Dissolved Oxygen in Natural and Waste Waters 1979 Version

Two Methods

Methods for the Examination of Waters and Associated Materials

Contents

London Her Majesty's Stationery Office

Warning to users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary. Local Safety Regulations must be observed. Laboratory procedures should be carried out only in a properly equipped laboratory. Field operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried. Care should be taken against creating hazards for others. Lone working, whether in the laboratory or field, should be discouraged. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specification. Specification for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

There are numerous handbooks on first aid and laboratory safety. One such publication is *Code of Practice for Chemical Laboratories* issued by the Royal Institute of Chemistry, London. Another such publication, which includes biological hazards, is *Safety in Biological Laboratories* (Editors E Hartree and V Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London.

Where the committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. It cannot be too strongly emphasized that prompt first aid, decontamination, or administration of the correct antidote can save life, but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radiochemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of the correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first aid, fire-fighting, and rescue equipment. If in doubt it is safer to assume that a hazard may exist and take reasonable precautions than to assume that no hazard exists until proved otherwise.

First published 1980

ISBN 0 11 751442 x

About this series

This booklet is one of a series intended to provide recommended methods for the determination of water quality. In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, has issued volumes of methods for the analysis of water and sewage culminating in *Analysis of Raw, Potable and Waste Waters*. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as individual methods, thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods being issued when necessary. The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users – the senior analytical chemist, biologist, bacteriologist etc – to decide which of these methods to use for the determination in hand. Whilst attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is one of the joint technical committees of the Department of the Environment and the National Water Council. It has nine Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
2.0 Instrumentation and on-line analysis
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General non-metallic substances
6.0 Organic impurities
7.0 Biological methods
8.0 Sludge and other solids analysis
9.0 Radiochemical methods

The actual methods etc are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committe. The names of those associated with this method are listed inside the back cover.

Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No 5, and the current status of publication and revision will be given in the biennial reports of the Standing Committee of Analysts.

TA DICK
Chairman

LR PITTWELL
Secretary

20 July 1977

Dissolved Oxygen in Natural and Waste Waters

General Introduction

The measurement of dissolved oxygen concentration in waters is important, for example to provide

(a) an indication of pollution of natural waters
(b) a control determinand in the activated sludge process
(c) an indication of potential taste and odour problems due to stagnation of potable water
(d) an indicator of stratification in reservoirs.

The measurement of dissolved oxygen is an essential part of the measurement of biochemical oxygen demand.

Two different analytical procedures are described.

The first method described is a titrimetric procedure based on the original work of Winkler[1] and is preferred as a procedure against which the second procedure is usually checked. The second method is an instrumental procedure and involves the use of an electrochemical cell (often called an oxygen electrode or sensor), the response of which is proportional to the thermodynamic activity of oxygen in solution in the water under test.

The greatest care must always be taken to prevent accidental aeration or de-aeration of the sample during sampling and/or measurement.

When using the titrimetric procedure, fixing of the dissolved oxygen at the time of sampling by oxidation of freshly precipitated manganous hydroxide in the sample bottle is widely practised, determination being completed later at some convenient time.

The instrumental method is usually preferred when large numbers of measurements have to be made, or where dissolved oxygen concentrations are required for polluted waters.

Solubility of Oxygen in Water

Solubility values based on the work of Montgomery *et al*[2] have been widely used in the past and a useful nomogram for obtaining such solubility values for oxygen in freshwater has been published by Hart[3]. Recent work by Murray and Riley[4] and by Carpenter[5] has provided more extensive and very accurate values for the solubility of oxygen in sea water or estuarine waters over a wide range of salinities. Mortimer[6] has discussed the continuing use of the tables of values given in all these references and it is considered that the recent tables published by UNESCO[7] represent the best data now available and these are conveniently summarized in Table 1.

The values in the table may be used as a secondary standard for calibration of oxygen sensors and also for converting dissolved oxygen concentrations in mg/l into percentage saturation values. The solubility values are for pure water in equilibrium with pure normal air, containing 20.94% V/V oxygen, saturated with water and at a total pressure of 101.325 kPa*.

The solubility at a given temperature varies with the pressure. If the atmospheric pressure at the time of sampling is not 101.325kPa then the saturation value will be given by:

$$S_x = \frac{S(P-V)}{(101.325-V)} \qquad (1)$$

$$\text{or less precisely } S_x = \frac{SP}{101.325}$$

* 101.325 kPa = 101·325 KN/m^2 = 1·01325 bar ≡ 760mm of mercury.

where S_x = solubility in mg/l at pressure P kPa
S = solubility in mg/l at 101.325 kPa
P = observed atmospheric pressure in kPa
V = saturation vapour pressure of water, kPa
} all values apply to a given temperature T°C

The appropriate value of S is taken from the table of oxygen solubilities.

The table also includes columns which permit a first order correction for the decrease of solubility resulting from the presence of dissolved electrolytes in sea water or in aqueous dilutions of sea water. The change of solubility with change of salinity is not exactly linear so that errors up to about 0.9% in calculated solubility values may occur when using these two correction columns. If more accurate values are desired they should be read directly from the tables in Reference 7.

If the concentration of dissolved oxygen in a water sample is found to be C mg/l at pressure P kPa and temperature T°C, then the % saturation of the sample is given by:

$$\% \text{ saturation} = \frac{C}{S_x} \times 100 \qquad (2)$$

Table 1 Oxygen solubility

Temperature °C	Solubility of oxygen in water in equilibrium with air at 101.325 kPa	Correction to be subtracted for each degree of salinity (expressed as g total salts per kg water)	Correction to be subtracted for each increment of +500 mg/l of chloride
0	14.59	0.0875	0.0787
1	14.19	0.0843	0.0759
2	13.81	0.0818	0.0736
3	13.44	0.0789	0.0710
4	13.08	0.0760	0.0684
5	12.75	0.0739	0.0665
6	12.42	0.0714	0.0643
7	12.12	0.0693	0.0624
8	11.82	0.0671	0.0604
9	11.54	0.0650	0.0585
10	11.27	0.0632	0.0569
11	11.01	0.0614	0.0553
12	10.75	0.0593	0.0534
13	10.52	0.0582	0.0524
14	10.28	0.0561	0.0505
15	10.07	0.0546	0.0491
16	9.85	0.0532	0.0479
17	9.64	0.0514	0.0463
18	9.44	0.0500	0.0450
19	9.25	0.0489	0.0440
20	9.07	0.0475	0.0427
21	8.90	0.0464	0.0418
22	8.73	0.0453	0.0408
23	8.55	0.0443	0.0399
24	8.40	0.0432	0.0389
25	8.24	0.0421	0.0379
26	8.08	0.0407	0.0366
27	7.94	0.0400	0.0360
28	7.80	0.0389	0.0350
29	7.66	0.0382	0.0344
30	7.54	0.0371	0.0334

The corrections for salinity are only applicable to sea-water or estuarine waters. Where the salinity is due to other electrolytes it may be necessary to determine the correction factors experimentally using the titrimetric method.

Method A Tritimetric Method

A 1 Performance Characteristics of the Method

(For further information on the determination and definition of performance characteristics see another publication in this series.)

