Dissolved Oxygen Electrodes

Young H. Lee
Department of Chemical Engineering, Drexel University
Philadelphia, PA 19104, U.S.A.

George T. Tsao
School of Chemical Engineering, Purdue University
West Lafayette, IN 47907, U.S.A.

Recent advances in theory, construction, operation, and application of dissolved oxygen (DO) electrodes are reviewed to assist those who use or intend to use them in such areas as biochemical engineering, microbiology, and environmental engineering. Basic operating principles of membrane-

covered DO electrodes and oxygen microelectrodes are presented together with methods of construction, electrode component selection, and general design considerations. Methods of calibration and effects of temperature and liquid film on electrode performance are also discussed. Sources of measurement errors due to probe characteristics and the reaction in the liquid are discussed to illustrate some of the limitations of DO electrodes. The spacial resolution of oxygen microelectrodes in local concentration measurements is also discussed. Finally, the application of DO electrodes in measuring aeration capacity and oxygen solubility is reviewed.

1 Introduction

Since its introduction by Clark in 1956[31], the membrane-covered dissolved oxygen (DO) electrode and its modified versions have been used widely both in research and in industry. Compared with wet chemical analysis[7] and other methods[5], the measurement of dissolved oxygen by the membrane-covered electrode offers several advantages: simplicity; less interference by other solutes in water; rapid, in situ measurements; and, above all, continuous measurement for real-time control of oxygen concentration in bioreactors or wastewater treatment units. Although the basic operating principles are the same, DO electrodes have been developed in different areas to meet requirements of the specific applications. Examples are:

steam-sterilizable DO probes for bioreactor applications[42],
oxygen microelectrodes for tissue oxygen measurements[136],
fast responding oxygen probes for respiratory gas analysis[39],
DO probes capable of measuring trace oxygen in boiler feedwaters[43], etc.

The wide applicability of the DO electrode can be illustrated by a number of related articles in such diverse areas as

biochemical engineering[142],
civil engineering[122],
microbiology[14],
medicine[20],
physiology[136],
chemistry[30],
chemical engineering[78],
mechanical engineering[113],
oceanography[70], etc.

In biochemical engineering, the laboratory data obtained from DO probe measurements not only give fundamental information on microbial physiology and kinetics[14] but also form the basis for bioreactor scale-ups[140], production yield calculations[5], and reactor control[126]. Thus, it is important to understand the operating principle and some of the limitations of DO electrodes in order to effectively use them in particular applications. Reviews on DO electrodes are available by a number of authors from different areas[14, 25, 34, 37, 42, 47, 61, 64, 84, 131, 136, 142]. This paper deals with a unified survey of the recent advances in theory, construction and application of DO electrodes to assist those who use or intend to use them in their research.

2 Historical Development

The reduction of dissolved oxygen at a noble metal surface negatively polarized with respect to a reference electrode was first observed in early 1897[136]. In 1942, Davies and Brink used this tech-

nique for measuring local oxygen tension in animal tissues[38]. They made various "open" and "recessed" electrodes with 25 μm platinum wire enclosed in glass insulation. These are so-called polarographic oxygen microelectrodes, which have been extensively used in tissue oxygen measurements[135]. In these applications, the oxygen microelectrode (cathode) and the reference electrode (calomel or Ag/AgCl) are separately immersed in a test medium which contains some sort of electrolyte. The major concern in electrode design has been the fast and accurate measurement of local oxygen tension in tissues. Electrodes having tip size of 1–2 μm with 95% response time of much less than 1 s were developed[135, 149]. Recently, these microelectrodes have been used for measuring oxygen concentration gradients in microbial slime layers[28, 66] and concentration fluctuations at the liquid surface[27, 86, 139].

The problem of calibration and measurement associated with early noble metal polarographic electrodes led to the introduction of the membrane-covered electrode by Clark in 1956[31]. In its original design, both the platinum cathode and the reference electrode (Ag/AgCl) were contained in a single electrode body containing the electrolyte solution, and the entire tip of the electrode body was covered with a single polyethylene membrane. The sensor is thus completely separated from the medium to be measured by a nonconducting membrane which is partially permeable to oxygen. The Clark electrode, now the basis of many commercially available polarographic DO probes (see Table 1), could be calibrated in different liquids and, furthermore, enabled dissolved oxygen measurements in non-conducting media. The major use of the early Clark electrodes was in blood oxygen measurements[131].

In 1959, Carrit and Kanwisher[30] used a modified version of the Clark electrode for measuring dissolved oxygen in Chesapeake Bay water. To increase the stability of the probe, they used Ag/Ag_2O instead of Ag/AgCl for the reference electrode and 0.5 M KOH for the electroylte. Since the temperature control of the bay water was not possible, they incorporated a thermistor in their probe to compensate for sensitivity change due to temperature. The Carrit-Kanwisher probe was further developed by Carey and Teal[29]. Hospodka and Caslavsky[65] used such a probe in microbial processes.

A different type of membrane-covered electrode, namely, the galvanic electrode, was first developed by Mancy et al. in 1962[106]. Noting the unstable behavior of a platinum surface as the cathode, they used silver as the cathode, and lead as the anode. Unlike the polarographic electrode, this galvanic probe did not require external voltage. The voltage generated by the silver-lead electrode pair was sufficient to cause a spontaneous reduction of oxygen at the cathode. The major advantage of the galvanic probe over the polarographic type (Clark electrode) was the long-term stability of sensitivity. However, the galvanic probe had a finite life time because of the gradual oxidation of the anode surface.

Mackereth[104] later modified the design so that the probe could be used continuously over several months without losing stability. The improvements were in the use of a silver tubing as the cathode for an increased current output, and a massive lead shot as the anode for an increased probe life.

An autoclavable galvanic probe for *biochemical engineering* work was first described by Johnson et al. in 1964[71] and was further improved by Borkowski and Johnson[21]. Unlike other probes, the elctrolyte chamber was vented to withstand repeated autoclaving. Also, to facilitate the fabrication of probes in the laboratory, the cathode was made from a silver wire wound in a spiral form and a flattened lead wire was used as the anode. The Borkowski-Johnson probe became popular[42, 120] because of its long life, ease of fabrication and relatively large current output. Brookman[24] later described a more rugged design which showed a linear response up to 10^3 mm Hg of oxygen partial pressure. Autoclavable versions of the Mackereth probe were described by Flynn et al.[49] and Harrison and Melbourne[59]. The modifications included decreased cathode area and the use of different membranes and electrolytes[49, 59]. The Mackereth probe has also been widely used for monitoring and controlling dissolved oxygen in cultivation media[60, 126].

In the area of medicine and physiology, improvements in the DO probe design have included the response time, the spacial resolution and the flow sensitivity. The *flow sensitivity* means that the output current of the probe changes depending on liquid velocity. Normally, a DO probe requires a high liquid velocity for a proper operation. This flow dependency decreases with decrease in cathode diameter. Silver[134] and Bicher and Knisely[18] described miniaturized Clark electrodes with tip diameters of 2–5 μm, which exhibited low flow dependency and fast response time (95% response of less than 0.5 s). These microelectrodes have been used for local oxygen tension measurements in tissues[136]. However, these electrodes have relatively poor stability[73] and extremely low current output requiring careful attention to amplification and instrumentation.

Several approaches have been used to incorporate the advantages of microelectrodes in macroprobe design. Fatt and Helen[48] and Siu and Cobbold[137] used a number of microcathodes in a single probe body. Others[13, 72, 75] used a thin band metal as the cathode. These probes showed relatively fast response time and low flow dependency and yet produced high current output since the total surface area of the cathode was large. Some of the commercial probes for use in deep waters employ thin band metal as the cathode[13, 72].

Other developments in DO probes were in the measurement of trace amount of dissolved oxygen and the *gas phase oxygen measurement.* Evangelista et al.[43] described a platinum-lead galvanic probe capable of measuring dissolved oxygen down to the parts per billion range for use in boiler feedwaters. DO probes for gas phase oxygen measurements were mainly developed in the area of physiology. The major application has been in respiratory gas analysis, which requires a fast probe response. Döhring et al.[39] obtained a 95% response time of 20 ms in their modified Clark electrode by employing a specially preparaed 0.4 μm thick Teflon membrane. However, a 95% response time of 0.1 s could be easily obtained by using a commercially available 3 μm membrane[51]. The accuracy of the gas phase oxygen measurement by the polarographic electrode was reported to be equal to or better than that obtained by the Scholander analysis or by the paramagnetic method[146].

3 Principle of Measurement

3.1 Polarographic Electrode and Galvanic Electrode

When an electrode of noble metal such as platinum or gold is made 0.6–0.8 V negative with respect to a suitable reference electrode (calomel or Ag/AgCl) in a neutral potassium chloride solution, the dissolved oxygen is reduced at the surface of the cathode. This phenomenon can be observed from a current-voltage diagram, called a *polarogram*, of the electrode. As shown in Fig. 1, the current increases initially with an increase in the negative bias voltage, followed by a region where the current becomes essentially constant. In this plateau region of the polarogram, the reaction of oxygen at the cathode is so fast that the rate of reaction is limited by the diffusion of oxygen to the cathode

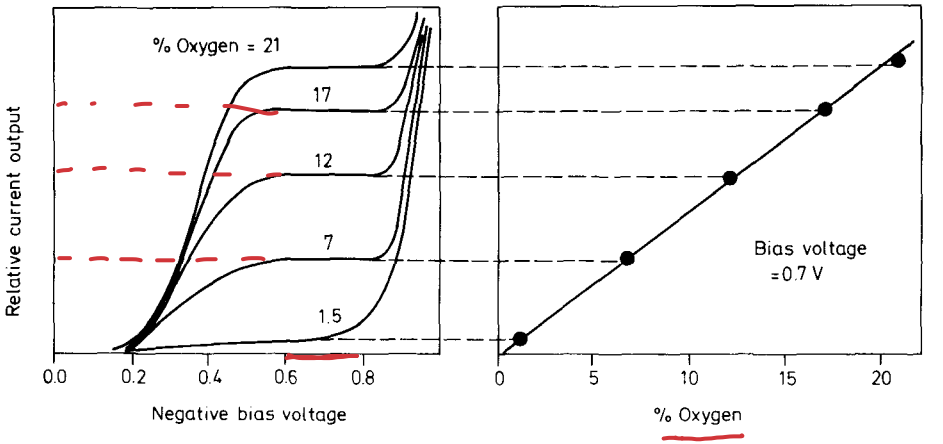

Fig. 1. Polarogram and calibration curve (Cobbold[34])

surface. When the negative bias voltage is further increased, the current output of the electrode increases rapidly due to other reactions, mainly, the reduction of water to hydrogen[37]. If a fixed voltage in the plateau region of the currentvoltage diagram is applied to the cathode, then the current output of the electrode can be calibrated to the dissolved oxygen (Fig. 1). It has to be noted that the current is proportional not to the actual concentration but to the activity or equivalent partial pressure of dissolved oxygen, which is often referred to as *"oxygen tension"*. A fixed voltage between −0.6 and −0.8 V is usually selected as the bias voltage (or polarization voltage) when using Ag/AgCl as the reference electrode[47].

