Before the advent of polarography, only classical wet-chemistry techniques were available for the determination of dissolved oxygen. The first standard test for dissolved oxygen was reported in 1888 by L.W. Winkler (1). The Winkler test is a titrimetric method based on the following reactions:

\[
\begin{align*}
\text{Mn}^{2+} + 2\text{OH}^- & \rightarrow \text{Mn(OH)}_2 \\
2\text{Mn(OH)}_2 + \frac{1}{2}\text{O}_2 + \text{H}_2\text{O} & \rightarrow 2\text{Mn(OH)}_3 \\
2\text{Mn(OH)}_3 + 6\text{H}^+ + 3\text{I}^- & \rightarrow 2\text{Mn}^{2+} + \text{I}_3^- + 6\text{H}_2\text{O} \\
\text{I}_3^- & \rightarrow \text{I}_2 + \Gamma \\
\text{I}_2 + 2\text{S}_2\text{O}_3^{2-} & \rightarrow 2\Gamma + \text{S}_4\text{O}_6^{2-}
\end{align*}
\]

Although the Winkler method is very precise when performed with strict control of pH and iodine concentration, it is still susceptible to errors. For example, ions such as Fe^{2+} and SO^{3-}, suspended solids, and some organics can produce gross inaccuracies. There have been numerous modifications of the method over the years to compensate for its shortcomings (2). Besides the errors inherent in the Winkler method, it is extremely labor intensive and slow (even when automated). In situ measurements are practically impossible and no real-time monitoring can be done (3). Furthermore, the Winkler method is suitable only for aqueous solutions, not solvents or gases.

Heyrovsky’s work with the dropping mercury electrode in the early 1920s was conducive to the acceptance of electrochemistry as a routine analytical tool (4). He identified the broad cathodic waves belonging to the reduction of dissolved oxygen at the dropping mercury electrode (DME) and established the direct relationship between dissolved oxygen concentration and limiting current.

In the decades following, many oxygen detectors were developed using a variety of materials and configurations. While the DME was instrumental in the determination of many biologically significant substances, the most practical working electrodes for dissolved oxygen detectors were solid electrodes that could be easily put to use in biological samples such as blood, tissue, and cultures. The two-electrode detectors usually consisted of a gold or platinum working electrode and the Ag/AgCl counter or reference electrode. (Note: Although “polarography” is usually reserved for those experiments done with a DME, much of the literature uses the term to describe voltammetric and amperometric oxygen detectors.)

The basis of amperometric oxygen electrodes is fairly straightforward. By placing the two electrodes in solution and applying a potential between them that is sufficient to reduce dissolved oxygen at the working electrode (typically ~ -650 mV vs. Ag/AgCl), one can determine the partial pressure of oxygen (pO_2) in an ideal solution because pO_2 is proportional to the limiting current.

This procedure would be sufficient if there were no other species in the solution that could reduce at
the reduction potential for oxygen. In addition, many real samples contain species which can adsorb onto the electrode surface and passivate, or block the effective working area of the electrode. For example, a metallic electrode in blood or other body fluid will become coated with a proteinaceous layer which will lead to a deterioration of the current response. Because of these complications, in situ measurements with bare electrodes are very difficult to perform without frequent calibration checks due to the changing electrode surface and/or the solution composition.

One of the first practical membrane-covered amperometric oxygen electrodes was invented in 1954 by Leland C. Clark, who also received the patent for the device in 1956 (5). By using a membrane that was permeable to gases, the selectivity of the oxygen electrode increased dramatically. Interfering anions and surface-fouling species could not permeate the membrane and foul the electrodes, thus the lifetime and stability of the detector increased.

Clark’s electrode overcame most of the limitations associated with classic chemistry methods, as well as those that plagued the early polarography experiments. Although the “Clark electrode,” as it is called in biological literature, had humble beginnings, it is still used in many clinical and industrial applications.

Applications

Widespread applications of the membrane-covered oxygen electrode exist in the clinical arena, where \( \text{pO}_2 \) measurements are needed routinely for both assessment and monitoring of patient health. Today, \( \text{pO}_2 \) measurements are performed in blood with electrochemical detectors that can be inserted via catheter or syringe. Most in vivo detectors are comprised of very thin wires encased in sealed Teflon tubing filled with supporting electrolyte.

The determination of \( \text{pO}_2 \) is essential in many environmental applications. Membrane-covered \( \text{pO}_2 \) detectors are often used in sewage treatment plants, either on sampling probes for intermittent checks or permanently placed in waste streams for continuous in-situ monitoring of aeration. Efficient aeration of sewage is essential for microbial breakdown of the waste.

Industrial uses include food science applications, such as spoilage determinations and quality control. The brewing and fermentation industries require dissolved oxygen monitoring to study the efficiency of yeast cultures and preservatives in beers and wines. Biosynthetic routes to some pharmaceuticals require controlled oxygen concentrations in order to avoid unwanted reactions.