A 1.1	Substance determined	Dissolved oxygen.		
A 1.2	Type of Sample	Natural and waste waters. See Section A2.		
A 1.3	Basis of method	Dissolved oxygen in the sample oxidizes freshly precipitated manganous hydroxide. Acidification in the presence of iodide liberates iodine in stoichiometric equivalence to the dissolved oxygen content. The free iodine is measured titrimetrically.		
A 1.4	Range of application	All degrees of solubility of oxygen in water including supersaturation.		
A 1.5	Calibration curve	Not applicable		
A 1.6	Standard deviation (within batch)	Dissolved oxygen concentration mg/l	Standard deviation mg/l	Degrees of freedom
		0.04 to 0.21 (a)	0.02 to 0.09	12
		8.5 to 9.0 (b)	0.03 to 0.05	9 to 11
A 1.7	Limit of detection (a)	0.08 to 0.46 mg/l (with 12 degrees of freedom).		
A 1.8	Sensitivity	1.00 ml of 0.0125M sodium thiosulphate is equivalent to 0.100mg dissolved oxygen, or for 250ml sample volume, is equivalent to 0.400 mg/l dissolved oxygen.		
A 1.9	Bias	Negative bias due to loss of free iodine during titration will not exceed 0.2% of liberated iodine if the Pomeroy–Kirschman–Alsterberg Reagent (see Section A 5.4.2) is used[4]. Iodine losses during transfer of solutions may increase this bias by up to 2.0%.		
A 1.10	Interference	Various oxidizing or reducing agents, for example, ferric and ferrous salts, residual chlorine, oxidizable sulphur compounds (for example, sulphites, sulphides, thiourea), nitrite and chromate. Readily oxidizable organic matter, and actively respiring systems also interfere.		
A 1.11	Time for analysis	10 minutes operator time for a single sample.		

(a) These data were obtained on de-aerated distilled water by North West Water Authority, Yorkshire Water Authority, Laboratory of the Government Chemist and Monsanto Ltd[19].

(b) These data were obtained on air-saturated distilled water and tap-water by North West Water Authority, ICI Ltd (Brixham), Yorkshire Water Authority and Monsanto Ltd[20].

A 2 Field of Application and Interferences

A 2.1 The method is suitable for river waters, lightly polluted waters, treated effluents and other aqueous samples free from interfering substances.

A 2.2 Interferences occur due to various oxidizing or reducing agents, either by liberation of or reduction of iodine. Examples of such interfering substances are ferrous and ferric salts, chromate, nitrite, residual chlorine, sulphites, sulphides and thiourea. Readily oxidizable organic matter also interferes. Modifications to overcome some of these interferences are discussed in Section A 9. The addition of sodium azide in the normal procedure (Section A 8) eliminates interference by nitrite in samples containing up to 3.0 mg/l nitrite as N.

A 2.3 In some circumstances there may be difficulty in obtaining a suitable sample (for example shallow streams) or in fixing the sample (for example actively respiring systems). (See Sections A7 and A9.)

A 2.4 When interference effects or sampling difficulties occur, the instrumental method for dissolved oxygen measurement (Method B) may be more appropriate.

A 2.5 The method is suitable for all concentration ranges likely to be encountered. Some samples may be supersaturated, that is they contain greater than 100% of the equilibrium dissolved oxygen content for water in contact with air at the temperature and pressure of the sample.

A 2.6 If the result is to be expressed as % saturation, it will be necessary to take the salinity of the sample into account.

A 2.7 This method is a reference procedure against which an oxygen sensor may be calibrated.

A 3 Principle

A 3.1 A precipitate of manganous hydroxide is produced in the sample under test. Dissolved oxygen in the sample rapidly reacts with the manganous hydroxide to form hydroxides of manganese in higher valency states. Subsequent acidification with sulphuric acid in the presence of iodide stoichiometrically liberates free iodine equivalent in amount to the original dissolved oxygen content of the sample. The released iodine is titrated with standard thiosulphate solution. The dissolved oxygen content is calculated from the titre.

A 3.2 Reactions

Note: Equations A 3.2.1 to A 3.2.4 are idealized reactions and the actual reactions are probably more complex.

A 3.2.1 Precipitation of white manganous hydroxide

$$Mn^{++} + 2OH^{-} = Mn(OH)_2$$

A 3.3.2 Oxidation of the white manganous hydroxide by dissolved oxygen

$$4\,Mn(OH)_2 + O_2 = 4\,MnO(OH) + 2H_2O$$

A 3.3.3 The brown basic oxide dissolves in sulphuric acid forming unstable manganic ions

$$MnO(OH) + 3H^{+} = Mn^{+++} + 2H_2O$$

A 3.3.4 The unstable manganic ions release free iodine from iodide

$$2\,Mn^{+++} + 2I^{-} = 2Mn^{++} + I_2$$

A 3.3.5 The liberated iodine is titrated with thiosulphate

$$I_2 + 2\,S_2O_3^{--} = S_4O_6^{--} + 2I^{-}$$

A 3.3.6 Nitrite interferes in the determination by liberating iodine from iodide ion and by catalysing the reaction between iodide and atmospheric oxygen during the titration.

Nitrite is destroyed initially by reaction with azide ion in acid solution added as part of the normal procedure (Section A 8).

$$N_3^- + NO_2^- + 2H^+ = N_2 + N_2O + H_2O$$

A 4 Hazards

The procedures described in this method involve the handling of strongly alkaline solutions and of strong acids. Reagents MUST NOT be pipetted by mouth. Face protection and appropriate protective clothing must be employed when preparing and using the various reagents.

Sodium azide solutions evolve toxic hydrazoic acid when rendered acid as in this procedure. Prolonged exposure to atmospheres containing more than 1 ppm of hydrazoic acid are hazardous so that these titrations should be carried out in a well-ventilated area.

If alkaline azide solution is swallowed, give the patient at least one pint of water or milk to drink and obtain medical attention urgently.

Although ionic azides are stable compounds, there are some co-ordination compounds, such as copper and lead azides which are highly explosive. These explosive compounds may be produced when aqueous azide solutions are discharged to drains or sinks. They may accumulate there and lead to subsequent serious explosions (for example when plumbers are making repairs).

Discarded solutions (reagents and treated water samples) should therefore be freed of residual azide, before discharge, by reaction with excess nitrite under slightly acidic conditions in a fume chamber. As a general guide, it would be wise to employ one part by weight of nitrite nitrogen for every two parts by weight of sodium azide to be destroyed.

A 5 Reagents

A 5.1 Except where otherwise stated, analytical reagent grade chemicals should be used. All solutions should be stored in glass containers. All are stable for at least one week, except where stated.

A 5.2 **Water**

Distilled or de-ionized water is suitable.

A 5.3 **50% m/V manganous sulphate tetrahydrate solution**

Dissolve 500 ± 10 g of manganous sulphate tetrahydrate in water and dilute with water to 1 litre. Filter if not clear. An equivalent solution may be prepared by dissolving 450 ± 10 g manganous chloride tetrahydrate in water and diluting to 1 litre. If not clear, filter the solution.

A 5.4 **Alkaline iodide–azide solutions** (notes a and b)

A 5.4.1 *Modified Alsterberg Reagent*[8]

Dissolve 500 ± 10 g sodium hydroxide (or 700 ± 10 g potassium hydroxide) in about 500 ml of water and cool. Dissolve 135 ± 10 g sodium iodide (or 150 ± 10 g potassium iodide) in about 100 ml water. Dissolve 2.00 ± 0·05 g sodium azide in about 40 ml water. Mix the three solutions together when all are at room temperature and dilute with water to 1 litre.

Note (a) Either of the alternative forms of alkaline azide solution may be used. The modified Pomeroy–Kirschman–Alsterberg Reagent is recommended for very accurate work because possible loss of iodine is minimized by the high iodide content. This reagent also gives a better titrimetric end point and the precipitated hydroxides dissolve faster when acidified with sulphuric acid. However, the reagent is very viscous and not suitable for field use.

