Parsons, 1968; Anderson and Foyn, 1969), with the most widely used being that of Carpenter (1965a and b). As a result of modern technology the method of end point determination has moved forward recently to automated methods using photometric (Williams and Jenkins, 1982) and amperometric techniques (Culbertson and Huang, 1987; Knapp et al., 1990). However the basic chemical pretreatment of the sample whereby iodine is liberated in direct proportion to the amount of dissolved oxygen present has changed very little.

The technique requires the collection of a known volume of water to which a solution of divalent manganese as manganous chloride is added. This is immediately followed by an excess of an alkaline iodide solution in the form of a concentrated solution of sodium hydroxide and sodium iodide. This forms a gelatinous precipitate of manganous hydroxide which reacts with the dissolved oxygen, and manganese is oxidised to a higher oxidation state. In acidic or neutral solutions divalent manganese is highly resistant to oxidation but in basic solution it is easily oxidised.

The reactions proceed according to the following equations.

$$Mn^{2+} + 2OH^- \text{------->} Mn(OH)_2$$
$$2Mn(OH)_2 + O_2 \text{------->} 2MnO(OH)_2$$

Since the manganese hydroxide is in excess, the quantity of the manganous compound formed is dependent directly upon the amount of dissolved oxygen in the sample. The manganese hydroxide precipitate is subsequently dissolved in sulphuric acid forming tetravalent manganese which in turn oxidises the iodide added initially to form free iodine according to the equation

$$2MnO(OH)_2 + 4I^- + 8H^+ \text{------->} 2I_2 + 2Mn^{2+} + 6H_2O$$

Using fully automated dispensing and detection techniques the free iodine is then titrated with standardised thiosulphate solution to form I^- and colourless tetrathionate according to the reaction

$$2I^{2-} + 4S_2O_3^{2-} \text{------->} 4I^- + 2S_4O_6^{2-}$$

Since the quantity of iodine liberated depends on the available quantity of the tetravalent manganous compound, which is itself limited by the amount of oxygen in the sample, the quantity of thiosulphate required for the titration is proportional to the dissolved oxygen content. Thus the detected presence of one molecule of dissolved oxygen results in the oxidation of four molecules of standard thiosulphate to two molecules of tetrathionate and from these relationships the quantity of dissolved oxygen in the original sample is calculated.

Unless careful attention is paid to sample collection, storage and analysis there are a number of serious systematic errors which can lead to misleading results.

a. Loss of iodine by volatilisation from the acidified solution during, or immediately prior to titration. This can be avoided by titrating the whole sample in the reaction vessel rather than transferring aliquots of sample to a separate receptacle. Special ground glass bottles with stoppers which displace sufficient of the sample to allow room for the titrant (see Section 4) are now used by most workers. In addition a solution which contains an excess of iodide which favours the formation of the less volatile tri-iodide complex is used.

b. Gain of iodine. Photoxidation of iodide can lead to high results if the acidified test solutions are exposed to strong light. Iodide can also react with atmospheric oxygen to form additional free iodine. The rate of both these processes increases with pH and so a pH of 2 to 2.5 should be maintained. It is preferable to carry out acidification and subsequent steps in diffuse light conditions.

c. Loss of oxygen. This is particularly important if there is any corrosion or oxidation of metal surfaces. The hydrographic bottles used by the JRC are fitted with epoxy coated metal springs and from time to time the coating can become chipped and corrosion occurs. It is important that the hydrographic bottle springs are checked periodically during a cruise.

d. A slight systematic error is introduced by failure to make allowances for the oxygen present in the Winkler reagents. For example Murray et al (1968) estimated an error of 0.017 ml l^{-1} at $20^{o}C$ using a sample volume at $20^{o}C$ and the reagents as described by Carpenter (1965b).

e. A further systematic error is from the presence of iodate in seawater. Fortunately the concentration is fairly constant equating to 0.065 ml O_2 l^{-1}. Most workers disregard this error.

f. One of the most significant gains of oxygen is from entrainment of atmospheric oxygen introduced during the sampling procedure. By carefully following the oxygen sampling procedure given in Section 3, this effect can be kept to a minimum.

g. Contamination is also a potential problem. Sample bottles must be scrupulously clean, since any contamination with residual amounts of manganese will lead to high results. In addition serious errors can be produced by impurities in the reagents. Contamination in the reagents can be accounted for in the reagent blank. The presence of iodate in the iodide used for the preparation of the alkaline iodide reagent will cause the blank to be positive. Reducing impurities in the reagents will give rise to negative blanks.

3 ANALYTICAL REAGENTS AND CALIBRATION STANDARDS

This section details the preparation of all analytical reagents and standards required for the measurement of dissolved oxygen in seawater. All solutions are prepared in class A Volax volumetric flasks using analytical grade chemicals (Merck) and 18 megohm cm^{-1} conductivity at $25^{o}C$ deionised water. Dry reagents are weighed to an accuracy and reproducibility of 0.01 g. Dry potassium iodate standard is weighed to four decimal places to an accuracy of 0.0001 g. Transfer pipettes for the preparation of working standards are air displacement Finnpipettes. All pipettes and volumetric flasks are weight calibrated in the laboratory to an accuracy of +/-0.1%.

The starch reagent is not required for routine photometric analysis but should be used at least once during a batch of amperometric titrations to ensure that the electrode is reading the correct endpoint. It is also required for semi automated visual endpoint titrations using the Metrohm Dosimat if there is a malfunction with both the photometric and amperometric automated systems. (See Section 7.)

All the reagents should be stored, ready prepared in brown glass screw capped reagent bottles.

3.1 Manganese II Chloride 3M

Dissolve 600 g of Manganese II Chloride $MnCl_2\ 4H_2O$ in 600 ml of deionised water in a 1 litre glass beaker. Filter the solution through a GF/F filter and make up to volume in a 1 litre volumetric flask using deionised water.

3.2 Sodium Hydroxide/Sodium Iodide 8M/4M

Dissolve 600 g of Sodium Iodide in 600 ml of deionised water in a 1 litre glass beaker. Ensure the solution is completely clear. **Very slowly** add 320 g of Sodium Hydroxide to the solution stirring continuously. Take great care, the reaction is very exothermic. Cover the solution and allow to cool. Filter through a GF/F filter and make up to volume in a 1 litre volumetric flask using deionised water.

3.3 Sulphuric Acid 5M

Slowly add 280 ml of concentrated Sulphuric Acid to 600 ml of deionised water. Again take great care, the reaction is very exothermic. Allow the solution to cool and make up to volume using deionised water.

3.4 Sodium Thiosulphate 0.02M

Weigh out approximately 5.0 g* of Sodium Thiosulphate $Na_2 S_2 O_3 5H_2O$ and dissolve in 800 ml of deionised water in a 1 litre volumetric flask. Make up to volume using deionised water.

*NB This quantity depends on the assay of the chemical. The molecular weight of sodium thiosulphate is 248 so if the assay is 100%, 5.0 g will give a solution marginally greater than 0.02M. Since the molarlity of the thiosulphate is determined accurately by subsequent titration, the weight need not be exact.

3.5 Potassium Iodate 0.01N

Dry a quantity of potassium iodate in an oven at 110°C for 3-4 hours. Cool in a desiccator. **Accurately** weigh out as near as possible 0.3567 g* of Potassium Iodate and dissolve in 800 ml of deionised water in a volumetric flask. Dissolve completely and make up to volume with deionised water.

*NB This quantity depends on the assay of the chemical. It is important that the weight of the iodate and the assay of the chemical is known accurately so that the normality of the iodate can be calculated accurately.

3.6 Starch 1%

Add 1 g of starch to a few ml of deionised water in a 100 ml glass beaker and mix to a paste. Slowly add 100 ml of boiling deionised water stirring continually until all the paste has dissolved.

