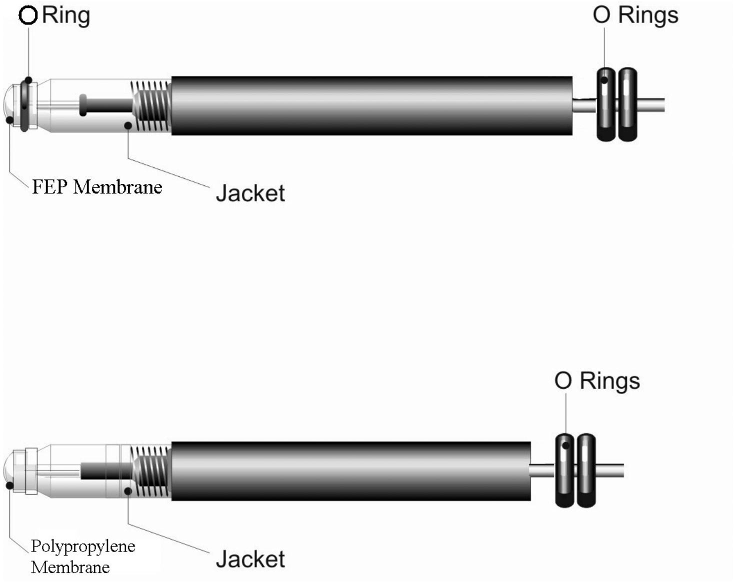
1302 Microcathode Oxygen Electrodes

Introduction

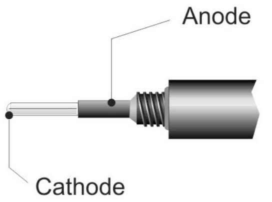
The 1302 oxygen electrodes are in use worldwide with Strathkelvin's 782 and 929 instruments and other products. They have the advantage of small size, high precision, and the ability to operate in either stirred or unstirred media.

They are supplied with pre-membraned polypropylene jackets, but for fast speed of response, a different jacket with FEP membranes should be used. With FEP membranes, they cannot be used unstirred and require vigorous water movement at the electrode tip. In all applications, the electrodes have to be housed in special electrode holders or accessories (see the Strathkelvin website: www.strathkelvin.com). These have been designed so that only the membrane at the electrode tip comes into contact with the medium. Failure to protect the electrode in this way can result in anode depolarisation and a shortening of the useful life of the electrode.

A full description of the 1302 electrode, including theoretical considerations and details of care and maintenance, is given below.



1. Principle of operation of Clark-type oxygen electrodes



The Clark-type oxygen electrode consists of a probe at whose tip is an exposed gold or platinum cathode and a silver or silver/silver chloride anode. When the anode and cathode are polarised so that the cathode is held at a voltage of -0.6 to -0.8 volts relative to the anode, and connected via a solution of electrolyte such as KCl, the following reaction will occur at the anode:

$$4Ag \rightarrow 4Ag^{+} + 4e \quad \text{and} \quad 4Ag^{+} + 4Cl^{-} \rightarrow 4AgCl$$

Simultaneously, at the cathode, any oxygen which is present is reduced:

$$O_2 + 2H_2O + 4e \rightarrow 4OH^{-}$$

Thus for each oxygen molecule reduced, 4 electrons of current flow in the circuit. Oxygen is therefore continually 'consumed' as it is reduced to OH^- at the cathode.

In practice, the anode and cathode are covered by an oxygen permeable membrane to exclude other species which would interfere. The KCl electrolyte is buffered to remove the OH^- produced in the cathode reaction.

As oxygen is removed at the cathode, a PO_2 gradient is set up which extends outwards into the surrounding medium. In unstirred water, oxygen therefore diffuses inwards along the PO_2 gradient. Because of the PO_2 gradient, the outside of the electrode membrane is effectively sensing a very much lower PO_2 than that in the surrounding water. For this reason, most Clark electrodes require the water to be stirred.
The size of the signal generated by the electrode is proportional to the flux of oxygen molecules to the cathode. This oxygen flux is proportional to:

1. The PO_2 of the water
2. The permeability of the membrane
3. The temperature of the water
4. The surface area of the cathode

Increase in any of these factors will therefore increase the size of the signal which is generated.

It is important to note that most Clark-type electrodes require the water to be stirred. Only in a microcathode electrode, fitted with a low permeability membrane, is stirring not required.