Note (b) If either of the alkaline azide solutions gives a blue colour with starch solution when acidified with sulphuric acid, it must be discarded.

A 5.4.2 *Modified Pomeroy–Kirschman–Alsterberg reagent*[9]

Dissolve 400 ± 10 g sodium hydroxide in about 500 ml of water and cool the solution to 40 to 50°C. Dissolve 900 ± 10 g sodium iodide in the sodium hydroxide solution, keeping the solution warm until all the iodide has dissolved. Cool, and dilute with water to 1 litre and stand overnight. Filter or decant if necessary and mix with a solution of 2.60 ± 0·05 g of sodium azide, dissolved in 300 ± 10 ml water.

A 5.5 **50% V/V sulphuric acid**

Add slowly and cautiously with stirring 500 ± 10 ml of sulphuric acid (d_{20} 1.84) to 500 ± 10 ml of water in a 2-litre beaker immersed in cold water.

A 5.6 **Starch solution**

Mix 5 ± 1 g of soluble starch powder to a thin smooth paste with cold water. Pour the paste into 500 ml ± 10 ml of boiling water and continue to boil for several minutes. Cool and dilute with water to 1 litre. The solution may be preserved for up to 1 week by the addition of 0·05 ml of toluene.

Suitable starch substitutes may be used, such as a 0.5% m/V solution of sodium starch glycollate in water, which is stable for several months.

A 5.7 **Standard reference solution of potassium iodate, 0.0042M**

Weigh 0.8920 ± 0.0005 g of potassium iodate (previously dried for 1 hour at 110°C, cooled and stored in a desiccator), dissolve in water and dilute with water to 1 litre in a calibrated flask. This solution is stable for at least 2 months.

20 ml 0·0042 M KIO_3 ≡ 40 ml 0.0125M sodium thiosulphate solution

A 5.8 **Solution of sodium thiosulphate pentahydrate, about 0.25M**

Dissolve 62.50 ± 0·01 g sodium thiosulphate pentahydrate in water and dilute with water to 1 litre. Preserve by adding 1 ml of chloroform. Store in a dark glass bottle.

A 5.9 **Standard solution of sodium thiosulphate, 0.0125M**

1.00 ml ≡ 0.100 mg dissolved oxygen

Dilute 50.00 ± 0.01 ml of 0.25M sodium thiosulphate solution with water to 1 litre in a calibrated flask. Add 1 ml of chloroform as preservative and store in a dark glass bottle. This solution should be standardized each day when required against 0.0042M potassium iodate.

Standardize the sodium thiosulphate solution (0.0125M) as follows:

Dissolve 2.0 ± 0·1 g potassium iodide (iodine free) in about 100 ml of water contained in a conical flask.

Add 2 ml of 50% V/V sulphuric acid, swirl, then add 20.00 ± 0.05 ml of potassium iodate solution. Dilute with water to about 200 ml and titrate with the sodium thiosulphate solution, adding 2 ml starch indicator when a pale straw colour is reached. Continue the titration till the disappearance of the blue colour. Reappearance of the blue colour on standing should be ignored. The sodium thiosulphate solution may be adjusted to exactly 0.0125M for convenience in use.

A 5.10 **90% m/m ortho phosphoric acid (d_{20} 1.75)**

A 5.11 **2% m/V sodium azide solution**

Dissolve 2.00 ± 0.01 g sodium azide in water and dilute with water to 100 ± 1 ml.

A 5.12 **40% m/V potassium fluoride dihydrate solution**

Dissolve 40.0 ± 0.5 g potassium fluoride dihydrate in water and dilute with water to 100 ± 1 ml.

A 5.13 **Potassium iodide (iodine free)**

A 6 Apparatus

A 6.1 All volumetric glassware used should be of grade B or better.

A 6.2 Clear glass narrow-necked bottles of good quality. The bottles should have well-fitting tapered glass or plastic stoppers (note c). The exact capacity is not critical but should be in the range 100 to 500 ml. New bottles should be rinsed with 50% V/V sulphuric acid then thoroughly with water. Bottles in regular use require only to be thoroughly rinsed with water after use. CHROMIC ACID OR DETERGENTS MUST NOT BE USED.

A 7 Sampling

A 7.1 **Collection of the sample:**

This operation requires care for the following reasons:

Turbulent flow into the sample bottle can affect the dissolved oxygen concentration.

Gases, including oxygen, may be displaced from solution as bubbles, and with opposite effect, air may be entrained.

Incorrect dissolved oxygen readings would result in either case.

There are several special water sampling devices which are designed to obviate these problems, they differ in their design but most have the following features in common.

(a) A delivery tube taking the water to the bottom of the sample bottle.

(b) An exit tube set in the top of the sampling device so that its orifice is higher than that of the delivery tube, see figure 1.

(c) Delivery and exit tubes closed by bungs or valves which are opened by remote control when the sampling device is at the required depth, by the operator at the surface.

(d) A watertight closure to the sampling device; the overall volume of the sampler allows a several-fold displacement of water through the sample bottle proper before flow ceases, with the displacement of all gas from the sample bottle. For measurement of dissolved oxygen, the sample bottle and the outer sample bottle container should both be purged with oxygen-free nitrogen just before the sample is taken.

Figure 1 illustrates the essential features of two commonly used displacement sampling devices[10].

In Type 1A the weight of the equipment is taken by a spring and the action of jerking the line causes the spring to extend, places the cord to the rubber bungs under tension and thereby removes the bungs from the inlet and exit tubes. Other designs, for example Type 1B, use springs and levers to similar effect or lead messenger weights which slide down the suspending line to operate levers which in turn open inlet and exit valves. Sampling devices may be top or bottom opening and constructed of either metal or plastic. Plastic or glass sampling equipment is generally to be preferred due to the danger of oxygen absorption if the plating on brass water bottles becomes corroded.

If the percentage saturation of the sample with oxygen is to be determined, the temperature of the water surrounding the bottles in the sampling device should be measured to the nearest 0.5°C and recorded at the time of sampling. For precise work the atmospheric pressure is required and the temperature should be measured to the nearest 0.2°C.

A displacement sampling device should be used wherever practicable. In those situations where the depth of water is too shallow for the use of the apparatus, the instrumental method should be used. If a dissolved oxygen sensor is not available, a sample bottle of convenient size should be held horizontally at the surface so that the water enters gently without bubbling and as the bottle fills it is brought gradually to a vertical position. When the bottle is completely filled the stopper is placed in it displacing a little of the sample in the process. This sampling method can introduce appreciable error and great care is required when using it.

Note (c) Plastic stoppers may be used provided tests show that the material is non-biodegradable, does not absorb released iodine, and is inert to reagents used in the test. All stoppers should be tapered so that they do not entrain in air bubbles when inserted into filled bottles.