When the cathode, anode, and the electrolyte are separated from the measuring medium with a plastic membrane, which is permeable to gas but not to most of the ions, and when most of the mass transfer resistance is confined in the membrane, the electrode system can measure oxygen tension in various liquids. This is the basic operating principle of the *membrane-covered polarographic DO probe* (Fig. 2a).

For polarographic electrodes, the reaction proceeds as follows[37]:

Cathodic reaction: $O_2 + 2\,H_2O + 2e^- \rightarrow H_2O_2 + 2\,OH^-$

$H_2O_2 + 2e^- \rightarrow 2\,OH^-$

Anodic reaction: $Ag + Cl^- \rightarrow AgCl + e^-$

Overall reaction: $4\,Ag + O_2 + 2\,H_2O + 4\,Cl^- \rightarrow 4\,AgCl + 4\,OH^-$.

The reaction tends to produce alkalinity in the medium together with a small amount of hydrogen peroxide[37]. Forbes and Lynn[50] postulated two principal pathways for the reduction of oxygen at the noble metal surface. One is a 4 electron pathway where the oxygen in the bulk diffuses to the surface of the cathode and is converted to H_2O via H_2O_2 (path a in Fig. 3). The other is a 2-electron pathway where the intermediate H_2O_2 diffuses directly out of the cathode surface into the bulk liquid (path b in Fig. 3). They stated that the oxygen reduction path changes depending on surface condition of

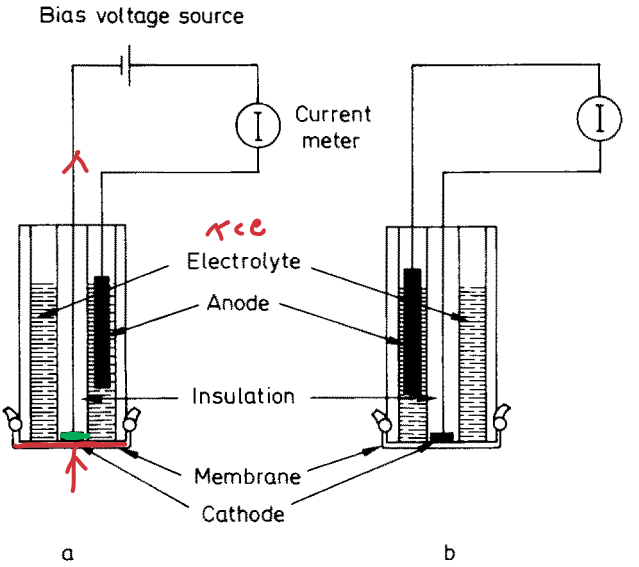

Fig. 2. Basic arrangements for (a) polarographic electrode and (b) galvanic electrode

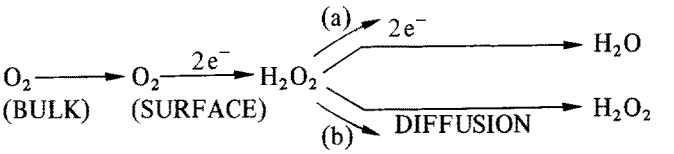

Fig. 3. Alternative pathways of oxygen reduction at the cathode surface (Forbes and Lynn[50])

the noble metal. This is probably the cause for time-dependent current drift of polarographic probes[9]. Since the hydroxyl ions are constantly being substituted for chloride ions as the reaction proceeds, KCl or NaCl has to be used as an electrolyte. When the electrolyte becomes depleted of Cl^-, it has to be replenished.

The *galvanic electrode* (Fig. 2b) is different from the polarographic type in that it does not require external voltage source for the reduction of oxygen at the cathode. When a relatively basic metal such as zinc, lead or cadmium is used as the anode and a relatively noble metal such as silver or gold is used as the cathode, the voltage generated by the electrode pair is sufficient for a spontaneous reduction of oxygen at the cathode surface. The electrode reaction of the silver-lead galvanic probe is as follows[104, 106]:

Cathodic reaction: $O_2 + 2\,H_2O + 4e^- \rightarrow 4\,OH^-$

Anodic reaction: $Pb \rightarrow Pb^{2+} + 2e^-$

Overall reaction: $O_2 + 2\,Pb + 2\,H_2O \rightarrow 2\,Pb(OH)_2$.

As shown above, the oxygen is reduced via four-electron reaction. Unlike the polarographic probe, the electrolyte does not participate in the reaction but the anode surface is gradually oxidized. Therefore, the probe life depends on the available surface area of the anode.

Whether the polarization voltage is applied internally (galvanic) or externally (polarographic), the operating principle of the electrode remains the same. For both types of probe, interference on measurement is expected when gases that reduce at 0.6–1.0 V are present in the test medium. Examples are halogens (Cl_2, Br_2, I_2) and oxides of nitrogen[13]. Hitchman[64] described in detail the electrochemistry of oxygen reduction.

3.2 Theory of Operation

The basic principle of measurement for the membrane-covered DO probes can be summarized as follows: provided that the oxygen diffusion is controlled by the membrane covering the cathode, the current output of the probe is proportional to the oxygen activity or the partial pressure in the liquid medium. The behavior of the probe can be predicted by using a simplified electrode model. For a mathematical analysis, the following assumptions are made:

1. The cathode is well polished and the membrane is tightly fit over the cathode surface so that the thickness of electrolyte layer between the membrane and the cathode is negligible.
2. The liquid around the probe is well agitated so that the partial pressure of oxygen at the membrane surface is the same as that of the bulk liquid.
3. Oxygen diffusion occurs only in one direction, perpendicular to the cathode surface.

This is the so-called one layer model[2, 7, 90] but it can be extended to include the effects of other layers as will be shown later.

Suppose the electrode is immersed in a well-agitated liquid and, at time zero, the oxygen partial pressure of the liquid is changed from zero to p_0. According to Fick's 2nd law, the unsteady-state diffusion in the membrane is described as follows:

$$\frac{\partial p}{\partial t} = D_m \frac{\partial^2 p}{\partial x^2} , \tag{1}$$

where D_m is the oxygen diffusivity in the membrane and x is the distance from the cathode surface (Fig. 4a). The initial and boundary conditions are:

$$p = 0 \qquad \text{at } t = 0 , \tag{2}$$

$$p = 0 \qquad \text{at } x = 0 , \tag{3}$$

$$p = p_o \qquad \text{at } x = d_m , \tag{4}$$

where d_m is the membrane thickness. The first boundary condition [Eq. (3)] assumes very fast reaction at the cathode surface. This condition was experimentally verified by Baumgärtl et al.[12].

The solution of Eq. (1) with the boundary conditions is (2):

$$\frac{p}{p_o} = \frac{x}{d_m} + \sum_{n=1}^{\infty} \frac{2}{n\pi} (-1)^n \sin \frac{n\pi x}{d_m} \exp\left(-n^2 \pi^2 D_m t / d_m^2\right) . \tag{5}$$

The current output of the electrode is proportional to the oxygen flux at the cathode surface[82]:

$$I = NFAD_m \left(\frac{\partial c}{\partial x}\right)_{x=0} = NFAP_m \left(\frac{\partial p}{\partial x}\right)_{x=0} , \tag{6}$$

where N, F, A, and P_m are the number of electrons per mole of oxygen reduced, Faraday's constant, surface area of the cathode, and oxygen permeability of the membrane, respectively. The permeability, P_m, is related to the diffusivity, D_m, by

$$P_m = D_m S_m , \tag{7}$$

where S_m is the oxygen solubility of the membrane. From Eqs. (5) and (6), the current output of the electrode as a function of time, I_t is derived as follows[2, 17, 90]:

$$I_t = NFA(P_m/d_m)p_o \left[1 + 2 \sum_{n=1}^{\infty} (-1)^n \exp(-n^2 \pi^2 D_m t/d_m^2)\right] . \tag{8}$$

The pressure profile and the current output under steady-state conditions can be obtained from Eqs. (5) and (8), respectively:

$$\frac{p}{p_o} = \frac{x}{d_m} \tag{9}$$

and

$$I_s = NFA(P_m/d_m)p_o . \tag{10}$$

At steady-state, the pressure profile in the membrane is linear (Fig. 4a) and the electrode current is proportional to the oxygen partial pressure of the bulk liquid. Equation (10) forms the basis for DO probe measurements.

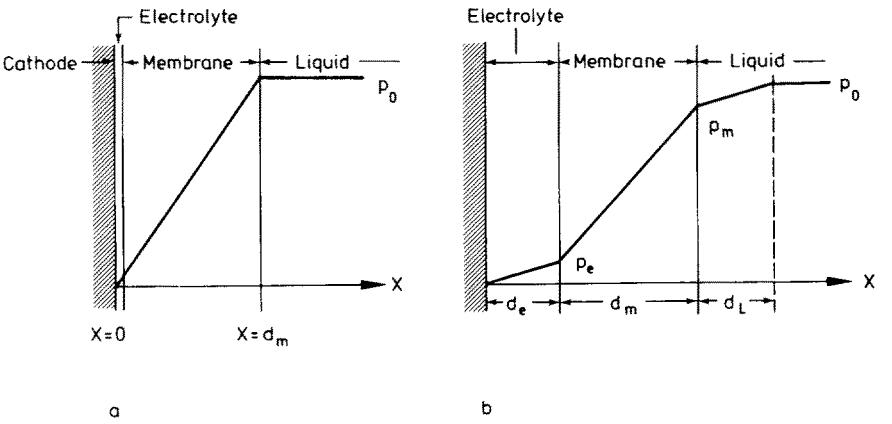

Fig. 4. (a) One-layer electrode model (b) three-layer electrode model

Another important consideration is the time response of the probe. According to Eq. (8), the probe response depends on the probe constant, k, defined as follows[15, 90]:

$$k = \frac{\pi^2 D_m}{d_m^2} \quad . \tag{11}$$

A large k, which means a thin membrane and/or a high D_m, results in a fast probe response. However, these conditions tend to weaken the assumption of membrane-controlled diffusion. Thus, a compromise has to be made for an optimum probe performance.