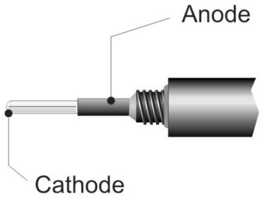
2. Principle of the 1302 Microcathode Electrode

The 1302 is a precision electrode with a very small diameter microcathode. Because of this, the rate of consumption of oxygen is extremely low, so that when used with the relatively low permeability polypropylene membranes, most of the resulting oxygen gradient is confined to the distance between the outside of the membrane and the cathode surface. Consequently there is no requirement for physical movement of the solution to replenish the oxygen at the outer surface of the membrane. There is a very small stirring effect, which would result in an error of 2 - 3% if the electrode has been calibrated in stirred solution and used in unstirred solution, and vice versa.

In order to use the microcathode electrode in unstirred solutions, it is necessary to use the polypropylene membranes, which are supplied as standard. These are relatively slow, about 18 sec for 90% change.

In applications where oxygen concentration changes rapidly (as with respiration of mitochondria), it will be necessary to use the fast responding, highly permeable FEP membranes. If working at very low temperatures, it may also be worth considering FEP membranes since speed of response varies inversely with temperature.
The FEP membranes are only 12.5µm in thickness and have to be used with a special electrode jacket, Part No SI035. This jacket has a pressure relief hole on the side. Without this hole the hydrostatic pressure produced as the jacket is tightened on the thread will cause the membrane to lift away from the cathode, thereby increasing the O_2 diffusion path and response time of the electrode. When used with fast-responding highly permeable FEP membranes, the flux of oxygen through the membrane is increased. In this case, the electrode behaves like a macrocathode electrode and it is then necessary to stir the solution. Regrettably, it is not possible to build an electrode with a fast response time but no stirring requirement.

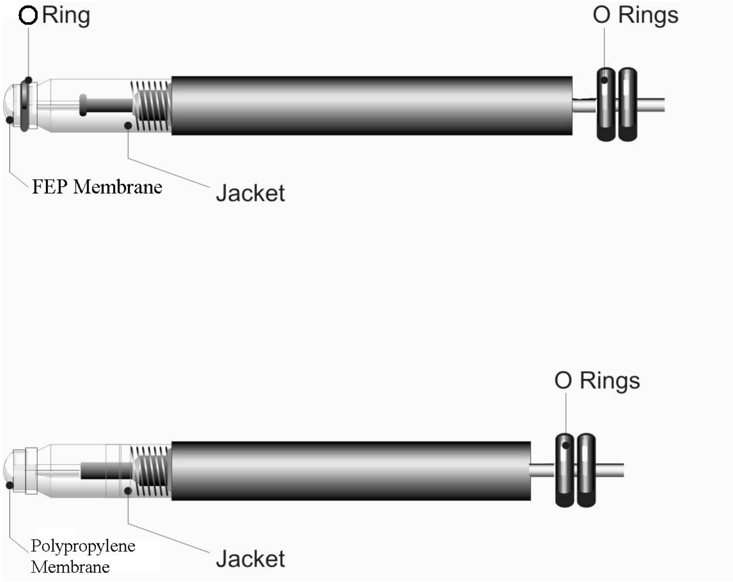
3. Electrode Construction



The electrode consists of a glass tube containing a 20µm platinum wire whose exposed tip forms the cathode. The anode is a band of chlorided silver, brown in colour, located a few millimetres from the tip.

Electrical connection between the anode and cathode is via an electrolyte of buffered KCl solution. The electrolyte is contained within a jacket over whose tip is stretched a thin polypropylene membrane. The polypropylene membranes are permanently fitted to the jackets (pre-membraned). When the membrane needs changed the whole jacket must be replaced. However the FEP membrane is not permanently attached to a jacket – the membrane needs to be attached to the jacket with an o-ring. When the FEP membrane needs changed a new membrane and o-ring are used.

The jacket is unscrewed from the body of the electrode in order to replace the membrane, electrolyte or jacket.



A. Fitting a pre-membraned polypropylene jacket

The electrode is normally supplied 'dry' and should be stored in this way if not in use for prolonged periods of time to prolong the life of the electrode.

1. Unscrew the electrode jacket and lay the electrode carefully on a paper tissue on a safe part of the bench. It is very vulnerable to damage when the jacket has been removed. Rinse the electrode with distilled water and blot dry with soft paper tissue.

2. Invert a new pre-membraned jacket and add electrolyte solution with a pasteur pipette up to the inscribed line. The jacket will then be about half filled. Tap the jacket to dislodge any bubbles and inspect under a strong light to **ensure that there are no air bubbles adhering to the inside of the membrane.**

3. Slowly insert the electrode into the jacket and screw down until tight, and the tip causes the membrane to bulge slightly. There will be an air bubble inside the jacket. Dry the outside of the electrode immediately with paper tissue.