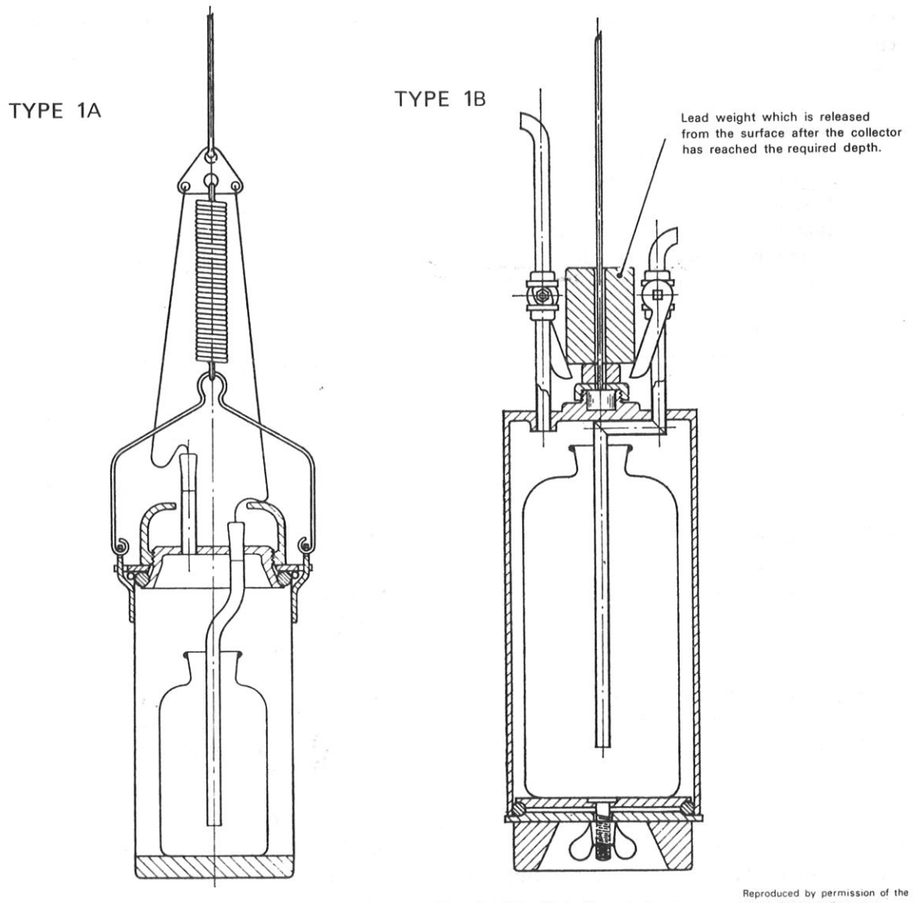

Figure 1 Immersion samplers for waters

A 7.2 **Fixing the sample**

The determination of the oxygen content must be carried out as soon as possible after sampling. In all cases steps A 8.1 and A 8.2 MUST be carried out as soon as the sample had been acquired in order to fix the oxygen. If delay in the subsequent steps of the analysis is unavoidable, the bottle is then carefully stoppered, taking care to exclude air bubbles. The 'fixed' sample may then be stored in the dark at 10–20°C for no more than 24 hours before proceeding with step A 8.3.

A 8 Procedure

A 8.0.1 READ SECTION ON HAZARDS BEFORE STARTING THIS PROCEDURE

A 8.0.2 The sample is the whole of the laboratory sample taken in Section A 7 and steps A 8.1 to A 8.3 of the determination must be carried out in the bottle used to collect the sample.

A 8.0.3 The following procedure applies to sample bottles of 250 ± 25 ml capacity. For bottles of other sizes, the reagent volumes added should be adjusted accordingly.

Step	Experimental Procedure	Notes
A 8.1	Carefully remove the stopper from the bottle containing the sample and add, using narrow tipped pipettes, 2.0 ± 0.2 ml of the manganous sulphate solution followed by 2.0 ± 0.2 ml of the alkaline iodide–azide solution, both just below the surface of the water (note d). Carefully replace the stopper and mix thoroughly by repeated vigorous inversion and rotation (notes e and f).	(d) The addition of 4 ml of reagents to a full sample bottle results in the displacement of sample and a correction is applied for this in the calculation. (e) The stopper must be inserted carefully to avoid the inclusion of air bubbles. (f) Sufficient sodium azide is present to eliminate the interference of up to 3.0 mg/l nitrite as N in the sample.
A 8.2	Allow the precipitate to settle to the lower third of the bottle and repeat the mixing. Then allow the precipitate to settle completely (note g).	(g) A clear supernatant liquid should be obtained. However, with saline water, the precipitate settles more slowly. Settlement may be improved by rotating the bottle carefully during the mixing process of step A 8.1, so as to blend the two reagents before shaking the bottle vigorously.
A 8.3	Carefully remove the stopper and add 4.0 ± 0.2 ml of 50% V/V sulphuric acid. Replace the stopper and thoroughly mix the contents by rotation or gentle inversion (note h).	(h) The precipitate should dissolve almost immediately. If it does not dissolve after standing for a few minutes, repeat the mixing. As a last resort, a few more drops of sulphuric acid may be added and the mixing repeated.
A 8.4	After acidification, immediately pipette a suitable volume, V_2 ml, of the mixed solution into a conical flask (note i).	(i) The entire sample can be titrated provided the volume of the sample bottle is known. This procedure will minimize the negative bias caused by loss of iodine during transfer.
A 8.5	Titrate the sample aliquot with 0.0125M sodium thiosulphate solution to a pale straw colour. Add 2 ml of starch indicator solution and continue the titration to the first disappearance of the blue colour. Record the volume, V_1 ml, of titrant used (note j).	(j) Reappearance of the blue colour in the titrated solution on standing should be ignored.

Calculation of results

A 8.6 Calculate the dissolved oxygen content (D.O.), of the sample from

(k) If interference by concentrations of nitrite (as nitrogen) greater than 3.0 mg/l is to be avoided, an additional 1.0 ± 0.1 ml of 2% m/V sodium azide solution will be added at step A 8.1 (see Section A 9.1). This 1.0 ± 0.1 ml must then be included in the value of V_4. Similar considerations apply if phosphoric acid or potassium fluoride solutions are used to prevent interference by ferric iron (Section A 9.2).

$$\text{D.O.} = \frac{V_1 \times 0.100 \times 1000 \times F \text{ mg/l}}{V_2}$$

Where V_1 = volume, in ml, of 0.0125M sodium thiosulphate used.

V_2 = volume, in ml, of sample titrated.

F is a dilution factor caused by addition of reagents to the sample.

$$F = \frac{V_3}{V_3 - V_4}$$

Where V_3 = Volume, in ml, of sampling bottle.

V_4 = combined volume, in ml, of reagents added to the sample bottle at step A8.1 (note k) but not including the sulphuric acid added at step A8.3.

A 9 Special Cases

Note that the procedures in Sections A 9.1 and A 9.2 refer to measurements made in bottles of 250 ± 25 ml capacity.

A 9.1 *Samples containing nitrite nitrogen in the range 3.0 to 18.0 mg/l NO_2 as N*

For samples containing less than 3.0 mg/l of nitrite nitrogen, the standard reagents contain sufficient azide to ensure that the error in the dissolved oxygen is less than 0.05 mg/l.

If higher concentrations of nitrite are present up to a maximum of 18.0 mg/l as nitrite nitrogen in the sample, this interference may be eliminated in an equally effective manner by the addition of 1.00 ± 0.05 ml of a freshly prepared solution of sodium azide (2% m/V). This should be added to the sample in the bottle immediately prior to the addition of the manganese sulphate and alkaline azide in step A 8.1 of the Procedure. It is necessary that this addition should be made using a small diameter, graduated pipette placed so that the point of discharge is about 10 cm below the liquid surface.

A 9.2 *Samples containing ferric iron (up to 175 mg/l)*

Interference due to dissolved ferric iron can be kept below the equivalent of 0.05 mg/l dissolved oxygen by one of two procedures:

A 9.2.1 The sulphuric acid used in step A8.3 is replaced by an equal volume of 90% m/m phosphoric acid.