In reality, the assumptions 1 and 2 made earlier are not entirely satisfactory. Often, there exists a finite thickness of electrolyte layer between the cathode and the membrane because of the roughness of the cathode surface. Also, a stagnant liquid film always exists outside the membrane even at very high liquid velocity. A more realistic model of the electrode has to be the one shown in Fig. 4b, where all three layers, namely, the electrolyte, the membrane and the liquid film are considered. Several authors[15, 17, 106] used two layer models incorporating the electrolyte layer and the membrane, while others[92, 128] discussed the effect of liquid film.

The effect of different layers on electrode behavior can be estimated by using the "one layer" model. At steady state, the oxygen flux, J, through each layer in Fig. 4b becomes identical:

$$\begin{aligned} J &= K_o p_o \\ &= k_{LM}(p_o - p_m) \\ &= k_m(p_m - p_e) \\ &= k_e p_e \quad , \end{aligned} \tag{12}$$

where K_o is the overall mass transfer coefficient and small k's represent individual mass transfer coefficients corresponding to the liquid film (k_{LM}), the membrane (k_m) and the electrolyte (k_e), respectively. The overall mass transfer resistance, $1/K_o$, is then expressed as the sum of the individual resistances:

$$\frac{1}{K_o} = \frac{1}{k_{LM}} + \frac{1}{k_m} + \frac{1}{k_e} \quad . \tag{13}$$

Equation (13) can be rewritten by using the oxygen permeability and the thickness of each layer:

$$\frac{1}{K_o} = \frac{d_L}{P_L} + \frac{d_m}{P_m} + \frac{d_e}{P_e} \quad , \tag{14}$$

where d_L, d_e, P_L, and P_e are liquid film thickness, the electrolyte thickness, the oxygen permeability of the liquid film and that of the electrolyte layer, respectively. A completely stagnant liquid film was assumed here, although it is more accurate to use the convective mass transfer coefficient, k_{LM}, directly[92].

The condition for a membrane-controlled diffusion becomes:

$$\frac{d_m}{P_m} \gg \frac{d_L}{P_L} + \frac{d_e}{P_e} \quad . \tag{15}$$

This means that a relatively thick membrane with a low oxygen permeability is required, which contradicts the requirement for a fast probe response. For a given cathode geometry, the resistance of the electrolyte is more or less fixed. Also, since the electrolyte is contained inside the membrane, it does not affect the measurement. Therefore, the condition for accurate measurements of dissolved

oxygen becomes:

$$\frac{d_m}{P_m} + \frac{d_e}{P_e} \gg \frac{d_L}{P_L} \quad . \tag{16}$$

When the individual resistances are taken into account, the steady state current output can be written as,

$$I_s = NFA(P_m/\bar{d})p_o \quad , \tag{17}$$

where $\bar{d}$ is defined as,

$$\bar{d} = d_m + \frac{P_m}{P_L} d_L + \frac{P_m}{P_e} d_e \quad . \tag{18}$$

In this case, the probe constant, k, is modified as follows:

$$k = \frac{\pi^2 D_m}{\bar{d}_t^2} \quad , \tag{19}$$

where

$$\bar{d}_t = d_m + \sqrt{\frac{D_m}{D_L}} d_L + \sqrt{\frac{D_m}{D_e}} d_e \quad . \tag{20}$$

Equations (17) and (19) show that the steady-state current decreases and the probe response time increases when there is a significant mass transfer resistance in the liquid film around the membrane. Normally, probes are operated such that the effect of liquid film resistance is negligible: this is achieved by using membranes of low oxygen permeability and by a vigorous agitation of the liquid around the probe.

Sometimes, the assumption of one dimensional diffusion (assumption 3) is not satisfactory, especially when the cathode diameter is small compared to the membrane thickness. Often, the probe response shows hysteresis[15, 61, 79] and doubling of the cathode area does not result in doubling of the output current[131]. Electrode models incorporating lateral or sideways diffusion[69, 80] and multi-layer, multi-region models[93] are helpful for understanding the electrode behavior in these situations.

3.3 Oxygen Microelectrodes

The measurement of local oxygen tension in microbial pellets and films is often necessary to understand the mass transfer mechanisms involved[28, 66]. The capability of measuring local oxygen concentration inside the liquid diffusion boundary layer enhanced our understanding of the oxygen transport process[27, 86, 87, 139]. Oxygen microelectrodes have been used for these purposes, which have long been applied in physiology for tissue oxygen measurements[136].

Figure 5 shows a basic arrangement for oxygen microelectrode measurements. This is similar to the membrane-covered polarographic probe described earlier except that the cathode and the reference electrode (anode) are used separately. Thus, an electrolyte is required in the medium for dissolved oxygen measurements. The analysis of the membrane-covered microcathode is similar to that

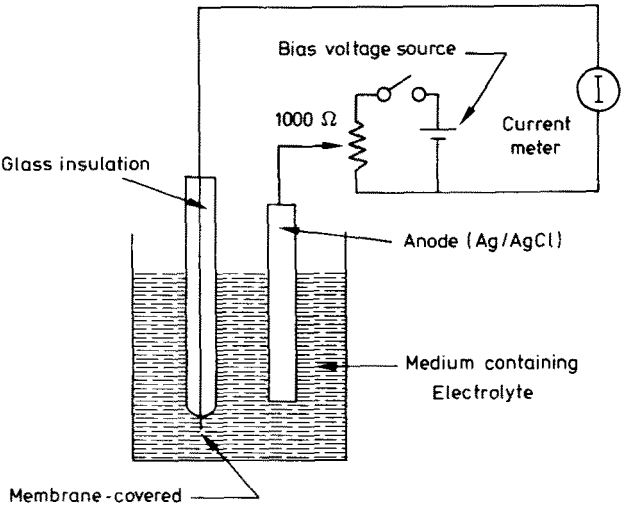

Fig. 5. Basic arrangement for oxygen microelectrode

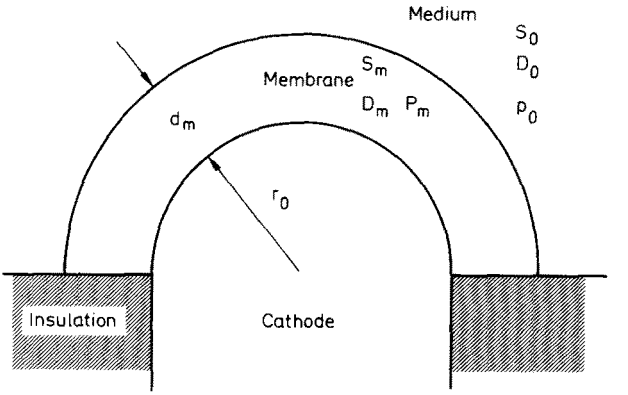

Fig. 6. Model of microcathode

of the macroprobes given earlier. Since the shape of the cathode tip is normally conical[86], a half spherical cathode with a radius of r_0 is used as a model. Suppose the cathode is immersed in an infinite medium whose undisturbed oxygen partial pressure is p_0 (Fig. 6). Considering the symmetry of the half-sphere, the governing diffusion equations with boundary conditions are:

$$\frac{\partial p}{\partial t} = D_m \left(\frac{\partial^2 p}{\partial r^2} + \frac{2}{r} \frac{\partial p}{\partial r} \right) \quad \text{for} \quad r_0 \leqslant r \leqslant r_0 + d_m \ , \tag{21}$$

$$\frac{\partial p}{\partial t} = D_0 \left(\frac{\partial^2 p}{\partial r^2} + \frac{2}{r} \frac{\partial p}{\partial r} \right) \quad \text{for} \quad r \geqslant r_0 + d_m \ , \tag{22}$$

$$p = 0 \quad \text{at} \quad r = r_0 \ , \tag{23}$$

$$p = p_0 \quad \text{at} \quad t = 0 \ , \tag{24}$$

$$p = p_0 \quad \text{at} \quad r = \infty \ , \tag{25}$$

$$-P_o\left(\frac{\partial p}{\partial r}\right)_{r=(r_o+d_m)^+} = -P_m\left(\frac{\partial p}{\partial r}\right)_{r=(r_o+d_m)^-} . \tag{26}$$

The analytical solutions to these equations[56] describe the transient behavior of the electrode. Steady-state solutions are:

$$p = p_o\left(\frac{r_o+d_m}{d_m}\right)\left(\frac{s}{1+s}\right)\left(1-\frac{r_o}{r}\right) \quad \text{for} \quad r_o \leqslant r \leqslant r_o+d_m , \tag{27}$$

$$p = p_o\left[1-\left(\frac{1}{1+s}\right)\left(\frac{r_o+d_m}{d_m}\right)\right] \quad \text{for} \quad r \geqslant r_o+d_m , \tag{28}$$

where

$$s = \frac{P_o\, d_m}{P_m\, r_o} . \tag{29}$$

The steady state current output can be obtained from Eq. (6):

$$I_s = 2\,NFP_m r_o\left(\frac{r_o+d_m}{d_m}\right)\left(\frac{s}{1+s}\right)p_o . \tag{30}$$

Equations (27) and (28) are plotted in Fig. 7 for different P_o/P_m when the membrane thickness is twice the diameter of the cathode. It is shown that the pressure gradient becomes confined within the membrane with increasing P_o/P_m. The condition for 99% of the pressure gradient to be confined inside the membrane can be obtained from Eq. (27):

$$\frac{P_o\, d_m}{P_m\, r_o} \geqslant 99 . \tag{31}$$

When this condition is satisfied, the local oxygen tension can be measured with a high spacial resolution and, for liquid phase measurements, the probe output is not affected by liquid velocity. In this case, the steady state current becomes independent of the oxygen permeability of the medium:

$$I_s = 2\,NFP_m r_o\left(\frac{r_o+d_m}{d_m}\right)p_o . \tag{32}$$

This equation shows that the current is proportional to the oxygen partial pressure of the medium but it is not exactly proportional to the surface area of the cathode.

For a cathode with membrane-controlled diffusion, the 95% response time of the probe, $\Gamma_{95\%}$, is given approximately as follows[57]:

$$\Gamma_{95\%} = \frac{d_m^2}{2D_m} . \tag{33}$$

Equation (33) shows that the response time of the probe is inversely proportional to the probe constant, k, defined by Eq. (11). The probes in this category have cathode diameters of around 1 μm and, with a proper membrane, $\Gamma_{95\%}$ is much less than 1 s[11, 18, 87, 134, 149].