4 SAMPLE COLLECTION, FIXING AND STORAGE

Seawater samples are collected from depth using General Oceanic 10 litre Niskin bottles fitted to a twenty four bottle CTD rosette package. The bottles are subsampled for oxygen analysis as soon as the CFC subsamples have been taken. It is imperative that oxygen is not entrained into the sample during subsampling, fixing and subsequent analysis. For this reason precalibated flasks (referred to as oxygen bottles throughout this document) with ground glass stoppers which form an air tight seal when closed are used. The flasks are approximately 100-120 ml capacity and each is weight calibrated to determine its exact internal volume when the stopper is in position. The stopper is of adequate size to displace sufficient volume to allow the addition of titrant and sulphuric acid at the analysis stage.

Fixing reagents are dispensed into the oxygen bottles using Anachem bottle dispensers which have been adjusted and weight calibrated to deliver 1 ml of the reagent. The reproducibility of the delivery is quoted by the manufacturers as 0.1%. Since excessive variations in temperature can significantly alter the dissolved oxygen content of the reagents due to temperature solubility effects it is important that the reagents are brought out on to deck just prior to subsampling. When not in use the reagents should be stored in the constant temperature laboratory.

It is necessary to measure the temperature of the water sample at the time of fixing so that the correct density can be used for mass calculations. This is usually achieved by collection of a second water sample into a separate dissolved oxygen bottle and measuring the temperature using an electronic temperature probe with an accuracy of +/- 0.1^{o}C.

It is imperative that the dissolved oxygen bottles are thoroughly clean. After each set of analyses wash the bottles well and check for chips and cracks. If the bottles are to be stored for a long period of time wrap the top in tissue before replacing into the bottle.

4.1 Collection of Subsamples

Water is transferred from the Niskin bottle to the precalibrated bottle using a six to eight inch piece of tygon tubing fitted over the end of the Niskin bottle tap. When not in use the tubing should be stored completely filled and immersed in seawater to ensure that all surfaces are wetted. If not bubbles tend to adhere to the inside of the tube and cause contamination of the sample. It is important that the following procedure for filling the oxygen bottles is followed carefully.

☐ Place the tygon tubing over the Niskin bottle tap.

☐ Open the tap by pushing inwards and allow the water to flow freely through the tubing to expel air. It may be necessary to pinch the tubing to remove any air bubbles.

☐ Rinse the oxygen bottle twice with the water sample and then insert the tube to the bottom of the flask.

☐ Allow the bottle to fill and overflow slowly checking that the neck area and sampling tube are completely clear of bubbles.

☐ Tap the bottle gently to dislodge any bubbles adhering to the glass.

☐ Allow the bottle to continue overflowing until at least four bottle volumes have been flushed.

4.2 Fixing

☐ Immediately the sample has been taken, add 1 ml of the Manganese II Chloride solution followed by 1 ml of the Sodium Hydroxide/Sodium Iodide reagent. It is important that the tip of the dispenser is below the neck of the flask when the reagent is added so that additional air is not injected into the sample.

☐ Stopper the flask with a firm twisting motion making sure that the top does not 'pop back' and vigorously shake the sample about 30 times to ensure that all the dissolved oxygen rapidly oxidises an equivalent amount of manganous hydroxide.

☐ Take duplicates of at least 10% of the samples to test the reproducibility of the method.

4.3 Storage

☐ Transfer all samples to the constant temperature laboratory and leave for approximately 30 minutes.

☐ Shake the samples vigorously and leave for a further hour to allow the precipitate to settle again and to equilibrate to laboratory temperature.

☐ If possible samples should be titrated within two hours of collection. They should not be left for more than four hours as after this time they will deteriorate.

5 PHOTOMETRIC ENDPOINT DETERMINATION

This section gives details of the automated photometric titration equipment and its operation. The chemistry of the method is as detailed in Section 3. All dissolved oxygen samples should be prepared as detailed in Section 4.

5.1 Analytical Equipment

Automated photometric determinations of dissolved oxygen are carried out using a Sensoren Instrumente Systeme (SIS) Oxygen Plus Winkler Titrator. The system comprises a Metrohm Dosimat 665 autotitrator, an LED, photodiode, stirrer assembly which holds the dissolved oxygen sample, and an electronic communications box which is coupled to the RS232 port on the autotitrator and to an IBM PS/2 computer. Figure 2 shows the orientation of the equipment and Figure 3 the configuration of the electrical connections. The Dosimat 655 is weight calibrated and has a reproducibility and absolute accuracy of 0.0005 ml. The minimum titrant addition from the 10 ml exchange unit is 0.001 ml. Consequently it provides a very accurate and precise method of titrant delivery.

5.2 Principle of Operation

Photometric endpoint detection is based upon the increase in light transmitted through the sample as the iodine is converted to the colourless tetrathionate. The light source is a solid state device modulated to discriminate between the iodine signal and daylight effects. The transmittance of light is measured at a preset wavelength selected to the absorption maximum of iodine.

The addition of titrant from the Dosimat is software controlled. Initially the titrant is added at a high rate until a preset transmittance is reached. At this point the rate is decreased to incremental feeding so as not to overshoot the endpoint of the titration. The addition of titrant stops automatically when no further increase in transmittance is detected by the photometer. Algorithms within the software determine the exact end point of the titration. The result is given as a titre volume from which the oxygen concentration is determined using the calculations described in Section 8.

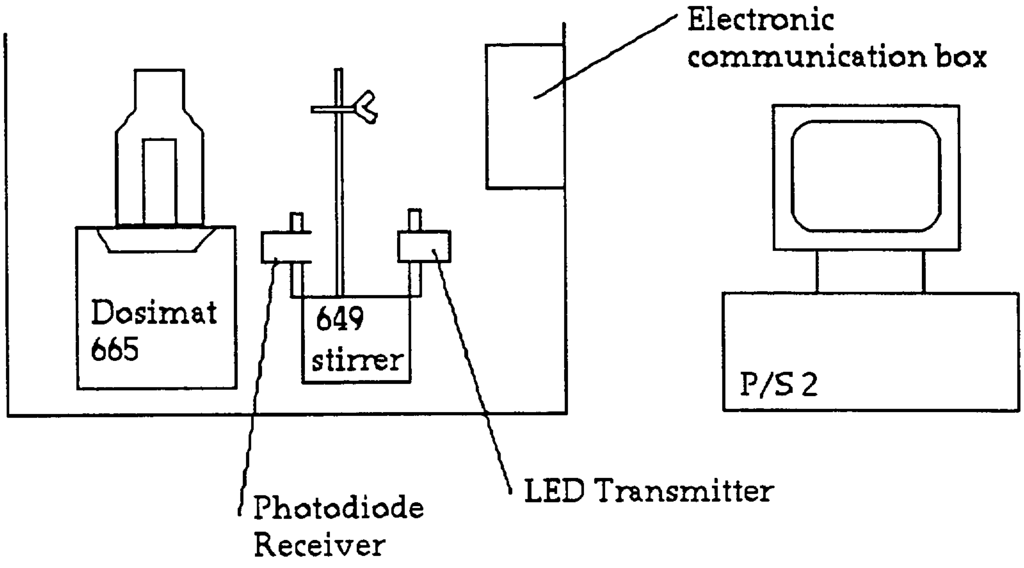

Figure 2. Orientation of the photometric system.