A 9.2.2 At the commencement of step A 8.1, add 1.00 ± 0.05 ml of 40% m/V potassium fluoride dihydrate solution, the addition being made just below the surface of the liquid.

The following sources of interference have been minimized in the past by chemical procedures, but in general such procedures are not very satisfactory. Reference is still made here to these chemical methods, but it is strongly recommended, if such interferences are present, that the instrumental method is used.

A 9.3 *Samples containing ferrous iron*

Ferrous iron interferes by reducing iodine to iodide. The procedure described in Section A8 will tolerate up to 1 mg/l ferrous iron[11]. The permanganate modification[12] can be used to overcome interference by ferrous iron but generally the instrumental method is now preferred.

A 9.4 *Samples containing sulphites, thiosulphates and polythionites*

The presence of any of this group of compounds in waste waters interferes by reducing iodine to iodide. Although the modification of Theriault and McNamee[13], using a preliminary oxidation with hypochlorite, has been proposed in the past to overcome interference, it is now preferred to use the instrumental method.

A 9.5 *Samples high in suspended organic solids (e.g. activated sludge)*

Suspended organic solids or flocs which exert an iodine demand or rapid oxygen demand will interfere and such samples should only be analysed for dissolved oxygen content by the instrumental method.

Method B Instrumental Method

B 1 Performance Characteristics of the Method

(For further information on the determination and definition of performance characteristics see another publication in this series.)

B 1.1	Substance determined	Dissolved oxygen.
B 1.2	Types of sample	Natural and waste waters.
B 1.3	Basis of method	An electrochemical oxygen sensor covered with a gas-permeable membrane is immersed in the water and the current generated is used to measure the partial pressure of oxygen in equilibrium with the water at the membrane surface. This partial pressure may be used as a measure of the dissolved oxygen concentration. The sensor may be either a galvanic or a polarographic (voltammetric) cell, the latter requiring an applied voltage. In each case the cell reaction involves the reduction: $O_2+2H_2O+4e^-=4OH^-$
B 1.4	Range of application	Oxygen contents corresponding to 0–100% saturation of the water with air; most instruments permit measurement of higher values.
B 1.5	Calibration curve	The relation between instrument reading and partial pressure of oxygen is normally linear (see Section B 6.3).
B 1.6	Standard deviation (within batch)	Dissolved oxygen concentration / Standard deviation / Degrees of freedom 0.17 to 0.28 mg/l (a) / 0.05 to 0.08 mg/l / 12 8.0 to 10.3 mg/l (b) (i) / 0.02 to 0.06 mg/l / 10 100.3% saturation (b) (ii) / 0.4% saturation / 10
B 1.7	Limit of detection	0.25 to 0.43 mg/l (with 12 degrees of freedom) (a).
B 1.8	Sensitivity	Typically 1% of full-scale reading corresponds to 0.10 mg/l oxygen.
B 1.9	Bias	No evidence of bias in the absence of interferences.
B 1.10	Interferences	(1) Any material that attacks, dissolves or obstructs the gas permeable membrane (see Section B 2.2.1). (2) Volatile species and gases other than oxygen which diffuse through the membrane and affect the operation of the electrochemical cell (see Section B 2.2.2).
B 1.11	Time required for analysis	Not more than 5 minutes for 1 sample, zero setting and calibration typically take about 20 minutes.

(a) These data were obtained on de-aerated distilled water by North West Water Authority, Yorkshire Water Authority, Laboratory of the Government Chemist and ICI Ltd (Brixham)[21].

(b) These data were obtained by North West Water Authority, Yorkshire Water Authority, Severn–Trent Water Authority, Monsanto Ltd and ICI Ltd (Brixham) on (i) air-saturated distilled water in 5 laboratories using Clark type sensors and (ii) air-saturated tap water in 1 laboratory using a Mackereth type sensor. The one result for the latter sensor can be regarded as typical[22].

B 2 Field of Application and Interferences

B 2.1 Dissolved oxygen sensors are particularly valuable in the following situations:

B 2.1.1 In the presence of high concentrations of ferric and ferrous iron or when nitrite, sulphite, thiosulphate, polythionite or excessive colour or turbidity are present in the sample, that is, when the titrimetric method is not recommended.

B 2.1.2 In systems containing respiring micro-organisms and in conjunction with BOD measurements, when it is possible to make a dissolved oxygen measurement without significantly affecting the concentration of oxygen in the sample.

B 2.1.3 For *in situ* measurements and continuous monitoring of dissolved oxygen in waters, thus avoiding the errors which may be introduced in taking a discrete water sample.

B 2.2 Apart from the necessity to ensure that the effects of temperature, pressure and salinity have been properly compensated for (see Instruction Manual supplied with the instrument in use) the following are the main sources of interference with the method:

B 2.2.1 Solvent attack or accumulation of insoluble debris, oil or biological growth on the membrane can influence the rate of diffusion of oxygen into the electrochemical cell, and hence the measured current.

B 2.2.2 Gases other than oxygen will diffuse through the membrane and may or may not have an effect on the measured current. The following information may be used as a guide to possible interferences[23]:

(a) Inert gases, hydrogen, ammonia, carbon monoxide and carbon dioxide do not normally interfere. On occasions carbon dioxide may cause problems by slow reaction with the cell electrolyte.

(b) Gases which undergo reduction at the cathode at the same potential as oxygen (-0.6 to -1.0 v) may be recorded as if they were dissolved oxygen. Examples are: chlorine, nitrous oxide, nitric oxide, formaldehyde and hydriodic acid.

(c) Hydrogen sulphide results in a reduction of the observed dissolved oxygen value for a water and may even lead to negative readings, desensitization and/or slow response characteristics. The effects observed vary with different forms of sensor and some commercial sensors are claimed to recover rapidly from the effects of exposure to hydrogen sulphide. Little firm information is available so that if hydrogen sulphide is present in a water, the instrument readings must be treated with caution.

B 3 Principle

B 3.1 The dissolved oxygen content of water is determined by immersing in it a sensor which consists of a small electrochemical cell confined by a membrane[14]. The current flowing through this cell is proportional to the partial pressure of oxygen in the sample.

The membrane is selectively permeable to dissolved gas and is usually a special film of polyethylene or polytetrafluoroethylene. The use of a membrane reduces the tendency for electrode fouling to occur, and while the membrane itself may become fouled, it is readily replaced.

In all cases, the sensor configuration is cylindrical, but the membrane may either constitute part of the curved surface of the cylinder (being formed from tubular film) or may cover one of its flat end-faces. Oxygen diffusing through the membrane is reduced at the cathode to hydroxyl ions.

B 3.2 The electrochemical cell which constitutes the sensor may be one of two types:

B 3.2.1 Galvanic[15, 16], for example the Mackereth type[17] consisting of a perforated, cylindrical, inert silver cathode surrounding a lead anode immersed in an alkaline electrolyte which is in most cases potassium hydroxide solution (sometimes saturated with potassium hydrogen carbonate to eliminate interference by carbon dioxide). No applied potential is required and diffusion of oxygen into the cell results in a galvanic current proportional to the partial pressure of oxygen in the water.

B 3.2.2 Polarographic, for example the Clark type[18] comprising an inert cathode (platinum or gold) associated with a reference electrode which is usually silver/silver chloride. The electrolyte is in this case potassium chloride solution and it is necessary to apply a potential difference to the two electrodes in order to bring about polarographic reduction of the diffused oxygen.