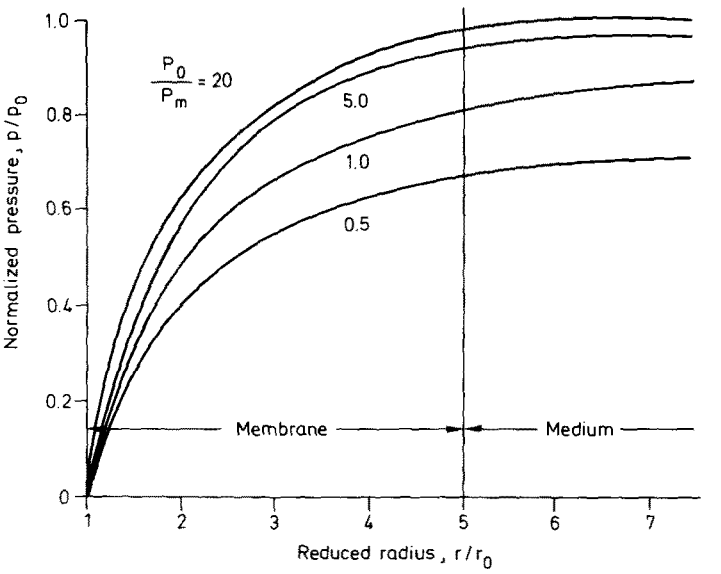

Fig. 7. Effect of (P_0/P_m) on steady-state pressure profile (Lee et al.[87])

4 Design of Electrodes

Although the membrane-covered DO electrode basically consists of a cathode, an anode, and the electrolyte, numerous designs of the probe have appeared in the literature during the past 20 years[47]. In designing DO probes, the following requirements are generally considered:

the calibration has to be stable over a long period;
the current output of the probe has to be sufficiently large and linear with DO;
the effect of liquid flow on probe performance has to be small;
the response time has to be fast;
the measurement has to be independent of temperature change of the medium;
and the probe must withstand high pressure and repeated autoclaving.

In actual probe design, some of the above requirements are emphasized more than others depending on specific applications.

Over 20 companies in the U.S. offer different types of DO probe for different applications. Some of them are listed in Table 1. Fatt[47] gave a comprehensive review of various probe designs. Shown in Table 2 are comparisons of six representative probes of the original design. Details of construction methods, selection of electrode components and design considerations are given below.

Table 1. Some oxygen electrode manufacturers

Type	Maker	Address	Cathode	Anode	Remark
Polarographic	Beckman Instrument	Irvine, California	Pt	Ag	Laboratory and field use
	Delta Scientific	Lindenhurse, New York	Au	Ag	Field use
	Instrumentation Laboratory	Lexington, Massachusetts	Pt	Ag	Steam-sterilizable probes
	Orbisphere	York, Maine	Au	Ag	Low drift, low DO; laboratory and field use
	Yellow Springs Instrument	Yellow Springs, Ohio	Au	Ag	Laboratory and field use
Galvanic	Biomarine Industries	Malvern, Pennsylvania	Au	Pb	Hospital and industrial use
	Electronic Instrument Ltd.	Surrey, England	Ag	Pb	Mackereth type
	New Brunswick Scientific	Edison, New Jersey	Ag	Pb	Borkowski-Johnson type, steam-sterilizable
	Precision Scientific	Chicago, Illinois	Ag	Pb	Mancy type; field use
	Rexnord Instrument	Malvern, Pennsylvania	Pt	Pb	Low DO; boiler feedwater applications
Microprobe	Transidyne General	Ann Arbor, Michigan	Pt	Ag	Local oxygen measurement

Table 2. Comparison between different types of DO electrodes

Electrode type	Clark	Mancy	Mackereth	Borkowski-Johnson	Kimmich-Kruezer	Microelectrode
Principle	Polarographic	Galvanic	Galvanic	Galvanic	Polarographic	Polarographic
Electrode metals (cathode-anode)	Pt–Ag/AgCl	Ag–Pb	Ag–Pb	Ag–Pb	Pt–Ag/AgCl	Pt–Ag/AgCl
Cathode: shape	Disk	Disk	Tubing	Spiral, disk	Thin band ring	Half-spherical
size	2 mm dia.	6 mm dia.	3.6 cm dia. 5.8 cm long	6 mm dia.	3 μm width 1 mm dia. ring	0.2–1.0 μm dia.
Membrane (thickness)	Polyethylene 25 μm	Polyethylene 25 μm	Polyethylene 75 μm	Teflon, FEP 50 μm	Teflon, FEP 6 μm	Polystyrene 1 μm
Electrolyte	sat. KCl	1 M KOH	sat. K_2HPO_4	pH 3, acetate buffer	pH 8, phosphate buffer	0.2 M KCl
Current output: (25 °C), μA						
Air saturation	6	10	200	10	0.12	$3(10^{-4})$
Zero-oxygen	0.001–0.01	0.2	negligible	0.001	0.0005	$1(10^{-5})$
Response time (25 °C)	15 s (95%)	5.6 s (95%)	60 s (90%)	60 s (95%)	0.4 s (95%)	0.1–0.05 s (95%)
Stability	2% drift/day	No loss of sensitivity for 3 weeks	No change in calibration for 6 months	0.5% drift/day 1 year life	2% drift/day	1–2% drift/hour
Ref.	31, 131	105	104	21, 71	75	11, 86

4.1 Construction Methods

4.1.1 Clark-type Electrode

As shown in Fig. 8a, this probe is characterized by a flat disk type cathode and a pool of electrolyte in which a reference electrode (Ag/AgCl) is immersed. Although the size of the cathode, the membrane material and the electrolyte differ widely, this design is most popular in commercial DO probes for use in the laboratory or in the field.

Clark-type electrodes often show current drift during initial "break-in" periods. After that, the current output remains relatively stable although the response and the calibration may change with use[15, 67, 80, 88]. The probe malfunction is caused by AgCl deposition on the anode surface[88], a deposition of silver on the cathode[131], a depletion of Cl^- from the electrolyte, or a loose membrane. However, with proper cleanings of the electrode, membrane replacements, electrolyte replenishments and frequent calibrations, these probes can be used for a long period.

With a 25 μm Teflon membrane, 95% response time of 15–20 s is usually obtained. But these sensors show a response hysteresis: the response to an increased oxygen tension is faster than that to a decreased oxygen tension. This phenomenon is caused by the electrolyte acting as a reservoir of dissolved oxygen[80] and/or the accumulation and slow decomposition of hydrogen peroxide in the vicinity of the cathode[99].

Some of the well-designed Clark-type probes showed very stable calibration and extremely low residual current. Orbisphere polarographic probe[117], which uses gold as the cathode, showed a drift of less than 0.01 ppm during 60 days of continuous measurement. Orbisphere (York, Maine) also manufactures a polarographic probe capable of measuring parts per billion range of DO. The improved performance of the probe came from careful choice of materials, good mechanical design and the use of extremely stable amplifier circuit.

4.1.2 Mancy Electrode

The galvanic probe originally designed by Mancy et al.[106] is shown in Fig. 8b. The major difference from the Clark electrode is the elimination of the electrolyte chamber. Instead, a thin film of electrolyte is placed between the cathode and the membrane. This is probably the reason why this electrode did not show the response hysteresis. Due to a relatively large diameter (0.6 cm) cathode employed, a microammeter could be directly connected to the probe. However, the probe showed very high flow dependency of the output current: stirred/unstirred current ratio of 20 was reported[105].

An improved probe stability was reported compared with earlier polarographic probes but the useful probe life may be somewhat restricted because the available surface area of the anode is relatively small. The anode surface is gradually oxidized with use until the probe cease to function.

4.1.3 Mackereth Electrode

Noting that the earlier Clark electrode and the Mancy electrode lacked long term stability and produced small currents (on the order of μA), Mackereth[104] designed a

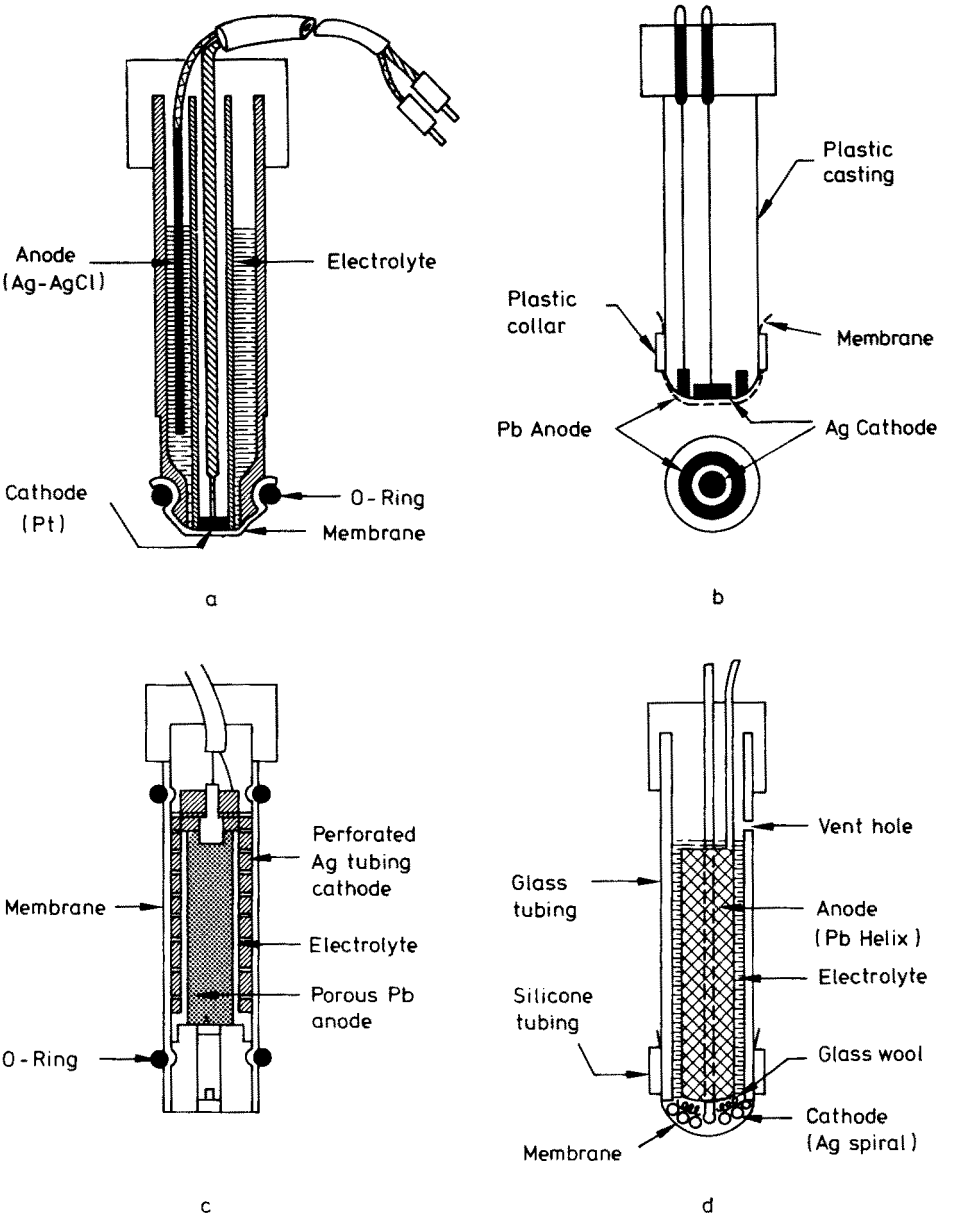

Fig. 8. Construction of various electrodes: (a) Clark[31], (b) Mancy[105], (c) Mackereth[104], (d) Borkowski-Johnson[21]

probe to solve these problems. In his design, a perforated silver tubing was used as the cathode and a massive shot of porous lead was used as the anode (Fig. 8c). The current output was much higher than those of other probes so that an ordinary current meter can be directly connected to the probe without amplification. The sensitivity was stable over many months of continuous operation. With a 25 μm Teflon FEP membrane, this probe gave a 90% response time of about 1 min. Harrison[61] reported hysteresis in the probe response due to the electrolyte solution acting as a reservoir for dissolved oxygen. This observation is similar to that of the Clark electrode described earlier.