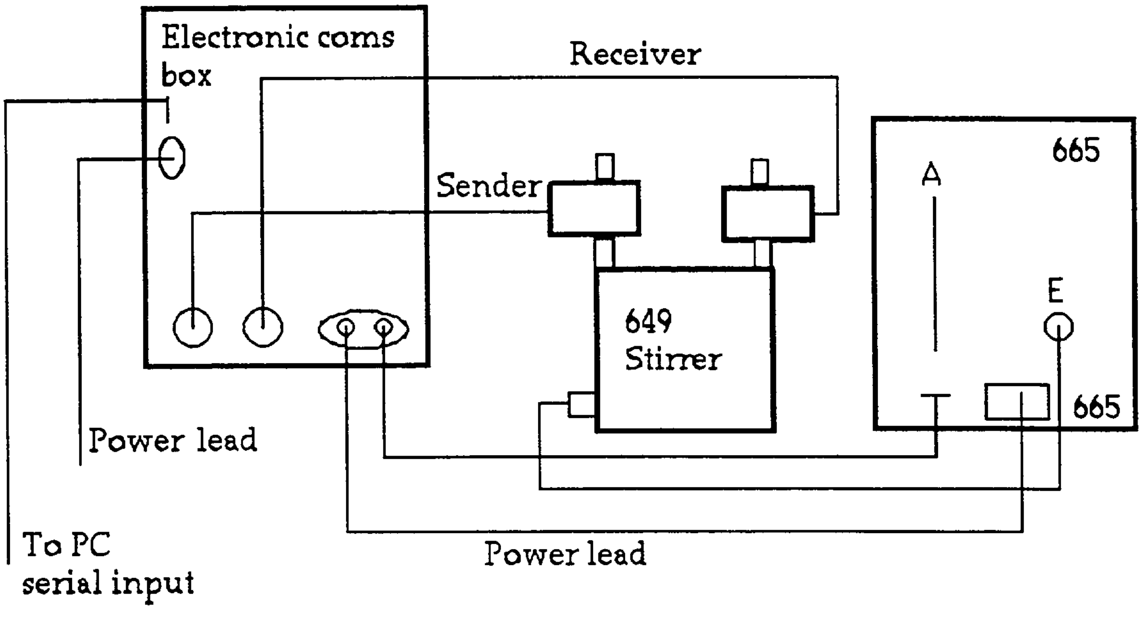

Figure 3. Configuration of the electrical connections in the photometric system.

5.3 Assembly

The assembly of the SIS equipment is relatively straightforward, since most of the components are housed in a purpose made box . However the glassware and exchange units of the Dosimat are normally transported separately in special polystyrene cartons and these need to be positioned and all electrical connections made. Assembly procedure is as follows. (Refer to Figure 3 for further clarification.)

☐ Connect the PTFE flat cock to the reagent delivery tip, the syphon screw connector and the 10 ml burette cylinder using three lengths of PTFE tubing. Take particular care with the plastic screw connections to ensure that they are not cross threaded. Tighten the screws using the torque wrench supplied with the equipment.

☐ Position the exchange unit on top of the Dosimat by pushing it forward along the rail until the projection on the top of the Dosimat marries with the depression on the metallic base plate of the exchange unit and clicks into place.

☐ Connect the data output socket on the side panel of the electronic communications box to the serial input of the PS/2 Personal computer.

☐ Connect the cable marked Sender to the LED source.

☐ Connect the cable marked Receiver to the Photodiode detector.

☐ Connect the data input socket on the base panel of the communications box to the data output socket A on the 665 Dosimat.

☐ Connect the power output socket on the communications box to the input socket on the 665 Dosimat.

☐ Connect the stirrer output socket E on the 665 Dosimat to the power input socket on the rear corner of the 649 Stirrer unit. This connection provides the 9 volt power supply required to drive the stirrer bar.

5.4 Filling the Dosimat

It is essential that the Dosimat is filled correctly since the presence of bubbles will lead to incorrect aliquots of titrant being dispensed. Also care must be taken to ensure that the thiosulphate has not crystallised out either in the titrant reservoir or in the tubing.

☐ Unscrew the top of the 1 litre brown Dosimat reservoir bottle and fill with thiosulphate. Replace the top.

☐ Lightly swirl the bottle to ensure the solution is well mixed.

☐ Set the Dosimat fill speed to a slow setting (~3dV/dt); this will minimise air bubbles entering the system during filling.

☐ Press the **Fill** button and then **Go** to draw liquid into the tubing and allow to flush to waste.

☐ Ensure there are no bubbles visible either in the PTFE tubing or over the piston.

☐ Repeat the exercise at least six times.

If the Dosimat is filled, but left standing for any length of time it is important that at least two barrel volumes of thiosulphate are flushed to waste prior to use.

5.5 Operation

All operation is controlled by the SIS software program. It controls the dispensing of the titrant, monitors the output from the photodiode and determines the endpoint. All operation is controlled by highlighting the appropriate option on a menu screen.

Prior to the titration of seawater samples, reagent blanks must be determined and the thiosulphate solution standardised. The SIS software package has a blanking procedure but this does not meet WOCE specifications and so reagent blanks are measured in the titration mode and the blank value calculated and entered into the SIS programme.

☐ Turn on the power supply to the electronic communications box and PC.

☐ At the commencement of a cruise only. Place a piece of white paper exactly half way between the LED tube and the photodiode receiver tube. A sharp purple spot should be clearly seen. If the spot is diffuse, refer to Section 5.6.1.

☐ Check that the dv/dt setting on the front of the Dosimat is set to 3.

☐ Load the SIS software and highlight the **titration** option as detailed in the next section.

5.5.1 Software Operation

☐ From the dos prompt C\: call up the tree **C;dosshell** and highlight the file system.

☐ Use the arrow keys to highlight the **OXY** directory and highlight the program **oxy.exe**.

The main menu screen contains six options.

☐	Bottles	A master file of bottle numbers and volumes is inserted by the operator into the software before each cruise. This option allows the operator to view, add or delete the bottle numbers and volumes held in memory.
☐	Titre normality	Allows for the standardisation of the thiosulphate solution as described in Section 5.5.3. The operator will be prompted for details of the iodate standard normality and volume before the titration is performed.
☐	Blank value	The determine blank value option should not be used (see section 5.5.2).
☐	Titration	The option is used for all routine titrations. The operator is prompted for details of the sample. Ensure the correct bottle number is entered for each titration. Enter a filename for data storage; this is usually the station number.
☐	Files	Viewing of complete data set or table.
☐	Quit	Exit the system.

5.5.2 Measurement of Blanks

Blank values must be determined for each new batch of reagents to determine the titration volume required by impurities in the pretreatment reagents. Two sequential titrations of potassium iodate standard are required. The first is performed with the addition of pretreatment reagents, the second titration without. The difference in the two titre volumes is equal to the blank. (The value should be very small.)

☐ Thoroughly wash a sample flask with deionised water and fill to approximately two thirds of its volume. Wipe the outside of the flask.

☐ Place the flask on the stirrer and insert the spinbar. Make sure that any writing on the bottle does not interfere with the light path.

☐ Add 1 ml of sulphuric acid to the flask.

☐ Add 1 ml of sodium hydroxide/sodium iodide solution. Make sure the solution is clear. If not start again.

☐ Add 1 ml of manganese chloride. Make sure the solution is clear. If not start again.

☐ Insert the titrant delivery tip into the solution so that the end of the tip is just above the spinbar. Make sure the tip is not in the light path.

☐ Add exactly 5 ml of potassium iodate standard.

☐ Highlight **start titration** and press **enter.**

☐ Record the volume of titrant required for the titration as indicated by the Dosimat (value a) and by the software (value b).

☐ Repeat the titration procedure by adding a further 5 ml of potassium iodate standard to the flask.

☐ Highlight **start titration** and press **enter.**

☐ Record the volume of the titrant required for the titration as indicated by the software (value c).

The blank titre volume is the value of the first titration minus the second; but it is important to account for the excess titre (overshoot) already present in the reagent flask when the second aliquot of standard is added. This can be done mathematically as follows.

$$a - b = x$$
$$x + c = y$$
$$a - y = \text{blank}$$

This value should be no greater than 0.01 ml. The blank value should then be calculated in ml l^{-1} and converted to micromols ml^{-1} by multiplying by 0.04466. The blank should then be entered into the software program in the input blank value memory.

* The standard is normally dispensed using a 5 ml adjustable air displacement Finnpippette with an accuracy of 0.3 -0.4%. Alternatively it can be delivered using a Dosimat fitted with a 5 ml exchange unit. This would allow the standard to be delivered to an accuracy of 0.05% and hence increase accuracy and precision. However experience has shown that this method is not ideal since the delivery of the Dosimat is too slow.

5.5.3 Standardisation of Thiosulphate

☐ Thoroughly wash out the flask and fill to the neck with water.