B 3.3 **Reactions**

B 3.3.1 The general electrode reactions, applicable to both galvanic and polarographic oxygen sensors, are as follows:

At the anode $M^{o} = M^{n+} + ne^{-}$

At the cathode $O_2 + 2H_2O + 4e^{-} = 4OH^{-}$

B 3.3.2 In the Mackereth type[15] galvanic cell, these specific reactions would be:

At the lead anode $2Pb^{o} + 6OH^{-} = 2PbOOH^{-} + 2H_2O + 4e^{-}$

At the silver cathode $O_2 + 2H_2O + 4e^{-} = 4OH^{-}$

The overall cell reaction being:

$$2Pb^{o} + 2OH^{-} + O_2 = 2PbOOH^{-}$$

B 3.3.3 In the Clark type[15] polarographic cell the reactions are:

At the silver anode $4Ag^{o} + 4Cl^{-} = 4\,AgCl + 4e^{-}$

At the platinum cathode $O_2 + 2H_2O + 4e^{-} = 4OH^{-}$

The overall cell reaction being:

$$4Ag^{o} + 4Cl^{-} + O_2 + 2H_2O = 4AgCl + 4OH^{-}$$

B 3.4 For both types of sensor, by ensuring close contact between cathode and membrane, the oxygen concentration within the sensor itself is effectively zero at all times. The current measured is determined by the rate of diffusion of oxygen through the membrane and is a function of the partial pressure of oxygen in equilibrium with the water[24].

Layers of water in a natural water body are often not in equilibrium with one another (for example with regard to temperature, salinity, and dissolved oxygen). When an oxygen sensor is placed in such a water layer, however, the partial pressure of oxygen at the membrane surface is that pressure which exists in true equilibrium with the oxygen dissolved in that water layer. The meter reading is therefore proportional to the partial pressure of oxygen in that water layer and can be related to dissolved oxygen concentration.

Henry's Law for ideal solutions states that the mass of gas (m) dissolved by a given volume (v) of solvent is proportional to the partial pressure (p) of that gas in equilibrium with the solution.

$$\frac{m}{v} = K.p$$

Where K is the Henry's Law constant and is dependent on the nature of the gas, the temperature, the nature of the solvent and salinity in the case of aqueous solutions.

Since the electrode directly measures the partial pressure of oxygen for waters containing dissolved oxygen, the electrode response can be calibrated to measure dissolved oxygen concentration. See Sections B 3.6, B 3.7 and B 3.8.

The sensor rapidly detects changes of oxygen concentration in the water but complete response requires a finite time because of the need to re-establish a new steady state for the diffusion process through the membrane.

B 3.5 **Effect of flow**

The electrochemical reaction within the sensor removes oxygen from the thin layer of water in contact with the membrane so that it is necessary to renew this layer continuously if steady and meaningful readings are to be obtained. This may be achieved by moving the sensor through the water, by the natural water flow or by means of a suitable stirrer (often an integral part of the sensor). This flow must be induced without entrainment of air bubbles. It is found that the output of the sensor rises to an essentially constant value with increasing flow and, for satisfactory results, the flow must be high enough to reach this 'plateau' region. Normally this requires a flow rate of about 100 mm/s for cylindrical membrane sensors and 300 mm/s for end-faced sensors. However, the flow rate required must be determined for each sensor used.

B 3.6 **Effect of temperature**

Both types of sensor produce an electric current which is measured on a suitable meter. The magnitude of the current is proportional to the partial pressure of oxygen (or activity) in the water surrounding the membrane. Variations in water temperature result in changes in the current of the order of 6–7% per °C due to variations in the membrane permeability to oxygen but, in most instruments, these changes are automatically compensated by a thermistor circuit. In this case the meter may be scaled in partial pressure units such as '% dissolved oxygen', or '% saturation'. Other instruments contain additional circuits providing compensation for the change in oxygen solubility with temperature (see General Introduction). In this case the meter may be scaled in concentration units such as 'ppm oxygen' or 'mg/l dissolved oxygen'.

In each case the observed meter reading may have to be corrected to compensate for a significant difference between the conditions of calibration and those of measurement. Pressure is the crucial factor in instruments reading in partial pressure units (see Section B 3.7) and salinity for those reading in concentration units (see Section B 3.8).

B 3.7 **Effect of pressure**

In air, or in air-saturated water, the partial pressure of oxygen (assuming that the air has an oxygen content of 20.94% V/V and is saturated with water vapour) is given by:

$$pO_2 = 0.2094\ (P - v)$$

Where pO_2 = partial pressure of oxygen
P = air pressure
v = saturation vapour pressure of water.

The degree of saturation of a water sample with air is often referred to as '% saturation' and is usually defined by:

$$\%\ \text{Saturation} = \frac{pO_2 \text{ in sample}}{(pO_2 \text{ in water saturated with air at same temperature and pressure as sample})} \times 100 = \frac{pO_2 \text{ in sample}}{0.2094\ (P - v)} \times 100$$

As this definition includes pressure, direct indication of '% saturation' would require an instrument having a pressure transducer to provide pressure compensation.

Consequently instruments with '% saturation' scales commonly indicate a different quantity, here termed % Sat (obs), which is defined with respect to the standard atmospheric pressure of 101.325 kPa, that is:

$$\%\ \text{Sat (obs)} = \frac{pO_2 \text{ in sample}}{0.2094\ (101.325 - v)} \times 100$$

Therefore, if true % Sat is required, it will be necessary to correct readings of % Sat (obs) for which P is significantly different from 101.325 kPa as follows:

$$\% \text{ Sat} = \% \text{ Sat (obs)} \times \frac{101.325 - v}{P - v}$$

Since v is usually small compared with P, a convenient approximation of this formula is:

$$\% \text{ Sat} = \% \text{ Sat (obs)} \times \frac{101.325}{P}$$

The dissolved oxygen content of deep waters is normally expressed in concentration units; if '% saturation' values are used, they are referred to water at the surface.

B 3.8 Effect of salinity

It follows from Henry's Law that the concentration of dissolved oxygen in a water sample is directly proportional to the partial pressure of oxygen in equilibrium with that water sample at constant temperature, and hence:

$$C = \frac{S \times pO_2 \text{ in sample}}{0.2094\ (101.325 - v)}$$

where C = oxygen concentration in sample water
S = oxygen solubility, that is oxygen concentration in sample water when this is saturated with air at 101.325 kPa pressure.

Thus: $C = \% \text{ Sat (obs)} \times \frac{S}{100}$

Both C and S are usually measured in mg/l.

However, in addition to decreasing as the temperature increases, the value of S also decreases as the concentration of dissolved species in the water increases. Thus S depends upon the salinity of the water, for example if samples of distilled water and sea-water are saturated with air at 20°C and 101.325 kPa pressure, then the respective dissolved oxygen concentrations will be 9.1 and 7.4 mg/l, although the partial pressures of oxygen in equilibrium with these saturated solutions will be identical and both samples would produce the same current output from a given oxygen sensor.

Therefore, it will be necessary to correct readings of C obtained from samples whose salinity differs significantly from that of the pure water used for calibration. Correction factors have been determined for saline waters (see Table 1 General Introduction) but are strictly only applicable to sea- or estuarine waters; if the salinity exceeds that of sea-water, or is due to the presence of other dissolved species, then different factors may be needed.

B 4 Reagents

B 4.1 Internal electrolyte solution for sensor

Oxygen sensors require filling with an electrolyte solution, but the composition of this solution will vary with the type and model of sensor used: the electrolyte should be prepared according to the manufacturers' instructions, or obtained as proprietary solutions.