B. Fitting a FEP membrane

The electrode is normally supplied 'dry' and should be stored in this way if not in use for prolonged periods of time.

1. Unscrew the electrode jacket and lay the electrode carefully on a paper tissue on a safe part of the bench. It is very vulnerable to damage when the jacket has been removed. Pour off the electrolyte and carefully prise off the retaining 'O' ring with the points of fine forceps. Rinse the electrode and jacket with distilled water and blot them dry with soft paper tissue. Stand the electrode jacket with its broad end on the bench. Now take an 'O' ring and tension it by sliding it on to the conical applicator. Stand the applicator on the bench and carefully push the 'O' ring down to the farthest edge.

2. Take a FEP membrane in the left hand and place over the tip of the jacket, pressing down a little to tension it **very slightly.** Now press the recessed end of the applicator over the membrane, and, with finger and thumb, press the 'O' ring downwards until it snaps into position in the 'O' ring groove in the jacket.

3. With fine scissors trim away excess membrane surrounding the 'O' ring, leaving a small frill. Check the appearance of the membrane. It should be taut but not deformed and there should be no holes or blemishes visible.

4. Invert the jacket and add electrolyte solution with a pasteur pipette up to the inscribed line. The jacket will then be about half filled. Tap the jacket to dislodge any bubbles and inspect under a strong light to **ensure that there are no air bubbles adhering to the inside of the membrane.**

5. Slowly insert the electrode into the jacket and screw down until tight, and the tip causes the membrane to bulge slightly. There will be an air bubble inside the jacket. Dry the outside of the electrode immediately with paper tissue.

4. Housing the electrode

The 1302 electrode is designed to operate with only the outer face of its tip in contact with medium. If therefore has to be housed in a special holder - such as those provided with the MT200, MC100, RC300, RC350, RC400 and TC500 accessories, or in an EH100 or FC100 electrode holder. Each of these has a precision-engineered tip to provide a face seal against the tip of the electrode. It is not possible to make a satisfactory seal using the membrane 'O' ring since this will often disturb the membrane tension and, in addition, solution will often penetrate past the 'O' ring via microchannels in the membrane folds.

The area behind the tip of the electrode must remain dry. This is to avoid earth leakage currents via the molecular film of liquid extending beneath the 'O' ring, which would lead to depolarisation of the anode. It follows from this that ***on no account should the electrode be immersed directly in the solution.***

5. Electrode Maintenance

When in regular use, the electrode should be kept in water-saturated air or the tip immersed in saline solution, to prevent drying out of the electrolyte. If the electrode is in daily use, keep it connected and polarised. If the electrode will not be used for several weeks rinse the inside of the jacket and electrode with distilled water, dry and refit the jacket and store dry in the electrode box.

The 1302 electrode is a very high impedance sensor and can be easily and irrecoverarably damaged by water or saline getting into the back of the electrode or the cable at the plug end. It must always be used enclosed in an electrode holder and care must be taken not to allow water into the back of the holder or onto the connector.

Changing the membrane / pre-membraned jacket

The intervals at which this should be done vary with usage. Generally, the membrane becomes coated with organic materials with time, and its permeability, and hence the electrode output will fall and response time will increase. The membrane may be carefully wiped with a soft paper tissue and this could be done once a day. However, if there are any abrasive particles on the membrane, it could become scratched and damaged and the electrode output will rise. If the electrode is used with blood or other solution which may deposit protein aggregates on the membrane, the electrode should be left with its tip in a mild proteinase solution overnight.

In normal usage, the membrane will last for several weeks. Fit a new membrane / pre-membraned jacket only if it is suspected that the electrode output has changed or the speed of response has increased. It is advisable to use a new 'O' ring if the membrane has not been changed for several weeks.

The electrode membrane will need several hours to stabilise each time the electrode is replaced in the holder/respiration cell. During this stabilisation period the measured oxygen values will drift slightly. Thus it may be a good idea to change the membrane at the end of the working day.

Cleaning the Cathode

Cleaning the cathode should be undertaken at intervals of about 3 - 4 weeks, if the electrode is in continuous use, or when changing the membrane.

Remove the electrode from its jacket, rinse with distilled water and dry the tip with paper tissue. Polish the tip with **a few light** strokes of the polishing paper provided in the service kit. Add new electrolyte to the jacket and screw the jacket back on to the electrode body. Take care not to finger the anode during this operation.