☐ Place the flask on the stirrer and insert the spinbar. Check that the spinbar is spinning freely and centred at the base of the flask.

☐ Select titre normality from the software menu and highlight the **determine titre normality** option. Press return.

☐ Add exactly 1 ml of sulphuric acid reagent to the flask.

- ☐ Add exactly 1 ml of sodium hydroxide/sodium iodide reagent to the flask. At this stage the solution should be completely clear.
- ☐ Add exactly 1 ml of manganese chloride reagent to the flask. The solution should remain clear, if not discard and start again.
- ☐ Insert the titrant delivery tip into the solution so that the end of the tip is just above the spinbar.
- ☐ Add exactly 10 ml of potassium iodate standard.
- ☐ Press enter on the PC.
- ☐ Allow the titration to go to completion. The normality of the titrant will be calculated and displayed.
- ☐ Repeat the standardisation procedure until two consecutive standardisations agree to within 0.2%.
- ☐ Repeat standardisation every few days.

5.5.4 Analysis of Samples

- ☐ Wipe the outside of the sample flask and place it on the stirrer. Insert the spinbar and check it moves freely at the base of the bottle.
- ☐ Add 1 ml of sulphuric acid reagent to the flask.
- ☐ Insert the titrant delivery tip into the solution as described above.
- ☐ Select the titration mode from the software menu, highlight **start titration** and press enter.
- ☐ The above procedures should be carried out fairly rapidly to minimise volatilisation losses.
- ☐ The titration will proceed automatically. The volume of titrant required for the titration and the calculated dissolved oxygen concentration will be displayed. However to ensure dissolved oxygen content is calculated according to WOCE specification, JRC operators prefer to note the titre volume and perform the calculations described in Section 8 using PC spreadsheets.

5.6 Common Operational Problems

The majority of errors associated with the system appear to come from operator inconsistency rather than failure of the actual equipment. It is important that the steps given in this manual are followed very carefully to ensure precise and accurate determinations. However equipment errors have occurred and practical suggestions on how to rectify them follow.

5.6.1 The Value of the Transmission at the End of the Titration is Below 50 Units

The transmission value displayed on the PC screen at the end of the titration is variable, since each glass flask will be slightly different in composition. The value however should always be above 50. If it does not reach this value, then it is likely that either the optical path of the light source has become misaligned, or an electronic component requires adjustment.

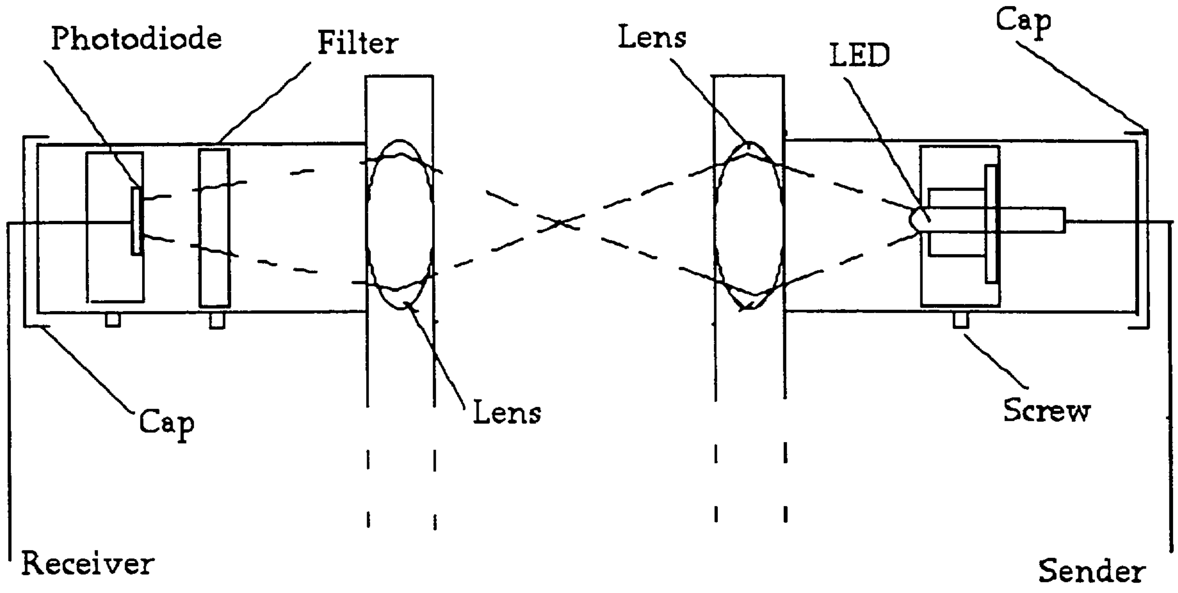

Figure 4. Alignment of optical path.

- ☐ Place a piece of paper half way between the LED tube and the photodiode receiver. A blue spot should be visible on the paper indicating the diode is transmitting, if not it will need replacement.
- ☐ If the diode is set correctly there will be a sharp rectangular array clearly visible on the paper. Ensure that the image is exactly central between the emitter and receiver.
- ☐ If not then the diode must be adjusted according to the following four steps (see Figure 4 for components).
- ☐ Remove the end cap of the transmitter and loosen the screw holding the transmitter diode.

☐ Adjust the position of the LED unit so that the sharp image is visible exactly half way between the transmitter and receiver.

☐ Tighten the screw and replace the end cap.

☐ Load the software and select the titration option. Set primary shot to zero. Start titration procedure without a bottle in the light path. If the proper adjustments have been made, the transmission should read approximately 80.

☐ If the value is not of the order 80, then the receiver unit or electronics will require adjustment. Proceed as follows.

☐ From the C:\OXYGEN subdirectory call up the program O2READ.EXE, which allows the output of the photometer to be monitored continuously.

☐ Figures will scroll from the top to the bottom of the PC screen. If the transmitter has been adjusted correctly, then the value should read approximately 30,000.

☐ Cover the receiver with a piece of paper and the value on the screen should drop to between 5 and 20.

☐ If the initial value is below 20,000. Remove the end cap of the receiver and unscrew the screw holding the photodiode.

☐ Slide the photodiode out from the block and place it directly into the light path, so that the light source shines directly onto the receiver. The output value should increase.

☐ If the value does not increase, there is a hardware defect in the electronics or photodiode which can only be rectified by the manufacturer.

☐ If the value does increase to the correct figure then the problem is either electronic or receiver misalignment.

☐ To adjust the receiver, put the photodiode back into the block and optimise the reading by moving the block in the tube. Whilst a figure above 25,000 will allow the unit to work, it is better to work as close to 30,000 as possible.

☐ To adjust the electronics, open the grey plastic housing and connect an oscilloscope to TP1 and GND. (See Figure 5).

☐ Start the program O2READ.EXE and ensure light path is completely clear.

☐ Adjust the potentiometer P1 to set the amplitude to 3V. Ensure that the curve shape does not alter from that shown on Figure 5.

☐ Using the potentiometer P3 adjust the value on the screen until it reads as close to 30,000 as possible.

- ☐ Darken the receiver and using potentiometer P2 adjust the value on the screen to 10.
- ☐ When all the adjustments have been made, lock the potentiometers.