B 4.2 Air-Saturated water for use in sensor calibration or in checks on sensor linearity

Into a 2-litre beaker, place 1500 ml of distilled or deionized water, previously adjusted to within ± 1°C of ambient temperature. Gently bubble clean air, free of organic vapours, through the water from a fully immersed sintered-glass diffuser, porosity 4 (range of maximum pore diameter 5 to 15 μm) for 1 hour ± 10 minutes. The attainment of saturation equilibrium may be monitored by the use of the sensor itself, but it should be completely removed from the water at least 10 minutes before aeration is stopped. Allow the water to stand for 15 minutes, with occasional stirring to avoid supersaturation, and record the water temperature to the nearest 0.5°C. This water should then be used immediately.

B 4.3 Oxygen-Free water for checking zero point of sensor

Oxygen-free water is obtained by dissolving 50 ± 1 g of sodium sulphite heptahydrate in 1 litre of water and adding to it 0.022 ± 0.002 g of cobalt chloride hexahydrate dissolved in 10 ml of water. This solution is stable for about 2 months in a stoppered bottle.

B 5 Apparatus

The general form of the oxygen sensors has been described in Section B3 but it is convenient here to illustrate the arrangement of the electrodes in typical commercial examples of the Mackereth and Clark cells, figure 2.

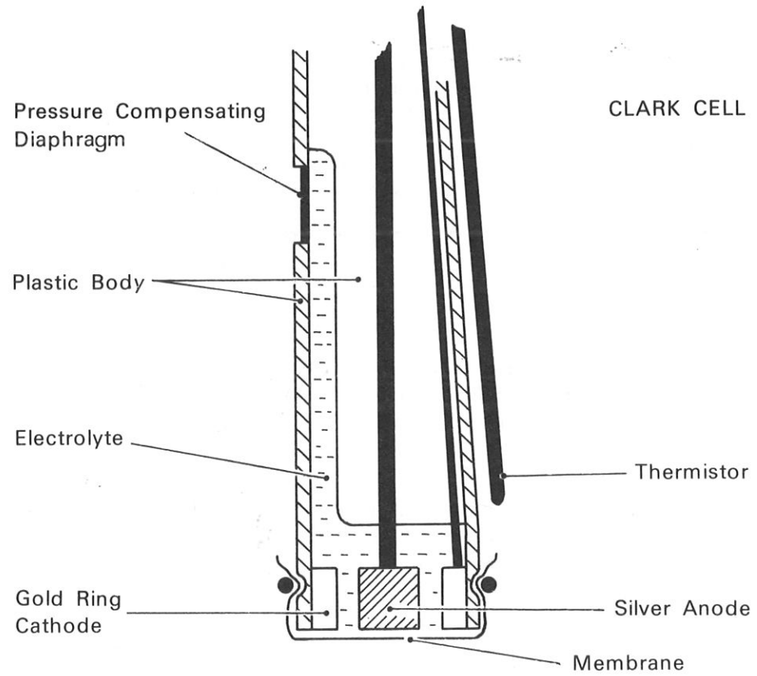

Figure 2 Dissolved oxygen sensors

When dissolved oxygen measurements are to be made in bottles (as part of a biochemical oxygen demand measurement) the necessary stirring of the solution may be achieved by placing a magnetic stirrer bar in the bottle and standing the bottle on a magnetic stirrer motor unit. Alternatively, dissolved oxygen sensors are now available with attached stirrers, the whole unit being of suitable size to enter the neck of a bottle used for biochemical oxygen demand (BOD) measurement.

In general, oxygen sensors should be stored in moist conditions but the manufacturers recommendations should be followed.

B 6 Procedure

B 6.0.1 In general, the procedure to be used will be described in the Instruction Manual supplied with the instrument. However, the essential procedure, particularly for calibration, is defined here.

B 6.0.2 The sensor should only be used for *in situ* measurements, except for BOD measurement, when the water is contained in a bottle.

Step	Experimental Procedure	Notes
B 6.1	Analysis of waters	
B 6.1.1	With the instrument prepared for use, the zero checked as in steps B 6.5.2 to B 6.5.4 and using a recently calibrated sensor, place the sensor in the water and ensure that adequate flow of water past the sensor membrane is promoted (note a).	(a) The required linear velocity will be indicated approximately by the instrument manufacturer but should be confirmed by practical test (see Section B 3.5).
	This flow may be achieved either by manual movement of the sensor, by mechanical stirring or by pumping the water past the sensor (note b).	(b) Ensure that the flow promoting process does not lead to cavitation or to the entrainment of air bubbles.
B 6.1.2	Allow time for the sensor to attain the water temperature. Measure the water temperature (note c) and, if appropriate, adjust the temperature compensating dial on the instrument.	(c) Many instruments are capable of measuring the water temperature using a thermistor in the sensor body.
B 6.1.3	Record the atmospheric pressure (note d).	(d) This step is not necessary *if* the instrument is calibrated to read in concentration units (for example mg/l) *and* the final results are also to be expressed in such units.
B 6.1.4	If the salinity is unknown determine it by chloride titration or by conductivity measurement. See appropriate methods in this series (note e).	(e) The salinity need not be known *if* the instrument is calibrated to read in partial pressure units (for example % saturation) *and* the final results are also to be expressed in such units.
B 6.1.5	Allow time for the sensor to stabilize with the instrument switched to the 'READ', or equivalent, position. Record the instrument reading.	
B 6.2	Calculation of results	
B 6.2.1	The calculation required will depend on whether the instrument used was calibrated to read in concentration or partial pressure units and also on which of these is to be used to express the final results.	
	In calculating the results attention must be paid to differences between the calibration conditions and the measurement conditions (note f). During the process of calculation it is always helpful to remember that the sensor actually responds to the partial pressure of oxygen in equilibrium with the water under test.	(f) See Sections B 3.6, B 3.7 and B 3.8 and also General Introduction.

Step	Experimental Procedure	Notes
B 6.2.2	Use the instrument readings from step B 6.1.5 and the appropriate pressure and/or salinity values from steps B 6.1.3 and B 6.1.4 to calculate the oxygen contents of the samples in the required units.	
B 6.3	Calibration of sensor and instrument	
B 6.3.1	It is usually found that the output of a sensor increases linearly with the dissolved oxygen content over the range from zero to air-saturated water. It is essential to check the linearity of response of any new sensor before it is taken into routine use and thereafter at regular intervals. This can be done by analysing a series of samples, with different dissolved oxygen contents, by both the instrumental and the titrimetric method and using the results to prepare a calibration graph.	
B 6.4	Preparation of calibration graph	
B 6.4.1	Completely fill a 1-litre borosilicate glass aspirator with distilled or deionized water, insert an immersion heater and heat to boiling; alternatively, completely fill the aspirator with boiling water. Loosely stopper the aspirator with a bung with a small hole drilled through it or, preferably, bubble oxygen-free nitrogen or argon gently through the water. Allow this deoxygenated water to cool to ambient temperature.	
B 6.4.2	Prepare about 800 ml of air-saturated water in a 1-litre beaker (note g).	(g) Use the procedure given in Section B 4.2.
B 6.4.3	To six 250-ml bottles (note h), labelled A to F, add 0, 50, 100, 150, 200 and 250 ml respectively of air-saturated water, then immediately stopper them.	(h) These bottles should be of glass and their mouths must be wide enough to accept the sensor under test. They must be fitted with tapered stoppers that do not trap air on insertion.
B 6.4.4	Carefully fill up bottle A with deoxygenated water (note i), insert a magnetic stirrer bar and immediately determine the oxygen content of the water by the instrumental method (note j), that is steps B 6.1.1 to B 6.1.3 and B 6.1.5 (note k).	(i) Every effort must be made to minimize the absorption of oxygen by the deoxygenated water, for example by using a tube on the tap of the aspirator and discharging the deoxygenated water at the bottom of the bottle. (j) If the sensor is not a close fit in the mouth of the bottle it should be fitted with a suitable stopper or shield to minimize ingress of air during determination. (k) In this case the sample is mechanically stirred using the magnetic stirrer bar.
B 6.4.5	Remove the sensor and immediately determine the oxygen content of the water by the titrimetric method, that is steps A 8.1 to A 8.5 inclusive.	
B 6.4.6	Repeat steps B 6.4.4 and B 6.4.5 for each of the other bottles B, C, D, E and F in turn.	
B 6.4.7	Calculate the concentration of oxygen in each bottle, in mg/l, found by the instrumental method (step B 6.2.2) and by the titrimetric method (step A 8.6).	
B 6.4.8	Plot the two results for each sample against each other; the resulting points should lie on a straight line.	