Bubbles in the electrolyte

Whilst it is undesirable to have an air bubble trapped on the membrane adjacent to the cathode, an air bubble elsewhere in the electrolyte will do no harm providing that there is good electrical connection between anode and cathode through the electrolyte. In fact it is quite normal for the electrolyte to lose water through the membrane and for air bubbles to appear.

Temperature and pressure effects

Ensure that the medium is not subjected to changes in pressure (as would happen, for instance, if a sample of blood or water is drawn vigorously into a syringe before injection into the MC100 microcell) nor exposed to bubbles of air. Remember that oxygen measurements have to be made at the same temperature as calibration and that the temperature control should be within +/- 0.05°C, since the signal from the electrode varies with temperature.

When working at a different temperature, it will be necessary to recalibrate the electrode. Allow the tip to come to temperature equilibrium (10 to 15 minutes) before calibrating. Do not expose the electrode to sudden increases in temperature particularly if there is a bubble in the electrolyte. The associated pressure change could cause the membrane to bulge and the response time of the electrode would increase. If an air bubble is present, it is best to top up the electrolyte level, before raising the temperature.

6. Checking electrodes

6.1 Checking Electrode Output

It is possible to check the state of the electrodes by observing the electrode output in picoamps (pA), which is displayed on the meter during calibration. Electrodes vary in their outputs (which is why they have to be calibrated) but the output may also change with time. There are no absolute values to work with, but observation of the outputs will alert you to potential problems.

High Point output

Standard **polypropylene membranes**, the output at 20ºC in air-saturated water is normally in the range of 200 - 600 pA.
FEP membranes, the output at 20ºC in air-saturated water is normally in the range of 800-1200 pA.
The output changes by 2 - 3% with each degree Centigrade of temperature change, increasing as the temperature increases.

Zero output

In zero oxygen, there is usually a very small residual current, called the dark current. During Calibration, this signal is compensated for, so that a zero reading is obtained in zero oxygen. The interface displays this dark current during zero oxygen calibration. Normally the value shown will be between 0 and about 50 pA. Very high dark currents may indicate a short circuit within the electrode.

7. Troubleshooting

7.1 High dark current

A high dark current displayed on the interface during calibration with the electrode in zero oxygen solution could be due to your solution having become oxygenated again. So first check the solution by adding more sodium sulphite to it. If the problem persists, it may indicate an internal short circuit within the electrode

Such a short circuit is most commonly caused by water or saline through the back of the electrode or into the plug on the electrode cable. To check if this has happened:

1. Unplug the electrode from the meter and start a Calibrate Zero. Note the meter reading (usually 0 pA)
2. Remove the jacket from the electrode, wash the exposed glass cathode area and **carefully** dry it with a soft tissue. Leave the jacket off.
3. Plug the electrode back into the meter. The displayed electrode current should not change.

If the reading in 3 above increases, a short circuit is present. This may be due to either deposits on the exposed glass area or the presence of water inside the electrode or connector.

1) To remove any deposits **gently** wipe the exposed glass with acetone or a similar acid using a soft cloth.
2) Rinse the cathode with water and dry using a soft tissue.
3) Using polishing paper provided in the Strathkelvin Instruments electrode service kit, gently rub the cathode to remove any film that may be present on the tip or the sides of the exposed glass.
4) Wash the exposed glass area again and dry with a soft tissue.

7.2 High output current in air saturated water

If the current in zero oxygen solution is not high then:

Polypropylene membrane: If it is more than 400 pA at 20°C, the possible causes are:
1. Damage to the membrane (e.g. pin holes).
2. Enlargement of the cathode by plating on of silver. Remove by polishing with the blue lapping film.
3. Air bubbles in the electrolyte at the cathode.
4. Overpressure in the electrolyte.

Both the last two can be relieved by holding the electrode tip down, unscrewing the jacket a little, tapping the jacket to displace air bubbles and retightening. Do this several times.

FEP membrane: If it is more than about 1200 pA at 20°C, the causes may be the same, except for 4. The pressure relief hole in the jacket prevents overpressure.

7.3 High output current in zero oxygen solution

This may be due to membrane damage or to damage to the electrode. To check for the latter:

1. Remove the jacket from the electrode.
2. Wash the exposed glass probe with water.
3. Carefully dry the glass probe with a tissue.
4. Measure the output current of the electrode.