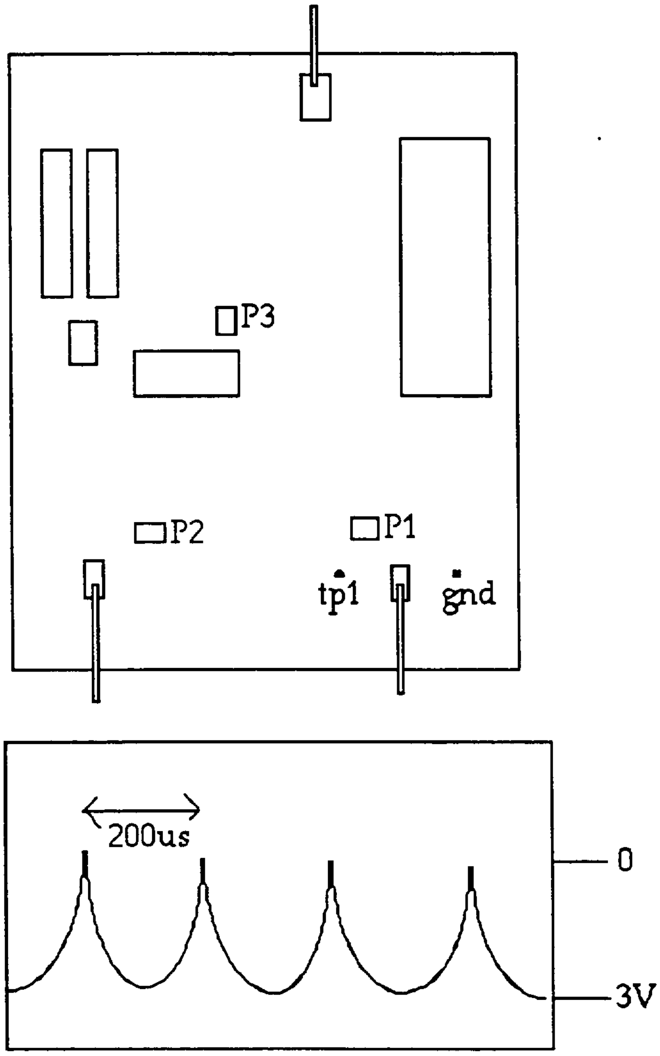

Figure 5. Electronic connections and expected signal output from potentiometer PI.

5.6.2 Poor Reproducibility

If there is poor reproducibility between duplicate samples or during standardisation, it is important to establish whether this is due to analytical error rather than equipment malfunction. If the operator is sure that the analytical technique has not varied then it is likely that an air bubble has lodged in the burette cylinder. Air bubbles often form around dust particles which adhere to the inside of the cylinder and are not visible. To ensure that bubbles do not get trapped in the burette when filling, it is imperative that the speed control on the Dosimat is set to setting 3 so that it refills at a slow speed. Higher settings may draw air into the system and cause inconsistencies in the delivery of titrant.

If an air bubble is suspected, carefully remove the burette cylinder and completely wet the inside with titrant. Reinstall the cylinder and refill the unit. This will normally alleviate the problem.

6 AMPEROMETRIC ENDPOINT DETERMINATION

This section gives details of the amperometric titration equipment and its operation. The chemistry of the method is as detailed in Section 3. All dissolved oxygen samples should be prepared as detailed in Section 4.

6.1 Analytical Equipment

Amperometric determinations of dissolved oxygen are carried out primarily using a Metrohm 686 Titroprocessor; a multifunction microprocessor capable of controlling and monitoring equipment involved in titration analyses. The titroprocessor is coupled to a Metrohm E585 polarise to which is connected a Metrohm KF double platinum electrode (cat no 6.0341.100). The system also comprises a Metrohm Dosimat 665 autotitrator fitted with a 10 ml exchange unit, and a Metrohm E649 stirrer. Figure 6 shows the orientation of the equipment and Figure 7 the configuration of the electrical connections. The Dosimat 655 is weight calibrated and has a reproducibility and absolute accuracy of 0.0005 ml. The minimum titrant addition from the 10 ml exchange unit is 0.001 ml. Consequently it provides a very accurate and precise method of titrant delivery.

6.2 Principle of Operation

The amperometric endpoint detection is based upon the change in electronic potential of the solution as iodine is converted to the colourless tetrathionate. A dual plate platinum electrode monitors the increase of electrical potential as the iodine diminishes. The titroprocessor is capable of a number of different endpoint detection techniques but for the purpose of amperometric oxygen analysis it is programmed to control the Dosimat to deliver titrant until a set endpoint is reached.

At the start of the titration, the titrant is added at the maximum rate of the Dosimat and the Titroprocessor monitors the increase in the potential across the two plates of the electrode. At a preset point, the unit instructs the autotitrator to stop delivery and the voltage is allowed to stabilise. Further addition of titrant is in progressively smaller increments depending upon whether certain drift criteria are met. As the voltage approaches zero, the addition of the titrant decreases even further until the set endpoint is reached. The titration then terminates and the endpoint voltage and amount of titrant required to complete the process is noted. The relevant data are then used to calculate the concentration of oxygen from algorithms held in the memory of the titroprocessor. As with the photometric method it is necessary to standardise the thiosulphate and establish reagent blank values prior to the analysis of samples.

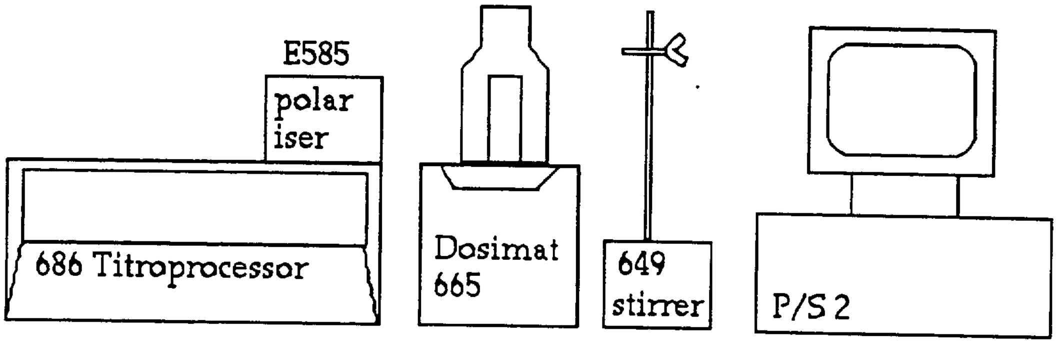

Figure 6. Orientation of the amperometric system.

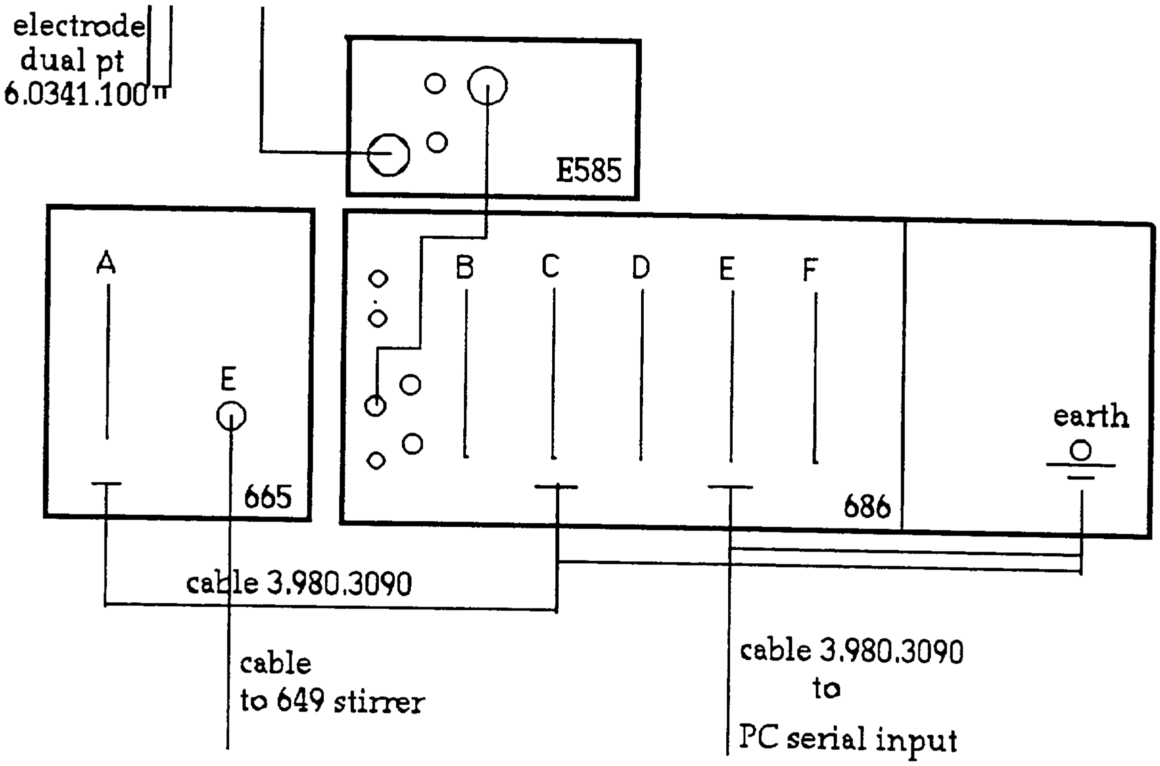

Figure 7. Configuration of the electrical connections in the amperometric system.