Experimental

The BAS oxygen electrode (F1) is initially prepared for use by polishing for a few minutes with 1-µm diamond polish on a polishing disk. After polishing, the electrode surface is rinsed with methanol and then with water. The next step is to prepare the Ag/AgCl reference electrode. This is done by placing a drop of reference coating solution (CF-2200) on the silver electrode. The solution is allowed to react with the silver for at least ten minutes to assure complete formation and coverage of the AgCl layer.
After preparing the Ag/AgCl electrode, the assembly can be completed as shown in F2. After soaking a nitrocellulose filter paper disk in a few drops of supporting electrolyte solution, the saturated filter paper is transferred with tweezers to the face of the electrode. The disk is positioned so that the platinum working electrode and the Ag/AgCl reference electrode are completely covered. One O-ring is placed in the assembly base and a PTFE membrane is laid on top of the O-ring and base. The oxygen electrode is centered over the PTFE membrane and O-ring in the assembly base and is gently pushed down through the O-ring until the electrode touches the bottom of the assembly base. The assembled oxygen electrode is then pulled out of the assembly and is ready for use.

A simple two-point calibration can be performed in which the current response is measured in an air-saturated sample solution (maximum) and in deoxygenated sample solution (minimum). F3 illustrates the current response of the BAS oxygen electrode for a water sample before and after purging with argon.

Deoxygenation is typically accomplished by purging the sample solution with an inert gas, such as argon or nitrogen. Alternatively, sodium sulfite can be used in solutions with pH > 6 to efficiently scavenge dissolved oxygen without the use of inert gas.

\[ 2\text{SO}_3^{2-} + \text{O}_2 \rightleftharpoons 2\text{SO}_4^{2+} \]

A sulfite ion concentration of approximately 0.1 M is usually sufficient. Although this approach is not viable at lower pH due to the reduction of hydrogen sulfite and sulfurous acid at the working electrode, it is an efficient way to remove dissolved oxygen in a wide range of samples (6). Hydrogen peroxide (7), NADH (8), and ascorbic acid have also been used for calibration of oxygen detectors.

**Discussion**

PTFE (Teflon®) is the favored membrane for oxygen detectors because of its inertness, relative strength, temperature resistance, and hydrophobicity. PTFE is also available in very thin sheets (less than a micron thick) and is crystalline and highly oxygen permeable. Other membranes reportedly used for amperometric oxygen detectors include polyethylene, silicone rubber (9), cellophane, collodion, and silica gel (10).

A disk of filter membrane is used to contain the electrolyte for many reasons. First, the filter paper provides for a reproducible electrolyte layer between the PTFE membrane and the electrode body. Second, the filter paper support minimizes the loss of electrolyte and extends the detector lifetime. Third, the use of such a support allows the electrode to operate with the minimum of electrolyte, thus decreasing the residual current that is produced from the reduction of dissolved oxygen in the bulk of the electrolyte. Fourth, by making the diffusion layer as thin and as uniform as possible, a filter paper support increases the diffusion current as well as shortens the response time of the detector (12). Nitrocellulose was chosen as a suitable support due to its high degree of hydrophilicity and because perpendicular and lat-
eral flow through the membrane is allowed. The nitrocellulose membranes are also very stable and do not react with the electrodes.

Although membrane-covered amperometric oxygen electrodes have many advantages over classical wet-chemistry methods and bare electrodes, they are susceptible to interferences and complications. Sources of error, such as gaseous impurities, temperature, pressure, and flow variations, must be addressed to obtain reproducible data.

In some situations, a sample may contain gases that interfere with the detection of dissolved oxygen. For example, Cl₂, NO, and Br₂ have standard potentials more positive than oxygen. In most cases the concentrations of these gases are very small relative to oxygen and their effect on the current response is minimized by frequent background checks. Sulfurous gases, such as SO₂ and H₂S, are especially harmful to Pt and Ag working electrodes and must be removed from the sample before analysis.

Depending on the design of the detector, a 1°C increase in temperature can increase the current response by up to 6%. F4 illustrates the temperature dependence of the current response of the BAS sensor. In general, the operation of any oxygen electrode should be carried out under thermostatic conditions. Thermostatic conditions can be achieved by either controlling the sample solution temperature or by regulating the temperature of the electrode itself so that the membrane and diffusion layers are at a constant temperature throughout an experiment.

If thermostatic conditions are not feasible, temperature effects must be compensated for in order to obtain accurate pO₂ measurements. One example of this type of compensation is to measure the temperature of the solution near the membrane with a thermocouple and use a calibration curve to determine the dissolved oxygen concentration from the current response. Membrane-covered oxygen detectors are less susceptible to flow variations than bare electrodes. They do, however, need a sufficient flow of solution past the membrane to work in optimal fashion. F5 illustrates the flow dependence of the BAS Oxygen Electrode. After approximately 500 rpm, the stir bar creates enough flow past the membrane so that the current response is only dependent on the diffusion through the membrane material (Dₘ). Below this speed, the current response is flow dependent because the rate at which dissolved O₂ is being brought to the membrane is less than the rate of oxygen diffusion through the membrane. For accurate and reproducible pO₂ measurements, it is important to avoid a situation in which the diffusion layer of the detector is depleted. This is most easily achieved by stirring the sample solution.

Conclusions

Some basic principles and applications of amperometric oxygen electrodes were presented as background material for teachers and students using the BAS Oxygen Electrode Kit.

References