The current should be zero. If not then:

Current 1 – 100 pA: Try polishing all the exposed glass with the blue lapping film, rewashing and drying. This will remove any plated on salts. If this does not reduce the current to zero there may have been some water ingress. If no salts have been carried in then drying the electrode in a desiccator for 48 hours sometimes works. If it does not,
Or
Current more than 100 pA: the electrode is probably damaged beyond repair by water ingress and must be replaced.

Return the electrode to your distributor or to Strathkelvin Instruments for verification.

7.4 Low output

The output will fall as a result of fouling of the membrane by bacteria or adsorbed proteins. Wipe the membrane from time to time with fine paper tissue.

7.5 Continual downward drift

When the electrode is first connected to the interface and thereby subjected to the bias voltage, there will be a very high reading - which will gradually decline to a stable value, providing that the electrode is at a constant temperature and exposed to a constant oxygen concentration. After changing the membrane, the electrode may take several hours to settle down again. If the electrodes are going to be in daily use, keep the electrode connected to the interface which should be switched on. The electrodes will then be ready for immediate use as required.

The electrode might be expected to drift by no more than 0.5% over 12 hours. If the downward drift is greater than this, check the following:

1) Is the electrolyte being maintained at constant temperature? A drop in temperature will cause the signal to fall.

2) Is the electrode in an enclosed volume of water? In these circumstances, the reading can fall because the O_2 concentration in the chamber is declining due

to bacterial respiration. If checking the electrode in a situation like this, make sure the chamber has been sterilised and that the solution is sterile. Even distilled water from a distilled water reservoir can have a significant population of bacteria. Ideally, use freshly boiled then cooled and re-aerated saline and keep a bacteriocidal solution in the chamber when not in use.

If you are using an EH100, the best check of electrode stability is to clamp the EH100 with the electrode tip just below the water surface of the constant temperature water bath and keep the bath water well aerated.

If you are certain that the drift is not due to either of these two causes, go to rechloriding the electrode.

7.6 Continual upward drift

Check whether the temperature of the solution being measured has changed. This sometimes happens in respirometry experiments if full sunlight or other bright light is allowed to fall on the respiration chamber. The electrode output will change by about 3% for each °C change in temperature. If the temperature change can definitely be ruled out, go to rechloriding section.

7.7 Unstable Display

The reading flickers erratically over several digits. This can be caused by trouble with the membrane, the cathode or the anode. Try the following in turn:

1) Wipe the membrane with a soft tissue, first dry, then moistened with acetone or alcohol. If no improvement, change the membrane.
2) Remove the electrode jacket and rinse the inner electrode with distilled water. Carefully dry with a soft paper tissue. Clean the anode or cathode with a soft tissue moistened with acetone or alcohol to remove any grease which may have got on to these surfaces. Add more electrolyte to the jacket and screw on tight again.
3) Clean the cathode
4) Rechloride the anode

7.8 Response time becomes excessively high

Response time varies with a number of factors including temperature, and membrane type and thickness. If the response time increases, it is probably because the membrane has become coated and is therefore less permeable. Try wiping the membrane very carefully with a soft tissue. If you are measuring oxygen in high-protein solutions, dip the tip of the electrode (in its holder) into a proteinase solution when not being used.

Sometimes increased response time is due to an increase in the membrane to cathode distance. This could be due to the membrane becoming slack, in which case change the 'O' ring. It may also be due to the jacket not having been screwed on tightly.

7.9 Persistent malfunction

If there is a persistent malfunction in an electrode, send it, with a purchase order number and full description of the experimental set-up and the nature of the fault, to your distributor or to Strathkelvin Instruments. We will do our best to service and repair the electrode although where there is excessive dark current, repair is sometimes not possible.

8. Rechloriding electrode anode

The anode of the 1302 is a silver band with a dark brown layer of silver chloride deposited upon it. If there has been any leakage to ground from the electolyte, the anode may become depolarised and will appear very light and silvery in colour due to loss of most of the silver chloride. The most likely cause of this is that the electrolyte has been in electrical contact with the grounded solution. This can happen if there is a minute hole in the membrane, or if water gets into the electrode holder. If the anode has been stripped, it is usually possible to rechloride it successfully.

You will need:

1. A 1.5v battery and battery holder.
2. About 5cm length of silver wire, soldered to a length of copper wire, which is connected to the negative side of the battery holder.
3. Another length of copper wire connected to the positive side of the battery holder and terminating in a crocodile clip.
4. A small beaker of 5 - 6cm diameter, containing about 4cm depth of 0.1N HCl.