6.3 Initiation of the Titroprocessor

It is necessary to set the titration criteria for the analysis of samples, standardisation of the thiosulphate and determination of reagent blanks prior to using the titroprocessor. This involves the initiation of four method files; one each for samples and standardisation and two for blank determination. The formula for the final calculation of dissolved oxygen content in the seawater samples is incorporated in the sample method file. Within this formula there are two common variables; the standardisation titre volume and the reagent blank volume. It is necessary to set up these common variables within their respective method files so that they can be easily incorporated in the final calculation. Whilst the titration parameters are identical for the four files, the files deviate in the way provision is made for the individual titre volumes to be used, coded or stored. Calculation variables, endpoints and results are linked with arithmetical operations. The following three sections detail how each method file is set up. The procedure for setting the titration parameters in all four files is the same and so will only be detailed in the section covering the sample method file.

Once set the method files will require little adjustment for routine use. The main exception is for the sample method file where formula constant 1 will need amending to account for changes in the normality of the iodate standard (see below).

6.3.1 Sample Method File

To set the titration parameters:

☐ Press **SET** and then **enter**. The display will read **SET U ********.**

☐ Press the key labelled **parameters**. This is a rolling key which will display a different parameter each time it is pressed.

☐ Work through the parameter settings entering the appropriate values as outlined in Table 1. Press **enter** after each parameter is amended.

Table 1 Parameters for the four methods files

Parameter	Description	Setting for O2
EP1 U	setting of the first endpoint	-1 mv
dyn. U1	reagent feeding parameter voltage at which continual reagent feeding stops.	180 mv
drift 1	pulse range, a new feeding pulse is only emitted if this drift condition is met.	5.0 s
t(delay) 1	stop delay time. Stop condition	10 s
EP2 U	Setting of the second endpoint	OFF
temp.	Titration temperature.	20°C
stop V	Stop volume. If the endpoint is not reached, the titration stops at the set volume.	10 ml

The formula required for the calculation of dissolved oxygen is as follows (see Section 8 for further discussion)

F1 = (EP1 - C32) * C01 / (C31 - C32) - C02 ; 3 ; ml
F2 = RS1 / (C00 - C03)

where

F2 = Formula for concentration of dissolved oxygen.
EP1 = Endpoint 1. The volume of titrant in ml required to complete the titration.
C32 = Common variable 32, which is taken as the titrant volume of the blank titration stored in the blank file.
C01 = Formula constant 1. This is calculated as the product of the volume of the iodate standard, the normality of the standard and the equivalence factor of 5598. The value will change depending on the normality of the iodate standard and should be recalculated when a new batch of iodate standard is prepared. (The value is nominally 559.8 for a 0.01 N potassium iodate standard.)
C31 = Common variable 31, which is taken as the titrant volume of the standard titration stored in the standard titration file.
C00 = Input as the sample size (ie bottle volume).
C03 = The amount of reagent added to the sample during pretreatment. (ie 2 ml .)

C02 = Formula constant 2. This is a constant calculated as 1000 times the amount of dissolved oxygen added with the reagents. (=1.7.)

;3 = First delimiter specifies the number of decimal places to which the results are reported.

;ml = Second delimiter specifies the units in which the results are reported.

To input the formula:

❐ Press **2nd** followed by the **fmla** key.

❐ Press 1.

❐ Enter the formula by pressing each of the following keys
(EP 1 - C 32) * C01 / (C31 - C32) * (C00 - C03) - C02 ; 3 ; ml.

❐ Press **enter** to store the formula.

The formula constants specified in the formula must now be entered.

❐ Press **fmla const**. The system will display 'C01 = 0'.

❐ Enter the numerical value of the constant (eg 559.8) and press **enter**.

❐ Press **fmla const**. The system will display 'C02 = 0'.

❐ Enter **1.7** and press **enter**.

❐ Press **fmla const**. The system will display 'C03 = 0'.

❐ Enter **2.0** and press **enter**.

❐ Leave the formula inquiry by pressing **QUIT**.

❐ The criteria for automatic data output must now be specified. Press **def records**. The system will display 'report'.

❐ Enter **2** and press **enter**. 2 is the code number which instructs the processor to print out an extensive result profile at the end of each titration. The profile will list the start and endpoint voltages, the titration volume and any calculations which have been entered in the formula function.

❐ The system will display 'send'. Enter **2** and press **enter**. This instructs the processor to send the titration data via the communications board to the data storage software on the personal computer.

❐ Press **QUIT**.

The method is now complete and must be stored.

☐ Press **user methods** twice. The system will display 'store xxxxxxxx'.

☐ Enter the method name. The last digit of the storage command determines the filename the data will be stored under.

☐ Enter the method name as **03** (oxygen sample method). This will store all the data under ID1 (code number 3) which will be identified as the station name when individual sample details are identified at the start of a run.

6.3.2 Standardisation Method File

Within this method file, the standard titration volume is entered as a common variable **C31**. The value of this constant variable needs to be determined before any samples are analysed so that it can be used in the calculation of the oxygen content of the sea water samples.

☐ Set up the titration parameters for the standardisation method file as detailed in section 6.2.1.

To input the formula

☐ Press **2nd** followed by the **fmla** key. The formula for the standardisation file is simply the volume of titrant required to reach the endpoint.

ie F1 = EP1

where

F1 = Formula for the standardisation volume.
EP1 = Endpoint 1. The volume of titrant in ml required to complete the standardisation titration.

☐ Press **1**.

☐ Press **EP 1** and then **enter** to store the formula.

There are no formula constants in this formula so the next step is to define records, therefore go straight to define records.

☐ Press **def records**. The system will display 'report'.

☐ Enter **2** and press **enter**. The system will display 'send'.

☐ Enter **2** and press **enter**. The system will display 'm RS1:99'.

At this stage the method must be instructed to perform a mean calculation on the next two standard titres and store this value as the common variable 31.

☐ Change the value 99 to 02 by means of the cursor and press **enter**. The system will display 'com.var'.

☐ Enter the following:- **2nd mean 1 2nd ; 2nd C-val 31** then press **enter**.

☐ Press **user methods** twice.

☐ Enter the method name as: **13** (standard titration method).

6.3.3 Blank Method Files

The blank titration volume is entered as common variable 32 within the method file. Two titrations are necessary to determine the value of this variable, hence two blanking files.

In the first blank method file the titre volume is stored as constant variable 33.

☐ Set up titration parameters for blank method file 1 as detailed in section 6.3.1.

To input the formula

☐ Press **2nd** followed by the **fmla** key. The formula for blank method file 1 is simply the volume of titrant required to reach the endpoint.

ie $F1 = EP1$

where

F1 = Formula for the first blank titre.
EP1 = Endpoint 1. The volume of titrant in ml required to complete the first blank titration.

☐ Press **1**.

☐ Press **EP 1** and then **enter** to store the formula.

There are no formula constants in this formula so go straight to define records.

☐ Press **def records**. The system will display 'report'.

☐ Enter **2** and press **enter**. The system will display 'send'.

☐ Enter **2** and press **enter**.

The titration volume must now be stored as a common variable within the first method for use in the second blanking file.

☐ Press **enter**. The system will display 'com. var'.

☐ Press **2nd res 1 2nd ; C-val 33 enter**.