Because of its long term stability, this probe has been used for monitoring the dissolved oxygen tension in continuous cultivation which lasts several weeks[61, 107, 126].

When vertically inserted in an air-sparged cylindrical vessel, the Mackereth probe exhibited a "gross" response free from any effects of air bubbles ascending through the vessel, whereas other probes with cathodes at the tip showed interference due to bubbles touching the cathode[3]. Since the cathode area is relatively large, a vigorous agitation of the liquid is required for a reliable operation and the oxygen consumption by the probe must be considered when using this probe for measuring oxygen in a small liquid volume. The probe may not be suitable for a viscous medium. The need for a tubing membrane makes it difficult to fabricate the probe in the laboratory.

4.1.4 Borkowski-Johnson Electrode

The original design was by Johnson et al.[71] but it was improved later by Borkowski and Johnson[21] for a longer life and a better stability. As shown in Fig. 8d the cathode is made from a silver spiral and a flattened lead wire forms the anode. A low pH acetate buffer is used as the electrolyte to prevent interference by dissolved CO_2. This probe has been used widely in biochemical engineering applications[42, 61, 120]. The probe can withstand repeated steam sterilizations, is capable of operating for several months and has a linear response from below 0.02 to 150 mmHg of oxygen[21].

With a 50 μm Teflon membrane, 90% response time of 1 min was reported[71]. The response time varies depending on the direction of step change in oxygen tension. The upstep response is faster than the downstep response and the latter depends on the length of time the probe was exposed prior to the downstep[79]. A vigorous agitation of the liquid (at least 60 cm s^{-1} for water) is required for reliable measurements[42]. The probe may not be suitable for viscous liquids unless a thicker membrane is used.

4.1.5 Electrodes with Low Flow Dependency and Fast Response

The requirements of the probe for low flow dependency and fast response time oppose each other: a thicker membrane, needed for low flow dependency of probe sensitivity, gives slow response time. One solution to this problem is to use cathode with small diameters. For example, a 12 μm diameter platinum cathode covered with 6 μm Teflon membrane gives 99% response time of 1.2 s and requires very low flow velocity for proper operation[131]. The problems are that the current output is very low (on the nA range) and often the stability of the probe is poor[73].

Another approach is to use various shapes of narrow band cathodes. Locally, the narrowness of a thin band allows the advantages of small cathodes regarding the flow

dependency and fast response, but since the total surface area of the cathode is relatively large, the probe gives large output current (on the μA range) and a better stability. Figure 9 shows various shapes of thin band cathodes. Usually, metal foils are imbedded in a casting epoxy and ground flat to produce the desired form. Figure 9 a shows a ribbon type cathode employed by a Beckman probe[13], which requires only 5 cm s^{-1} of water velocity with a 25 μm Teflon membrane, compared with 30–70 cm s^{-1} for other probes. Kimmich and Kreuzer[75] used a ring type cathode (Fig. 9 b) which showed a 95% response time of 0.4 s and a flow requirement of 5 cm s^{-1} in blood with a 6 μm Teflon membrane. Jones et al.[72] further increased the cathode surface by using an S-shaped cathode in their disposable galvanic sensor (Fig. 9 c) for use in deep water.

A multicathode approach was used with some degree of success[48, 137]. Fatt and St. Helen[48] used 18 cathodes, each 25 μm in diameter, in a single probe body which was able to measure oxygen tension in the range of 0–1 mmHg with a linearity better than 1%. Siu and Cobbold[137] described a multicathode Clark-type probe that is fabricated by using integrated circuit technique. They were able to lay down 161 gold cathodes each 7 μm diameter and spaced 60 μm apart to give a sensor with a diameter of about 0.6 mm. The probe showed extremely low residual current, 0.8% linearity in the range of 0–760 mm Hg of oxygen partial pressure, and very low flow dependency of probe sensitivity (flow increased the output 1% above the static value).

One of the *steam sterilizable probes* of Instrumentation Laboratory (Lexington, Massachusetts) employed a double membrane, which showed both low flow sensitivity and a reasonably fast response time. The inner, current-determining membrane was a 25 μm Teflon membrane and the outer membrane was a highly permeable, 150 μm silicone membrane[26]. This probe, which had a 250 μm diameter platinum cathode, showed only 2% change in current between an agitated and non-agitated solution, and 98% response in 50 s.

Since the condition of membrane-controlled diffusion is readily obtainable with probes in this category, they can be used in viscous liquids with better accuracy compared with other probes.

4.1.6 Oxygen Microelectrodes

The major considerations with these probes are: a high spacial resolution and a fast response time. When the cathode diameter becomes less than 1 μm, even a bare metal cathode (Fig. 10a) becomes insensitive to liquid flow and measures local oxygen tension[136]. The probe performance is improved by covering the cathode with a membrane (Fig. 10b)

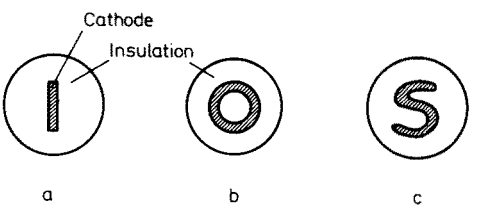

Fig. 9. Different shapes of cathode: (a) ribbon, (b) ring, (c) S-shape

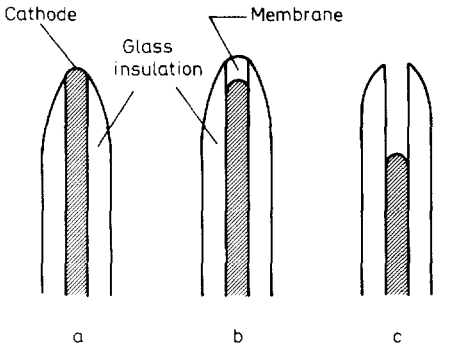

Fig. 10. Different shapes of microcathodes: (a) bare, (b) membrane-covered, (c) recessed (Lee[86])

or by extending the insulation (Fig. 10c) such that the diffusion gradient is confined inside the recess[148].

Details of construction of these probes were given elsewhere[18, 86, 134, 148]. Usually, a thick platinum wire is first etched in an electrolyte solution to a fine point of 0.2-1 μm diameter and then insulated with a thin layer of glass[86]. The membrane is applied by a dip coating. Polystyrene is most popular[136] but other materials have been used. The membrane coating has to be done carefully[129] to obtain optimum result.

Although the capability of local oxygen measurement is attractive, several problems exist with these microprobes: they are relatively difficult to make, requiring practice and experience; extremely fragile; unstable; and the useful probe life is short. Since the current output of the probe is extremely low (on the order of 10^{-10} to 10^{-11} A in air-saturated water), a special amplifier is required together with a careful grounding technique to prevent noise[86].

4.2 Electrode Metals

Sawyer and Interrante[127] studied the reduction of dissolved oxygen at Pt, Pd, Ag, Ni, Au, Pb, and other metal electrodes. They found that oxygen reduction at pre-oxidized metal electrodes is pH-dependent, whereas for pre-reduced metal electrodes, the reduction was pH-independent. They also showed that the reaction mechanism of oxygen reduction is complex due to an oxide film coating on metal surfaces except for Ag and Au. Evans and Lingane[44] reported that even the Au surface showed aging effects. Mancy et al.[106] preferred Ag for a better stability, but Ag is readily poisoned by sulfur impurities[64].

For polarographic probes, platinum[31], gold[76], silver[118], or rhodium[13] have been used as the cathode and Ag[118], Ag/AgCl[31], Ag/Ag_2O[30], or calomel[69] as the reference electrode. Gold is generally preferred to platinum as the cathode material, because it is less susceptible to poisoning by noxious gas, notably, H_2S[64]; the reaction at the cathode surface is less complicated[44, 127]; and surface aging is less pronounced[64]. However, gold may not be convenient for applications in steam-sterilizable probes and in microelectrodes since gold and glass cannot be fused together. Gold-plating method has been used in making microelectrodes to circumvent this problem[148].

The reference electrode has to maintain a stable reference voltage for a good performance of the polarographic probe. Also, it has to have a large surface area to avoid polarization[37]. Ag/AgCl is normally used as the reference electrode. However, Carrit and Kanwisher[30] preferred Ag/Ag_2O because it gave better stability for their probe. When the chloride containing electrolyte is used, the chloride ion concentration will fall as it is consumed by the anode reaction and will be replaced by OH^- generated by the oxygen reduction at the cathode. Consequently, Ag/AgCl will gradually change to Ag_2O, and since the reference voltage are different (+0.222 V for Ag/AgCl and +0.35 V for Ag/Ag_2O), this may cause a change in probe sensitivity unless the probe has a wide current-voltage plateau[37].

The *aging effect* of polarographic probes such as the change in calibration or unstable probe sensitivity is attributed to the deposition of silver ions on platinum[131], the oxidation of the catalytic surface[44] or an excessive deposition of AgCl on the reference electrode[88]. The cathode surface can be mechanically cleaned with soft scouring powders and a wet leather[131] or by tooth paste[47]. Excessive AgCl deposits can be removed by washing with 15% NH_4OH[88]. These procedures normally rejuvenate an aged probe. Kessler[73] reported unstable behavior of platinum microcathodes, but Barr et al.[9] showed that periodical anodization of Pt improved the stability.