Step	Experimental Procedure	Notes
B 6.4.9	If the calibration graph is non-linear by more than ± 1 standard deviation (Section B 1.6), repeat steps B 6.4.1 to B 6.4.8 inclusive to confirm the non-linearity (note 1).	(l) Confirmation of non-linearity should be regarded as evidence of malfunction. It is recommended that the advice of the equipment supplier be obtained if normal electrode servicing does not rectify the fault.
	See Footnote	
B 6.5	Zero Setting	
B 6.5.1	The following checks must always be carried out before any measurements are made; both instrument zero and sensor zero are included (note m).	(m) The procedure for the zero setting process may vary somewhat from instrument to instrument, but is usually very much as described here.
B 6.5.2	With the instrument switched 'OFF' check that the meter reads zero; if it does not, adjust the reading to zero with the screw on the meter face.	
B 6.5.3	With the sensor connected and the instrument controls set to the 'ZERO' position, adjust the meter reading to zero with the appropriate controls.	
B 6.5.4	Immerse the sensor in oxygen-free water (that is 50 g/l sodium sulphite solution – Section B4.3). With the instrument controls set to the 'READ' position, check that the meter reads zero (note n); a stable reading should be obtained in 2–3 minutes (this gives a useful indication of the sensor response time).	(n) A significant reading, for example greater than 1% full scale reading, indicates a sensor fault. Note, however, that a sensor which has been dismantled for replacement of the membrane, or replenishment of electrolyte, may need prolonged soaking in sulphite solution before a very low, stable reading is obtained.
B 6.6	Single-point calibration	
B 6.6.1	Single-point calibration of the sensor and instrument should be carried out at the beginning and end of each day's measurements. Three alternative procedures are given, as follows:	
B 6.7	Calibration using air-saturated water of known oxygen concentration as determined titrimetrically (note o).	(o) This procedure must be used for work requiring the highest accuracy. In many cases, however, the alternative procedure using air-saturated water (Steps B 6.8.1 to B 6.8.3) will give almost the same degree of accuracy with considerably less effort.
B 6.7.1	Prepare about 1500 ml of air-saturated water (note g).	
B 6.7.2	Carefully fill three 250-ml BOD bottles with this water by displacement so that no change of oxygen content occurs (note i). Immediately fix the dissolved oxygen, that is steps A 8.1 and A 8.2 of the titrimetric method.	
B 6.7.3	Then immediately and carefully fill three wide-mouthed bottles with the same water and stopper them immediately (note h).	
B 6.7.4	Complete the titrimetric determination, that is steps A 8.3 to A 8.6 inclusive, on the solutions already fixed in step B 6.7.2.	

Note: Water samples with known oxygen contents, for use in sensor calibration, may also be prepared by bubbling accurately defined mixtures of nitrogen and oxygen from cylinders (supplied and certified by suppliers of compressed gases) through water until equilibrium is attained at the prevailing atmospheric pressure and temperature. This procedure is an alternative to steps B 6.4.1 to B 6.4.4 inclusive, above, but must be followed by a titrimetric determination of the dissolved oxygen content (step B 6.4.5).

Step	Experimental Procedure	Notes
B 6.7.5	Record the mean value of dissolved oxygen concentration in mg/l (note p).	(p) If any one of the three results differs from the other two by more than 0.1 mg/l it should be discarded and the mean of the other two results recorded.
B 6.7.6	Take one of the bottles from step B 6.7.3, insert a magnetic stirrer bar and immediately determine the dissolved oxygen content of the water by the instrumental method, that is steps B6.1.1 to B6.1.3 and B 6.1.5 (note k).	
B 6.7.7	Using the mean oxygen concentration found in step B 6.7.5 either (i) set the instrument to read this concentration or (ii) calculate the value of % saturation appropriate to the recorded temperature and atmospheric pressure (note q) and set the instrument to read this value.	(q) See equation (1) in General Introduction and Sections B3.6 and B3.7.
B 6.7.8	Repeat step B6.7.6 with each of the remaining two bottles of water to check that the instrument reading set in step B6.7.7 is reproduced (note r)	(r) If readings differing by more than 0.1 mg/l are obtained the whole calibration procedure (steps B 6.7.1 to B 6.7.8 inclusive) must be repeated.
B 6.8	Calibration using air-saturated water	
B 6.8.1	Prepare air-saturated water (note g).	
B 6.8.2	Using this water as the sample, carry out steps B 6.1.1. to B 6.1.3 and B 6.1.5.	
B 6.8.3	Either (i) Calculate the dissolved oxygen concentration appropriate to the recorded temperature and atmospheric pressure (note s) and set the instrument to read this concentration, or (ii) calculate the value of % saturation appropriate to the recorded atmospheric pressure (note t) and set the instrument to read this value. The sensor and instrument are now calibrated.	(s) See equation (1) in General Introduction. (t) See Section B 3.7.
B 6.9	Calibration using water-saturated air (note u)	(u) This method is convenient for field use but the above methods using air-saturated water are more accurate. It may not be reliable to use this method of calibration with all types of probes (even if the method is described in the instrument manual). This procedure should therefore only be used with a particular probe after trials have shown that it gives identical results when also calibrated by the procedure of steps B 6.7.1 to B 6.7.8 above.
B 6.9.1	Place the sensor loosely in the neck of a bottle which contains a little fresh water. Ensure that the membrane surface is free of liquid water (wipe with a tissue).	
B 6.9.2	Allow at least 10 minutes for temperature stabilization and record the air temperature and atmospheric pressure.	
B 6.9.3	Calculate the dissolved oxygen concentration as in step B 6.8.3. The sensor and instrument are now calibrated.	

B 7 Special Cases

B 7.1 Concentration measurements in waters of abnormal salinity

The determination of oxygen concentration in waters having significantly greater salinity than sea-water, or whose salinity is due to the presence of other dissolved species, is best achieved by calibration with an air-saturated sample of the water in question, the oxygen concentration in this sample being determined by the titrimetric method.

This will not be possible, however, if any constituents of the water interfere with the titrimetric determination. In such a case, the dissolved oxygen content can only be expressed in partial pressure units, for example % saturation, using an instrument provided with such a scale.

B 7.2 Biological Growth

There may be occasions when rapid biological growth on the sensor membrane makes use of this type of sensor unsatisfactory, particularly for on-line operation. Under these conditions the use of a conductometric method based on oxidation of thallium metal[25] may be appropriate.

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Address for Correspondence

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