☐ Press **user methods** twice.

☐ Enter **23** to define method name (first blanking method).

It is now necessary to define the common variable 32 in the second method file.

☐ Set up the titration parameters for blank method file 2 as detailed in section 6.3.1.

☐ Press **2nd** followed by the **fmla** key. The formula for blank method file 2 is

F1 = C33-EP1

where

F1 = Formula for the final blank value.
C33 = Common variable determined during the first blank titration.
EP1 = Endpoint 1. The volume of titrant in ml required to complete the second blank titration.

☐ Press **1**.

☐ Press **C 33 - EP 1** and then **enter** to store the formula.

There are no formula constants in this formula so go straight to define records.

☐ Press **def records**. The system will display 'report'.

☐ Enter **2** and press **enter**. The system will display 'send'.

☐ Enter **2** and press **enter**.

The titration volume must now be stored as common variable 32 for use in the final calculation.

☐ Press **enter**. The system will display 'com. var'.

☐ Press **2nd res 1 2nd; C-val 32 enter**.

☐ Press **user methods** twice.

☐ Enter **33** to define method name (second blanking method).

6.3.4 Recall

After initiation the titroprocessor will have four methods contained in its memory. These will be defined as 03 for the sample method file, 13 for the standardisation file and 23 and 33 for the two blanking files. To select a method file after initiation

☐ Press **user methods** once. The system will prompt 'recall'.

☐ At the prompt enter the method name, for example, **03**.

6.4 Operation

☐ Turn on the power supply to the titroprocessor, the polariser and the Dosimat.

☐ Ensure the polarisation current on the front panel of the polariser is set to 200 mv.

☐ Check the fill speed setting on the front of the Dosimat is set to 3dv/dt.

☐ Fill the Dosimat with thiosulpate solution as outlined in section 5.4.

Prior to the analysis of seawater samples the blanking and standardisation procedures must be performed.

6.4.1 Measurement of Blanks

Blank values must be determined for each new batch of reagents to determine the titration volume required by impurities in the pretreatment reagents. Two sequential titrations of potassium iodate standard are required. The first is performed with the addition of pretreatment reagents, the second titration without.

☐ Press **user methods** once. The system will prompt 'recall'.

☐ Press **23 enter** to recall the first blank method file.

☐ Thoroughly wash a sample flask with deionised water and fill to approximately two thirds of its volume.

☐ Place the flask on the stirrer and insert the spinbar. Check the spinbar is freely spinning and centred at the base of the flask.

☐ Add 1 ml of sulphuric acid to the flask.

☐ Add 1 ml of sodium hydroxide/sodium iodide solution. Ensure that the solution is completely clear. If the reagents have become contaminated, the solution may be slightly tinged with a brown colouration. If so start again.

☐ Add 1 ml of manganese chloride reagent.

☐ Insert the titrant delivery tip and electrode into the solution so that the plates of the electrode are approximately 2 cm above the spinbar and the delivery tip is above the electrode plates. Support both the delivery tip and the electrode with the stand.

☐ Add exactly 5 ml of potassium iodate standard.

☐ Press **GO** and allow the titration to proceed. Note the titre volume.

☐ Press **user methods** once. The system will prompt 'recall'.

☐ Press **33 enter** to recall the second blank method file.

☐ Add exactly 5 ml of potassium iodate standard.

☐ Press **GO** and allow the titration to proceed. The titre volume will be printed out on the chart roll.

The difference in the two titre volumes is equal to the blank and the processor will store this value as a common variable C32. The value should be very small. Properly filtered reagents should produce a blank value of no greater than 0.01 ml l^{-1} oxygen or approximately 0.01 ml of titrant. Remember to reset common variable 32 after new reagent blanks are determined.

6.4.2 Standardisation

The normality of the thiosulphate solution must be determined for each batch of solution and checked every two to three days to ensure accuracy.

☐ Thoroughly wash a sample flask with deionised water and fill to approximately two thirds of it's volume.

☐ Place the flask on the stirrer and insert the spinbar. Check the spinbar is freely spinning and centred at the base of the flask.

☐ Press **user methods** once. The system will prompt 'recall'.

☐ Press 13 **enter** to recall the standardisation method file.

☐ Add exactly 1 ml of sulphuric acid.

☐ Add exactly 1 ml of sodium hydroxide/sodium iodide. Ensure that the solution is completely clear. If the reagents have become contaminated, the solution may be slightly tinged with a brown colouration. If so start again.

☐ Add exactly 1 ml of manganese chloride reagent to the flask.

☐ Insert the titrant delivery tip and electrode into the solution as described for the blanking procedure.

☐ Add exactly 10 ml of potassium iodate standard.

☐ Press **GO** and allow the titration to proceed. The titre volume will be printed out on the chart roll.

☐ Repeat the standardisation procedure until two consecutive titrations agree to within 0.002 ml.

☐ Reset C31 in the standard method file after each standardisation or if a new batch of thiosulphate or potassium iodate standard is used.

6.4.3 Analysis of Samples

☐ Press **user methods** once. The system will prompt 'recall'.

☐ Press **03 enter** to recall the sample method file.

☐ Place the sample flask on the stirrer and insert the spinbar. Check the spinbar is freely spinning and centred at the base of the flask.

☐ Add 1 ml of sulphuric acid.

☐ Insert the titrant delivery tip and electrode into the solution as describe for the blanking procedure.

☐ Press **GO** and allow the titration to proceed. The titre volume will be printed out on the chart roll. If the silo memory function is utilised (see section 6.4.4) then the concentration of dissolved oxygen in the sample will be printed out together with the titre volume.

When the titration is completed the titroprocessor resets displaying 03 so there is no need to recall the method file. Follow on with the next sample until all are analysed. At the end of an analytical run ensure that the plates of the electrode are stored covered with deionised water.

6.4.4 Silo Memory

Within the titroprocessor there is a silo memory function which allows the input of sample specific data prior to the analysis of a batch of samples. Successive entries in this memory are accessed as the samples are titrated and the oxygen content of the sample calculated directly by the titroprocessor and printed out at the end of each analysis. There are advantages and disadvantages to the use of the silo memory and its use is left to the operators discretion. The obvious advantage of direct calculation is overshadowed by the fact that for WOCE precision and accuracy a number of correction factors need to be applied and this can make the use of the silo memory tedious. Also if errors occur during the titration of samples (for example if an out of range message occurs) and it becomes necessary to press **Go** twice the subsequent entry in the silo memory is lost and sample numbering becomes out of sequence. Consequently, many operators prefer to note the titre volume and work out the results elsewhere using spreadsheet techniques. However for completeness details of the use of the silo memory follow. The silo memory must be accessed and data entered prior to the analysis of samples.

☐ Arrange the samples in titration order.

☐ Press **silo**. The LED above the key will come on.

☐ Press **smpl data**. The system will display 'silo line 1'.

☐ Press **smpl data**. The system will display 'method'.

☐ Press **03 enter** to enter the method id. The system will display 'id 1'.

☐ Enter the station number for example **62001 enter**. The system will display 'id 2'.

☐ Enter the sample number for example 1 **enter**.

☐ There is no sample specific calculation variable therefore press **smpl data**. The system will display 'C00 = 0g'.

- ☐ Enter the sample volume of the first sample for example **119.23 enter**.
- ☐ Press **smpl data**. The system will display 'silo line 2'.
- ☐ Successively press **enter** to work through the above procedures for all samples, changing the id for sample number and the sample volume for each successive line.
- ☐ Check all lines have been entered correctly.
- ☐ Press **report smpl data enter** to print sample data list.
- ☐ To delete an entire silo memory press **smpl data delete 99 enter**. When all entries have been cleared the system will display 'method'.

6.5 Common Operational Problems

6.5.1 Poor Reproducibility

If there is poor reproducibility between duplicate samples or during standardisation, it is important to establish whether this is due to analytical error rather than equipment malfunction. If the operator is sure that the analytical technique has not varied then it is likely that the problem is with titrant delivery. First check that the titrant delivery tube is positioned above the tip of the electrode so that the addition of titrant is rapidly "seen" by the electrode. If this appears correct check the Dosimat is functioning correctly (see Section 5.6.2). If the problem persists reduce the speed at which the titrant is added.