For galvanic probes, silver as the cathode and lead as the anode are most common[24, 104, 106, 118] but silver-aluminum[3], platinum-aluminum[54], platinum-lead[43, 123], gold-zinc[70], and gold-lead[19] pairs have also been used. Although the galvanic probes suffer less from poisoning and survive autoclaving with greater reliability[142], the probe life is limited by the available surface area of the anode due to gradual oxidation of anode surface. The useful life-time of a given probe depends on the current drain just like an electrical battery. In other words, a probe can be used much longer when it is used for monitoring low, rather than high oxygen tension. Dead probes can be rejuvenated by dissolving away the oxide layer on the anode surface with 20% CH_3COOH[104] or 20% HCl[126].

4.3 Electrolytes

Since the electrode reaction occurs in the electrolyte solution, the composition, pH, and the volume the electrolyte are directly related with probe stability. For polarographic probes, the electrolyte takes part in the reaction (depletion of Cl^-: see Sect. 3.1), so a refill of electrolyte is necessary in regular intervals. In general, pH of the electrolyte does not affect oxygen reduction on a clean metal surface but an adverse effect was observed for an oxidized metal cathode[127]. The solubility of the electrode metals in the electrolyte solution has to be low for probe stability. Sometimes, the polarization voltage may change depending on electrolyte concentration[29]. In this case, the change in electrolyte concentration due to evaporation or diffusion of water through the membrane affects probe stability.

Table 3 shows various electrolyte solutions used by different investigators. For polarographic probes, KCl is the most common electrolyte, which gives a constant calibration well over 48 h[100]. Sometimes, CO_2 present in the medium, such as blood or cultivation media, permeates through the membrane and alters pH of the electrolyte solution. To eliminate this small CO_2 effect, Severinghaus[131] used 0.5 M $NaHCO_3$ + 0.1 M KCl,

Table 3. Various electrolytes used in DO probes

		Electrolyte	Ref.
Polarographic	Clark	sat. KCl	31
	Lübbers et al.	0.2 M KCl	101
	Severinghaus	0.5 M $NaHCO_3$ + 0.1 M KCl	131
	Carrit and Kanwisher	0.5 M KOH	30
	Kimmich and Kreuzer	Phosphate buffer ar pH 8–9	75
	Pittman	NH_4Cl	119
Galvanic	Mancy et al.	1 M KOH	105
	Mackereth	sat. $KHCO_3$	104
	Rowley	sat. $NaHCO_3$	126
	Parker and Clifton	10% by wt. K_2HPO_4	121
	Borkowski and Johnson	acetate buffer	21
	Brookman	1 M K_2HPO_4	24
	Evangelista et al.	50% by wt. KI	43
	Harrison and Melbourne	30% by wt. K_2CO_3 + 10% by wt. $KHCO_3$	59

which had pH of 9. Buffered phosphate[75] has also been used for the same purpose. Carrit and Kanwisher[30] used 0.5 M KOH in their probe ($Pt-Ag/Ag_2O$) because it gave a better stability compared with KCl electrolyte. Since the hydroxide is a reaction product formed at the cathode, the hydroxide added initially as the electrolyte is expected to make the electrode reaction to start under a condition which is similar to that obtainable after a long period of use. A problem with a strong alkaline electrolyte is the interference by CO_2[64]. Pittman[119] used NH_4Cl in his steam-sterilizable polarographic probe because KCl caused an early failure of the silicone tubing used as the membrane.

For galvanic probes, Mancy et al.[105] preferred 1 M KOH to KCl, because KCl gave high residual current and it did not maintain a clean anode surface. The solubility of Ag is reported to be high in KCl[24]. In the original Mackereth galvanic cell[104], saturated $KHCO_3$ was used, but a mixture of saturated K_2CO_3 and $KHCO_3$ was found to be better by others[59, 126] in preventing the effect of CO_2 on probe stability. When bicarbonate solution is used as the electrolyte, a whitish deposit of basic lead carbonate builds up on the anode, in comparison with KOH which keeps a clean anode. Borkowski and Johnson[21] employed 5 M acetic acid + 0.5 M sodium acetate + 0.1 M lead acetate for the electrolyte in their steam-sterilizable galvanic cell. This electrolyte has a low pH (pH of 3) and hence the calibration and the cell life are not affected by the CO_2 permeating through the membrane during the monitoring of DO in cultivation media[42].

Brookman[24] tested most of the electrolytes listed in Table 3 and concluded that only KCl and K_2HPO_4 gave a linear response up to a high oxygen tension and also were steam-sterilizable. Since KCl gave a high residual current, he recommended using 1 M K_2HPO_4 as the electrolyte. He obtained a linear response of the electrode to oxygen partial pressure from 1.5×10^{-2} to 10^3 mm Hg. Evangelista et al.[43] used 50% by weight KI for the electrolyte in their galvanic probe which could measure parts per billion range of dissolved oxygen. Sawyer and Interrante[127] showed that, although the oxygen re-

duction is independent of pH, it changes with iodide concentration. They reported that the reaction involved formation of PtI_2 film on the platinum surface.

A rather serious problem with the electrolyte is the *loss of solvent through the membrane* by diffusion or evaporation. Several methods have been used to minimize this effect. One method was by having a large electrolyte reservoir, which can effectively supply solvent to the electrolyte film where the solvent loss occurs. In this case, the path between the reservoir and the electrolyte film has to be as long as possible to minimize the residual current[83]. Another method is to have the electrolyte in the form of gel or paste. An added advantage is that the residual current becomes smaller because the oxygen permeability is normally lower in more viscous media. A third method is to add a small amount of deliquescent salt to the electrolyte. Hitchman[64] showed that the addition of 0.01 M KH_2PO_4, which is a deliquescent salt, to 2.33 M KCl electrolyte prevented loss of solvent and lengthened probe life considerably. When not in use, the probe has to be stored in a water-saturated atmosphere or in water to prevent evaporation loss of electrolyte solution.

A recent development has been the use of a *solid electrolyte* instead of the conventional liquid form. Niedrach and Stoddard[114] described a probe using ion exchange membrane as the electrolyte. Certainly, this would lead to a more rugged construction of the probe.

4.4 Membrane

An ideal membrane for use in DO probes has to have a relatively low oxygen permeability and a high oxygen diffusivity. The permeability has to be low to ensure membrane control of oxygen diffusion [Eq. (15)], whereas a high diffusivity gives fast probe response [Eq. (11)]. The membrane has to be mechanically strong and chemically inert. Since the current output is directly related to the thickness and the oxygen permeability of the membrane [Eq. (10)], the probe sensitivity is directly affected by change in membrane properties. Membrane swelling or change in the apparent oxygen permeability results in change of probe sensitivity. Other important factors are CO_2 permeability and water permeability. The water permeability of the membrane has to be low to prevent loss of water from the electrolyte solution, which causes an increase in electrolyte concentration and early failure of the probe. Low CO_2 permeability of the membrane is also desirable for probes to be used in aerobic cultures and in blood.

Teflon[71], polyethylene[31], and polypropylene[131] have been most popular as the membrane material but silicon[119], polystyrene[135], and mylar[41] have also been used. The properties of various membranes are shown in Table 4. It has to be noted that data on gas permeability and diffusivity vary widely depending on the method of measurement[68]. According to the data shown in Table 4, polypropylene is better than Teflon in several aspects: it has a lower oxygen permeability; a lower CO_2 permeability; and yet a higher oxygen diffusivity. Polypropylene was recommended by Severinghaus for physiological works[131] and by Kinsey and Bottomley for bioreactor applications[76]. However, Teflon seems to be more popular in steam-sterilizable probes due to its higher

Table 4. Properties of various membranes

Membrane	O_2		CO_2		H_2O	Heat Resistance °C
	P_m [a]	D_m [b]	P_m [a] –	P_m [a] (vapor)	Absorption (24 h, %)	
Teflon FEP	4.4	1.07	9.9	29	0.01	227–274
Polypropylene	1.2	1.62	3.9	51	0.005	132–149
Polyethylene						
low density	3.0	–	16.0	95	0.01	82–93
med. density	2.4	–	10.4	51	0.01	104
hi. density	1.1	–	3.4	22	Nil	121
Polystyrene	2.1	–	5.3	624	0.06	80–96
Mylar [c]						
(polyester)	0.05	–	0.09	148	0.8	300
Silicone [c]	480		2,530	16,900	–	–

[a] 10^{-10} cc $s^{-1} cm^{-1} (cm\ Hg)^{-1}$
[b] 10^{-7} cm^2 s^{-1}
[c] From Ref. 131
[d] From Ref. 2
All others from Ref. 110

heat resistance. An added advantage of the Teflon is its extremely low water permeability. Polystyrene is popular for microprobes because it sticks well on the glass insulation and has a relatively low oxygen permeability. Polystyrene and polyethylene are not suitable for steam-sterilizable probes because of low heat resistance.

For steam-sterilizable applications, changes in membrane properties with temperature must be reversible. Thin membranes (10–25 μm) have problems because they do not withstand large pressure differences, and the sterilization temperature (120 °C) causes an irreversible change in membrane thickness[26]. Borkowski and Johnson[21] used a 50 μm Teflon membrane, which could withstand about 20–30 times repeated steam sterilizations[141]. The steam-sterilizable probe of the Instrumentation Laboratory[26] employed a special double membrane to withstand sterilizations. The inner membrane was a 25 μm Teflon film and the outer membrane was 150 μm thick silicone which was reinforced by thin steel netting.

Figure 11 shows a plot of current output vs. the number of sterilizations for both types of probe. It is interesting to note that, while the current output of the polarographic probe (Instrumentation Laboratory probe) increased upon repeated sterilization, the current decreased for the galvanic probe (Borkowski-Johnson probe). Reduced membrane thickness is probably the reason for the current change of the polarographic probe, whereas consumption of the anode may be the main factor for the galvanic probe.

In bioreactor applications, *growth of microorganisms or soil coating* on the outer surface of the membrane is the most common cause for faulty measurements. The soil layer behaves as an additional membrane and the growth of microorganisms inhibit oxygen supply to the cathode. Frequent calibrations are required for contaminated probes. The contamination may be detected from the response of the probe since all the contaminated probes respond slowly[26].

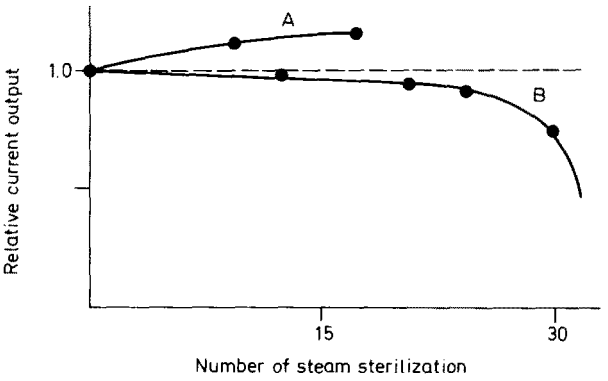

Fig. 11. Effect of steam-sterilization on probe current: polarographic probe (IL probe: Bühler and Ingold[26]) and galvanic probe (B–J probe: Tuffile and Pinho[141]). A: Instrumentation Laboratory probe B: Borkowski-Johnson probe

4.5 Instrumentation

Unless the current output is very large, some sort of current amplification is required for DO probes. Operational amplifiers which provide multi-range amplification and zero current suppression are normally used for this purpose. LaForce[85] described in detail the use of operational amplifiers for polarographic sensors. For an improved stability in current amplification, Orbisphere (York, Maine) employed chopper-stabilized amplifier in its DO meter[117]. Commercially available DO meters also incorporate temperature measurement and/or compensation circuits.