Method:

Position the silver wire vertically on one side of the beaker containing the 0.1N HCl. Unscrew the electrode jacket and rinse the electrode in distilled water. Dry the anode carefully with paper tissue and then carefully rub it with very fine (400 grit or finer) emery (or similar) abrasive paper until any residual silver chloride coating has been removed and it appears a uniform silver colour. Take care not to finger the anode. Rinse in distilled water again and dry with soft paper tissue.

Place a light above the beaker; connect the crocodile clip to the outside of the Lemo connector and insert the electrode into the beaker as far as possible from the silver wire, to a depth, which covers the anode.

Rotate the electrode in the solution for 15 seconds. You will see hydrogen bubbles on the silver wire and the anode will become a dark brown colour as silver chloride plates on to it. Remove the electrode from the solution and examine it. If the anode is not an even brown colour, repeat the process again.

Rinse the electrode in distilled water, dry with paper tissue and reassemble.

9. Calibration solutions

It is possible to calibrate the oxygen electrodes with gas mixtures, but most researchers tend to calibrate at the low end with a zero oxygen solution and at the high end with air-saturated water.

Air-saturated water This must be at exactly the same temperature (within 0.1 C) as the solution, which will be measured in the experiment, since the electrode is temperature sensitive. To obtain air-saturated water, bubble air through it, or stir vigorously for 15-20 mins. Make sure that this does not alter the temperature of the water.

Zero oxygen solutions An oxygen-free solution can be produced by adding a pinch of sodium sulphite to distilled water or saline in a beaker and swirling to dissolve. Alternatively make up a solution of 2% sodium sulphite in 0.01M sodium borate (3.81 g sodium borate in 1 litre distilled water) and keep in a stoppered bottle. The borate acts as a weak buffer to stabilise the solution. Some early literature suggested the use of sodium dithionate solution. Do not use this, since its breakdown products can impair the functioning of the cathode.

Rinse all traces of the sulphite from the electrode after zero calibration

10. Oxygen solubility tables

The concentration of oxygen in a solution will vary with temperature and the concentration of solutes dissolved in it. The solubility coefficient or Bunsen coefficient α expresses the concentration of oxygen in solution when in equilibrium with oxygen gas at a pressure of 760 torr (mm Hg), in mls O_2 (S T P) per ml solution.

For freshwater and seawater use the calculation facilities in the software, or refer to the detailed tables in references (1) and (2).

Mammalian Ringer solution:

Concentration of oxygen at 100% saturation with air and values for α (ml O_2 per ml solution, at PO_2 of 760 torr).

Temperature °C	**Concentration** (air saturation) ml O_2.1^{-1}	**Solubility Coefficient (α)** ml O_2.ml^{-1}
10	10.05	0.0480
15	7.12	0.0340
20	6.49	0.3100
25	5.97	0.0285
30	5.44	0.0260
35	5.13	0.0245
40	4.81	0.0230

From reference (3).

Mitochondrial medium:

Concentration of oxygen at 100% saturation with air.

Temperature °C	Concentration (air saturation) µmol $O_2.l^{-1}$	Concentration (air saturation) µg atoms $O_2.ml^{-1}$
15	288	0.575
20	255	0.510
25	237	0.474
30	223	0.445
35	205	0.410
37	199	0.398
40	190	0.380

From reference (3).

Conversion units

Pressure

1mm Hg = 1 torr = 0.133322 kilo Pascals = 0.001316 atm.

Oxygen concentration units

1mg = 0.700ml (at S T P) = 31.251 µmol = 0.0625 mg atoms.

11. Preparation of Electrolyte solution

Weigh out:	5.31g	disodium hydrogen phosphate dihydrate
	2.6g	potassium dihydrogen phosphate
	1.04g	potassium chloride

Make up to 100ml with distilled water. Add a few crystals of silver chloride to give a saturated solution. Finally drop in a small crystal of thymol, to inhibit fungal and bacterial growth. Shake vigorously and leave for 12 hours for the thymol to go into solution. Filter into a stoppered bottle.

12. References

1. Carpenter, J.H. (1966) New Measurement of oxygen solubility in pure and natural waters. Limnology and Oceanography 11, 267 - 277.

2. Green, E.J. & D.E. Carritt (1967) New tables for oxygen saturation of seawater. J. mar. Research 25, 140 - 147.

3. Clark, J.B. (1992) Electrochemical essays: the oxygen electrode. In Enzyme Assays (ed. Eisenthal, R. & Danson, M.J.). IRL Press, Oxford, 181 – 190.