7 MANUAL TITRATION

If either of the endpoint detection systems fail it is possible to use the 665 Dosimat for manual titrations. This is not ideal, since automatic detection is lost and endpoint determination becomes more subjective. However, titrant delivery is not affected and a careful analyst can still make accurate measurements. Sample collection, storage and pretreatment is as described in Section 4. The overall procedures for the standardisation of the thiosulphate solution, the determination of blanks and the measurement of samples are the same as for the automated methods, only the delivery of titrant and end point determination is changed. The procedure is as follows

❐ Unplug all peripheral units attached to the Dosimat.

❐ Plug the manual feed control into the sockets labelled D on the rear panel of the unit.

❐ Place a piece of white paper or cardboard behind the stage of the E649 stirrer.

❐ Place the sample flask on the stage and add the acid.

❐ Using the hand control and the dv/dt setting set to 10, immediately add the titrant and when the colour in the flask starts to fade, reduce the delivery to setting 3.

❐ When the solution becomes a pale straw colour, add 1 ml of the 1% starch solution. The solution will turn blue. Do not add the starch too early as this can effect reproducibility; it is important that nearly all the iodine in the flask has disappeared before addition.

❐ Change the dv/dt setting to 2 and continue the addition very slowly until all the blue colouration disappears.

❐ Record the volume of the titrant delivery.

❐ Dissolved oxygen content is then calculated as described in Section 8.

8 CALCULATION OF DISSOLVED OXYGEN CONTENT

This section describes the calculations which need to be performed to compute the dissolved oxygen content of seawater samples. The calculations are common to both analytical techniques described in this report and follow procedures described in the WHP Operations and Methods Manual (WHPO,1991).

8.1 Dissolved Oxygen Equation

The dissolved oxygen content of a seawater sample as determined by whole bottle titration can be calculated from the equation

$$O_2 = \frac{\dfrac{(V_{sam} - V_{blk.dw}) \times V_{IO_3} \times N_{IO_3} \times 5598}{(V_{std} - V_{blk.dw})} - 1000 \times DO_{reg}}{(V_{bot} - V_{reg})}$$

where

O_2 = Oxygen concentration (ml l^{-1}).

V_{sam} = Thiosulphate titre of sample (ml).

$V_{blk.dw}$ = Thiosulphate titre of deionised water blank (ml).

V_{IO_3} = Volume of iodate standard at the temperature of standardisation.

N_{IO_3} = Normality of Iodate standard (6 x molarity) at the temperature of standardisation.

5598 = This is the equivalence factor for ml O_2/equivalent. It is a fixed constant.

DO_{reg} = Amount of Oxygen added with the reagents. This is taken to be 0.0017 ml.

V_{std} = Thiosulphate titre of standard (ml).

V_{bot} = Volume of sample flask at the temperature of sampling (ml).

V_{reg} = Volume of sample (2 ml) displaced by reagents during fixing.

The equation differs from that of Carpenter (1965) in that the correction for oxygen added with the reagents is a function of the flask volume, whereas in Carpenter's equation a constant correction of 0.018 ml l^{-1} is used.

8.2 Correction Factors

8.2.1 Sample Volume

The temperature of water samples at the time of fixing varies depending on the depth from which the sample was taken. Such variation will effect the thermal expansion of glass and hence the volume of the oxygen bottle. It is therefore, necessary to correct sample volume referenced to 20°C according to the equation

$$V_{bot} = V_{(20)} \times [\, 1 + \alpha v \, (T_{sw} - 20) \,]$$

where

V_{bot} = Volume of oxygen flask at temperature of seawater.
$V_{(20)}$ = Calibrated volume of Oxygen flask referenced to 20°C.
αv = Cubical coefficient of thermal expansion of borosilicate glass = 1.0×10^{-5}
T_{sw} = Temperature of seawater at the time of fixing.

The change in volume of glass apparatus with temperature is small. For borosilicate glass, the volume changes by only 0.01% for a 10°C temperature difference.

8.2.2 Standard Buoyancy Factor

Since the weight of objects in air is less than that in a vacuum a buoyancy factor must be applied to the quantity of potassium iodate accurately weighed for the standard solution to account for air buoyancy. Assuming the density of KIO_3 is 3.89 g cm -1 then the buoyancy factor for KIO_3 is 1.000159. The normality (6 X molarity) of the KIO_3 can then be calculated as follows assuming its molecular weight is 214.001

$$N_{IO_3\,(tp)} = \frac{W_{KIO_3} \times f_{buoyKIO_3} \times 6}{V_{(tp)} \times 214.001}$$

where

$N_{IO_3\,(tp)}$ = normality of iodate standard (=6 molarity) at t_p °C
W_{KIO_3} = weight of KIO_3 in air
$f_{buoyKIO_3}$ = buoyancy correction for solid KIO_3 (=1.000159)

214.001 = molecular weight of KIO_3

t_p = preparation temperature of KIO_3 solution

$V_{(tp)}$ = volume of volumetric flask at temperature t_p °C

$V_{(20)}$ = volume of volumetric flask at 20°C reference temperature

and

$$V_{(tp)} = V_{(20)} \times (1 + \alpha v\ (tp - 20))$$

The normality of the KIO_3 standard at the reference temperature 20°C is calculated from the following equation

$$N_{IO_3\ (20)} = N_{IO_3}\ (t_p) \times [\rho_{w\ (20)} / \ \rho_{w\ (tp)}\ ]$$

It is quite acceptable to substitute the density of pure water for the density of dilute KIO_3.

8.2.3 Thermal Expansion

The thermal expansion of aqueous solutions is much greater than that of glass. Consequently the mass of solutions dispensed by burettes and pipettes depends on the temperature of the solution. The effects of thermal expansion can be kept to a minimum if all analyses and standardisations are carried out in a constant environment laboratory. For this reason it is the practice of the JRC to carry out all analyses in such an environment at sea. If such an arrangement is not practicable adjustments to thiosulphate titre volume and the volume of iodate standard dispensed are necessary.

For the thiosulphate titre volumes the error is small if temperatures during standardisation and sample analysis are close. This because in the final calculation (see Section 8.1) the volumes V_{sam} and V_{std} occur as a ratio and so mass changes due to thermal expansion of the thiosulphate cancel.

For the iodate standard the problem is more acute and so room temperature should be measured during each thiosulphate standardisation so that the mass of the iodate standard dispensed can be calculated (referenced to 20°C) using the following formula

$$V_{(tstd)} = V_{(20)} \times [1+\alpha v(t_{std}-20)]$$

and the iodate normality according to

$$N_{IO_3\ (tstd)} = N_{IO_3\ (20)} \times [\rho w_{(tstd)} / \rho w_{(20)}]$$

where

$N_{IO_3}(20)$ = Normality of iodate standard (6 x molarity) at 20°C

$\rho_{w(tstd)}$ = Density of pure water at standardisation temperature

$\rho_{w(20)}$ = Density of pure water at reference temperature 20°C

It is quite acceptable to substitute the density of pure water for the density of dilute KIO_3.

8.3 Units

Dissolved oxygen concentrations may be reported as ml l^{-1} or as µmoles kg^{-1}. The latter is the accepted unit of the WOCE Hydrographic Programme. Oxygen concentrations calculated in ml l^{-1} from the equation given in Section 8.1 can be converted to µmoles kg^{-1} using the following equation

$$O_2\ (\mu\text{moles kg}^{-1}) = \frac{44.660 \times O_2\ (\text{ml l}^{-1})}{\rho_{sw}}$$

where

ρ_{sw} = Density of seawater at the time of fixing or if unknown the density at the potential temperature of the seawater sample. (Remember to specify which density was used when reporting results.)

44.66 = 1000/molar volume of oxygen gas at STP.

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