Often, existing current amplifiers, voltmeters or strip-chart recorders are directly connected to DO probes. For polarographic probes, a bias voltage of 0.6–0.8 V (depending on the type of electrode) has to be externally applied as shown in Fig. 5. The selection of a polarization voltage is discussed in Sect. 4.6. A mercury battery is preferred as the voltage source because the voltage discharge characteristics are superior to other batteries. For galvanic probes, usually a resistor is connected in series with the probe and the voltage drop across the resistor is monitored with a voltmeter or a potentiometric recorder.

For both the polarographic and the galvanic probes, the input impedance of the current measuring circuit has to be low so as not to affect the cell potential of the probe[142]. The value of series resistance is shown to affect the sensitivity and the response time of the galvanic probes[61, 79]. The galvanic electrode pair spontaneously generate a voltage of around 0.7 V which is sufficient for oxygen reduction at the cathode. When a resistor is connected in series with the probe (Fig. 12a), the oxygen current flows through the resistance and causes a voltage drop, V, across the resistor:

$$V = IR_L \ , \tag{34}$$

where I is the oxygen current and R_L is the combined resistance of the parallel resistor and the input impedance of the measuring device. When the value of R_L becomes large, the increased voltage drop will significantly affect the cell potential. This is equivalent to change of polarization voltage in polarographic probes. Evidently, the probe calibration and the response will be affected. This effect will be more pronounced for large cathodes because the current output is high. The load resistance, R_L, has to be selected so that the voltage drop V is negligible compared with the cell potential. The same principle applies to polarographic probes.

A current meter (or amplifier) with zero input impedance is ideal for DO probes but a reasonably low value of R_L is acceptable. For example, if R_L = 100 Ω, the Mancy probe shown in Table 2 will give a voltage drop across R_L of 1 mV under air-saturated condition, which is negligible compared with 0.7 V cell potential. The probe would not function if the load resistance, R_L, is increased to 100 kΩ because the voltage drop due to R_L becomes 1 V, which is even higher than 0.7 V. Accord-

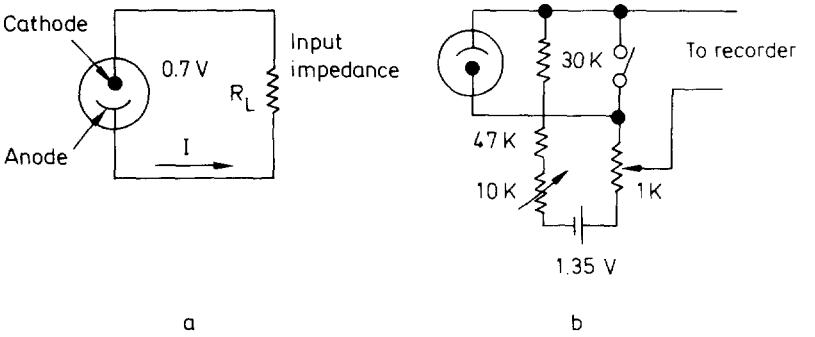

Fig. 12. (a) Equivalent circuit for oxygen electrode; (b) circuit used for measuring small changes in oxygen tension (Lipner et al.[97])

ing to Eq. (34), R_L may have high values when measuring low oxygen tension and when the cathode is small (since small cathodes produce low current).

Lipner et al.[97] showed that a simple bucking circuit shown in Fig. 12b could be used with the Mancy probe to measure very small changes in oxygen tension of a sample during enzyme reactions. Even if the residual current of the Mancy probe was relatively high, it could be effectively suppressed by an imposed opposing current.

4.6 General Design Considerations

One of the important considerations in DO probe design is to minimize the *residual current*, which is defined as the current output of the probe at zero oxygen level. Sometimes, it is called the background, dark, zero, offset or nitrogen current. Krebs and Haddad[83] listed four major sources which contribute to this residual current:

electrochemically active impurities in the electrolyte;
electrical leakage;
incorrect polarizing voltage;
and back diffusion of oxygen.

The effect of *reducible* or *oxidizable impurities* in the electrolyte is normally of short-term duration because they are scavanged by the cathode at the early stage of operation. Electrical leakage between the anode and the cathode through the insulating material is not normally significant for large cathode but becomes a problem for small cathodes. For example, the insulation requirement of the microelectrode shown in Table 2 has to be better than 7×10^{10} Ω to have a residual current of less than 1×10^{-11} A (resistance = polarizartion voltage/current). This is why glass is used as the insulating material for small cathodes. Epoxy resins, unless specially selected for their water resistance, often absorb water and increase current by forming an extraneous conducting path between the cathode and the anode.

The correct choice and the *stability of polarizing voltage* is important for stable performance of the probe. Figure 13 from Krebs and Haddad[83] shows three polarographic curves. If the polarizing voltage is chosen between points marked B and C and is main-

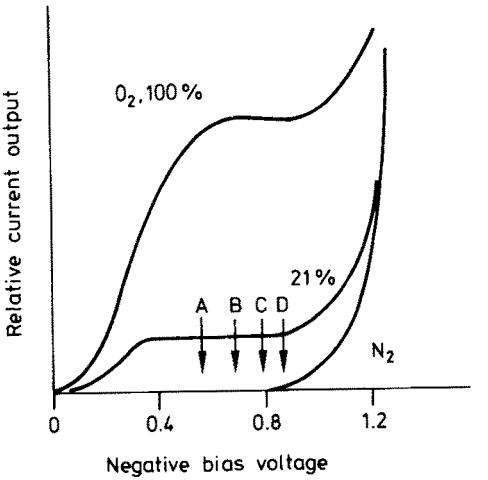

Fig. 13. Polarographic curves from a Pt-Ag/AgCl probe (Krebs and Haddad[83])

tained in this region, the probe current will depend only on the oxygen tension. However, when the polarizing voltage is larger than that indicated by point D, there will be residual current due to electrochemical reduction of water. If the polarizing voltage is less than that marked A, then the calibration of current vs oxygen tension will become non-linear. Since the shape of the polarographic curve changes depending on the electrode metal, the electrolyte concentration[29] and the size of the cathode[73], the optimum polarizing voltage has to be selected for each probe, separately. In general, large cathodes have wide and well-developed plateaus in their polarogram, whereas small cathodes have narrow and, often, very poorly-defined plateaus[73]. Probes with large cathodes, notably the galvanic probes designed to drive meters directly, may change their effective polarizing voltage if the combined resistance of the meter and the series resistor is relatively large. This was discussed in Sect. 4.5.

The fourth contributor to the residual current, namely, *back diffusion of oxygen* from the internal electrolyte is most pronounced for small cathodes. This is because the oxygen reduction due to back diffusion mainly occurs at the edge region of the cathode and, for small cathodes, the fraction of total area involved is relatively large. Mechanical designs which favor increased path length between the cathode and the electrolyte reservoir, as well as decreasing the thickness of the electrolyte film between the membrane and the cathode, minimize this effect[83]. Sometimes, oxygen dissolved in the plastic components of a probe diffuse back to the probe face when the probe is moved from an oxygen-containing environment to one free of oxygen[47]. Metal components for the sensor body reduce this type of back diffusion.

Mechanically well-designed probes with the above considerations in mind have very low residual current and are capable of measuring dissolved oxygen down to parts per billion (ppb) range. Orbisphere Model 2711 analyzer[117], which utilizes polarographic probe, has a 10 ppb full-scale range. Rexnord Model 3400 DO analyzer[123] employs a galvanic probe and has a 20 ppb full-scale range. Both probes are claimed to be extremely stable and rugged (for example, Orbisphere claims ±3% drift per year). These probe

can be applied in studying the effect of low dissolved oxygen tension on the metabolism of facultative microorganisms[61].

Galvanic probes have been preferred in bioreactor applications[3, 59, 141, 142] but the disadvantage is the expendable nature of the anode. Currently available designs of the steam-sterilizable galvanic probe could be improved considerably by further decreasing the cathode area, increasing the anode area and increasing the path between the electrolyte reservoir and the cathode. A well-designed polarographic probe was shown to withstand repeated steam-sterilization with a high reliability[83].

It would be fairly simple to fabricate working DO probes in the laboratory provided that proper design considerations are taken into account. The advantage of making the probe in the laboratory is that the probe can be designed to meet the requirements of the specific application.

5 Operation of Electrodes

5.1 Calibration

DO probes can be calibrated in three ways: % saturation, partial pressure of oxygen and actual concentration. Since the reliability of measurements depends on calibration, it is vital that the calibration be done as accurate as possible. In all calibrations, good temperature control (±0.1 °C) of the test medium is required because the probe sensitivity changes markedly with temperature as will be shown in Sect. 5.3.

5.1.1 Calibration Based on % Saturation

Gas-phase calibration is rapid and convenient. The probe output current in nitrogen is set at 0% saturation while that in air is set at 100%. Oxygen can be used instead of air. This method is simple but recommended only for a rough ealibration. Given the same oxygen partial pressure, the current output of the probe is lower in liquid phase compared with that in gas phase, because there exists a stagnant liquid film around the membrane even at high velocity. Equation (17) predicts such behavior.

For a better accuracy, the calibration has to be made directly in the liquid of interest. Air saturated liquid can be prepared by bubbling air through the solution at the desired temperature. A solution with zero oxygen content can be prepared either by stripping oxygen with nitrogen or by using chemicals such as sodium sulfite or sodium dithionite. These chemicals react rapidly with dissolved oxygen to provide anaerobic conditions. Addition of 2 volumes of saturated sodium sulfite to 100 volumes of liquid is sufficient for oxygen removal[37]. Dry powders can be directly added to a concentration of 0.01 M or higher for the same purpose[14].

5.1.2 Calibration Based on Partial Pressure

DO probes are known to measure the activity or the equivalent partial pressure of dissolved oxygen but not the actual concentration[106]. For example, the current output

from the oxygen probe is the same for air-saturated 1 M KCl and air-saturated pure water, whereas the actual oxygen concentration in 1 M KCl is only 73% of that in pure water. In practice, the current output changes slightly depending on different liquids but this tendency becomes negligible when the cathode area decreases[131]. The calibration based on % saturations can be directly converted to partial pressures when the barometric pressure is known.

