Microcathode Oxygen Electrodes

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:

$4Ag$ Õ $4Ag^{+} + 4e$ and **$4Ag^{+} + 4Cl^{-}$ Õ $4AgCl$**

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

$O_2 + 2H_2O + 4e$ Õ $4OH^{-}$

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. The outside of the electrode membrane effectively senses a very much lower PO_2 than that in the surrounding water. For this reason, most Clark-type 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 displayed on the oxygen meter.

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.

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 20µ 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. 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 membrane needs changed a new membrane and o-ring are used. The jacket for FEP also has a pressure relief hole on the side.
The jacket is unscrewed from the body of the electrode in order to replace the membrane, electrolyte or jacket.

4 Fitting the membrane

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

A) Pre-Membraned Jackets:

1. Unscrew the electrode holder and withdraw the electrode.

2. Take a new jacket from the service kit and half fill the jacket with electrolyte solution. Fill the jacket to just below the marker line which is cut into the jacket just below the threaded part. Tap the side of the jacket vigorously to dislodge any air bubbles left near the membrane. If there is an air bubble attached to the membrane, the output from the electrode will appear abnormally high.

3. Unscrew the jacket from the electrode, rinse the end of the electrode with distilled water and dry with soft tissue. Polish the very tip with a few circular strokes of the abrasive paper provided in the service kit. When the jacket is removed, the delicate glass interior of the electrode is vulnerable to damage, so carry out this operation quickly and carefully. Make sure that you do not touch any part of the electrode interior.

4. Screw the jacket on tight again and wipe away excess electrolyte on the outside with a paper tissue. Replace the electrode in the holder. Screw on the cap of the holder using medium pressure.

5. If possible change the membrane jacket at the end of the day as after re-connecting the electrode you will need to allow time for the electrode to stabilise.

B) Older Style Non-Membraned Jackets

1. Unscrew the electrode jacket and lay the electrode carefully on 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 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 until the jacket is filled to the line half way up the jacket. 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. During this operation, excess electrolyte will escape at the threaded upper part. Dry the outside of the electrode immediately with paper tissue.

5 FEP membranes

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 seconds for a 90% change). In applications where oxygen concentration changes rapidly (as with respiration of mitochondria in an RC300 or RC350 cell), it will be necessary to use the fast responding, highly permeable FEP membranes. If working at very low temperatures, it may be worth considering FEP membranes since speed of response varies inversely with temperature. The FEP membranes are only 12.5µ in thickness and have to be used with a special electrode jacket, P/N SI035. When using FEP membranes, remember that it is necessary to stir the medium.

6 Housing the electrode

The 1302 electrode is designed to operate with only the outer face of its tip in contact with medium. It therefore has to be housed in a special holder - such as those provided with the MC100, RC200, RC300, RC350, RC400 and TC500 accessories, or in an EH100 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 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.**

7 Electrode maintenance

When in regular use, the electrode should be kept with its tip immersed in water to prevent drying out of the electrolyte. If the electrode is in daily use, keep it connected to the meter and polarised (i.e. with the meter switched on and the standby button pressed). If the electrode will not be used for several days, rinse it and dry it and store in the electrode box. If it is to be stored for a longer period, remove the jacket and empty out the electrolyte. Rinse the inside of the jacket and electrode with distilled water, dry and refit the jacket and store dry in the electrode box.

a) Changing the membrane

The intervals at which the membrane should be changed vary with usage. Generally the membrane becomes coated with organic materials with time and its permeability and hence the electrode output and response time will fall. 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. 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 / jacket (see Section 2.4) only if it is suspected that output or speed of response has fallen. It is advisable to use a new O ring if the membrane has not been changed for several weeks.

b) 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.

c) 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 provided that there is good electrical connection between anode and cathode through the electrolyte.

Electrolyte Solution

Weigh out the following:

- 5.32 g disodium hydrogen phosphate dihydrate (or 4.24 g of anhydrous salt or 7.47g of the heptahydrate)
- 2.6 g potassium dihydrogen phosphate
- 1.04 g 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.

How to get the best out of your electrode

Housing your electrode

Microcathode electrodes are finely engineered precision electrodes and require care to be taken to obtain optimal results and to prolong their life in service. The only part of the electrode which should come into contact with the medium is the membrane-covered electrode tip. The other parts of the electrode should be kept dry, by enclosing it in an electrode holder (such as an EH100 or one of the Strathkelvin cells). Some users have simply put the electrodes through holes in rubber bungs to insert them into respirometer chambers. This is not to be recommended as it inevitably leads to a reduced life for the electrode as a result of bias current leak problems. Always ensure that the only part which is wetted is the electrode tip.

Know your electrode characteristics

Each electrode has slightly different characteristics with respect to response time, output at 100% saturation and the dark current when in zero oxygen solution. In order to gauge the relative performance of your electrode over time, it is worthwhile making a note of its output characteristics when new.

1. Output at 100% saturation.

Place the electrode in a fully saturated solution and switch to Coarse Gain position 2. Then turn the Fine Gain knob fully clockwise. With the units selector switch on the rear panel switched to % saturation and with standard polypropylene membranes fitted, the maximum output of a new electrode is normally about 25 at 20ºC. Note the output of your electrode.

2. Dark current.

Switch back to the normal coarse gain position 3. Place the electrode in a zero oxygen solution and wait until it stabilises fully. This may take 5 minutes. Zero the display using the Zero knob and then disconnect the electrode from the front panel. The meter will now display the offset current which is proportional to the dark current. This should be less than -2.0 and in a good electrode will be less than -1.0.

The electrode will still function satisfactorily at higher values, but if the dark current reads above about -8, it normally means that there is an internal short circuit.

Rechloriding the anode

The anode 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 electrolyte, the anode may become de-polarised 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 other side of the battery holder and terminating in a crocodile clip.
4. A small beaker of 5 - 6 cm 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 abrade it with very fine (400 grit or finer) emery abrasive paper until any residual 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 paper tissue.

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

Rotate the electrode in the solution for one minute. You will see hydrogen bubbles on the silver wire, and the anode will become a uniform dark brown colour as silver chloride plates on to it. The light is to stimulate the photochemical reaction which turns the silver chloride to its brown coloured form. Remove the electrode from the solution and examine it. If the anode is not a uniform brown colour, repeat the abrasive and subsequent steps again.
Rinse the electrode in distilled water, dry with paper tissue and reassemble.