When water is equilibrated with air at temperature T, the partial pressure of oxygen, pO_2, is expressed as follows[53]:

$$pO_2 = [pB - p(H_2O)] \times 0.2095 \quad , \tag{35}$$

where pB = temperature-corrected barometric pressure, $p(H_2O)$ = vapor pressure of water at a given temperature, 0.2095 = fraction of oxygen in atmospheric air.

The values of pB and $p(H_2O)$ can be obtained from Table 5. For example, when the barometric reading is 750 mmHg at 20 °C, the current output of the probe at 100% saturation corresponds to

$$pO_2 = [(750 - 2.44) - 17.515] \times 0.2095$$
$$= 152.9 \text{ mm Hg} \quad .$$

Note that this corrected value is slightly lower than that of the uncorrected one (750 x 0.2095 = 157.1 mm Hg).

Table 5. Temperature corrections for mercury barometer and vapor pressure of water (Gelder and Neville[53]) (All units in mm Hg)

Temp. °C	740 mm	750 mm	760 mm	770 mm	$p(H_2O)$
18	2.17	2.20	2.23	2.26	15.460
19	2.29	2.32	2.35	2.38	16.460
20	2.41	2.44	2.47	2.51	17.515
21	2.53	2.56	2.60	2.63	18.631
22	2.65	2.69	2.72	2.76	19.807
23	2.77	2.81	2.84	2.88	21.047
24	2.89	2.93	2.97	3.01	22.356
25	3.01	3.05	3.09	3.13	23.734
26	3.13	3.17	3.21	3.26	25.185
27	3.25	3.29	3.34	3.38	26.715

5.1.3 Calibration Based on Concentration

The calibration based on partial pressures can be convered to concentrations if the solubility of oxygen in the liquid is known. The solubility is often expressed as the Bunsen coefficient, α, which is defined as ml O_2 absorbed by 1 ml of solvent at 0 °C and 1 atm

pressure of O_2 [10]:

$$\alpha = \frac{V_g}{V_s} \frac{273.15}{T} , \tag{36}$$

where V_g, V_s, and T are volume of gas absorbed, volume of the absorbing solvent and absolute temperature, respectively. For a sparingly soluble gas such as oxygen, pressures can be converted to concentrations by using Henry's Law:

$$c = p_0/H , \tag{37}$$

where H is the Henry's law constant. The conversion of α to H is as follows[10]:

$$H = \frac{22{,}414(760)}{1000\alpha} . \tag{38}$$

The values of α for oxygen in water and other solvents are given in the literature[132]. The dissolved oxygen concentration changes markedly when the salt concentration in water increases. Such data are available in the literature[132] and a calculational method is also available[35]. However, a direct measurement of solubility is necessary when liquids of unknown composition are involved, or when the solubility data of the liquid are not available. The Winkler method[7] has been used widely for determining oxygen solubility in waters and wastewaters. Biochemists often use mitochondrial oxidation of NADH as the basis for DO probe calibration[14]. The oxidation of phenylhydrazine by ferricyanide was also used recently[109]. A DO probe method is given in Sect. 7.2.

In addition to these chemical methods, the mass spectrometric method, gas chromatographic method and manometric or volumetric method have also been used[10]. The manometric method of Van Slyke and Neill or that of Scholander[125] has been widely used for blood oxygen measurements and the advantage of this method for oxygen probe calibration is given by Elsworth[42]. Once the oxygen concentration in the solution is determined, then the probe output can be calibrated in terms of concentration. It has to be noted that the probe calibrated in one liquid cannot be used for measuring oxygen concentration in other liquids because oxygen solubility changes depending on the liquid composition.

5.1.4 Calibration for Long-Term Continuous Measurement

Probe calibration becomes a problem when it is necessary to monitor the dissolved oxygen continuously during a long-term cultivation. One problem is the drift of the probe sensitivity which occurs at approximately 2% per week[77]. Mclennan and Pirt[107] reported that after 5 weeks of continuous monitoring in cultivation media, the oxygen probe showed marked decrease in sensitivity and the calibration became non-linear at high dissolved oxygen tensions, although the residual current of the probe remained unchanged.

Recently, a DO probe capable of in situ calibration was introduced[81]. Kjaergaard[77] showed a simple method for recalibrating a DO probe during a long-term process by utilizing the known aeration capability of the bioreactor.

5.2 Response Time

The transient response of the DO probe becomes important when it is required to measure relatively fast concentration changes. Examples are: dynamic measurements of the volumetric oxygen transfer coefficient in bioreactors[8, 15, 40, 91]; oxygen respiration rate measurements in microbial suspensions[63]; concentration fluctuation measurements[87], low-level DO measurements[79], respiratory oxygen measurements[39, 51], etc. Because of these wide applications, the dynamic response of the DO probe has been studied extensively[15, 17, 57, 62, 80, 90–96, 124, 147].

The response time of the probe is the time required for the probe to reach a certain fraction of the steady-state current to a step change in oxygen concentration. The fractional response, Γ is experimentally determined as follows:

$$\Gamma = \frac{I_t - I_0}{I_s - I_0} \quad , \tag{39}$$

where I_0 is the current output at time zero. Frequently, 90 or 95% response time is quoted in the literature.

Theoretically, the response characteristics of the probe can be estimated by using an electrode model. For a single layer model shown in Sect. 3.2, the fractional response of the probe to a step change in oxygen tension is given as follows[15, 62, 91]:

$$\Gamma = 1 + 2 \sum_{n=1}^{\infty} (-1)^n \exp(-n^2 kt) \quad , \tag{40}$$

where k is the probe constant defined by Eq. (11). Linek rearranged Eq. (40) as follows:

$$\Gamma = 1 - 2 \exp(-kt) + 2 \sum_{n=2}^{\infty} (-1)^n \exp(-n^2 kt) \tag{41}$$

and showed that, for $\Gamma \geq 0.4$, the infinite series term can be neglected and k is the negative slope of a plot of $\ln(1-\Gamma)$ *vs.* t.

The factors affecting the response time of the probe can be estimated from Eqs. (11) and (40). A thinner membrane with a high oxygen diffusivity is expected to give a rapid response time. Evidently, varying d_m rather than D_m is more effective in obtaining a fast response. When more than one layer is involved in oxygen diffusion path, d_m in Eq. (11) has to be replaced with an effective membrane thickness $\bar{d}_t$ defined by Eq. (20). According to Eq. (20), a thick-liquid film with D_L comparable to D_m, is expected to slow down the probe response. In other words, the response time of the probe increases with decrease in liquid velocity (Fig. 14a) and with increase in liquid viscosity. The latter holds from the following relationship for Newtonian fluids[150].

$$\frac{D_1 \mu_1}{T_1} = \frac{D_2 \mu_2}{T_2} \quad , \tag{42}$$

where μ is viscosity and D is diffusivity.

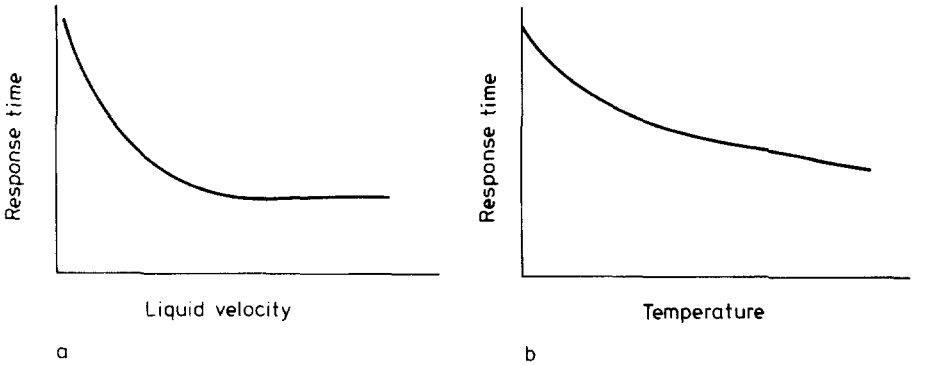

Fig. 14. Probe response time as a function of (a) liquid velocity and (b) temperature (Kimmich and Kreuzer[75])

The temperature also affects the response time since the oxygen diffusivity of the membrane, D_m, is a function of temperature[16]:

$$D_m = D_m^* \exp(-E_D/RT) \quad , \tag{43}$$

where D_m^*, E_D, R, and T are diffusivity at base temperature, activation energy for diffusion, gas constant and absolute temperature, respectively. Equation (43) shows that the diffusivity increases with increase in temperature. Schuler and Kreuzer[128] showed that 100 μm diameter cathode with a 12 μm Teflon membrane gave 95% response time of 2 s at 20 °C, but the response time decreased to 1.3 s at 50 °C (Fig. 14b). Severinghaus[130] operated a probe at an elevated temperature to obtain a fast response.

Often, DO probes show slowdown in the last 20% of the response to a step change in oxygen tension[15, 80, 93] and a marked difference in response time between the upstep response and the downstep response (Fig. 15). This phenomenon was observed for the Mackereth probe[61], Yellow Springs Instrument probe[15], Borkowski-Johnson probe[93], and other probes[80, 123]. These probes commonly employed a large pool of electrolyte very close to the cathode. The tailing of response was explained in terms of lateral diffusion of oxygen from the electrolyte reservoir[80]. Probes of other de-

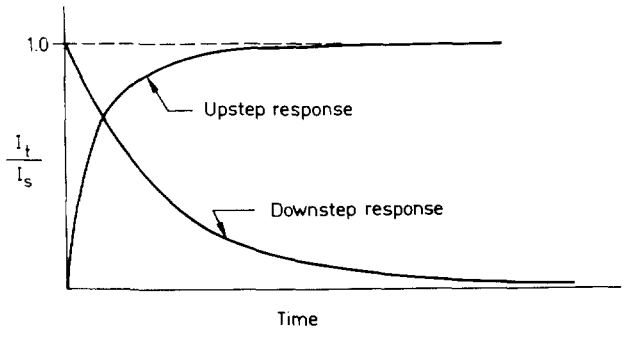

Fig. 15. Response hysteresis of oxygen electrodes (Harrison[61])

signs[93, 106] did not show such behavior. These probes either had small amounts of electrolyte or the electrolyte reservoir was located far from the cathode.

Several authors presented electrode models which take into account of the lateral diffusion[69, 80]. Linek and Benes[93] used a multi-layer, multi-region model for describing the tailing response but stated that such probes are not convenient because more than three parameters are needed for describing the dynamic behavior of the probe.

With a 25 μm Teflon or polypropylene membrane, 95% response time of 15 s is usually obtained in water at room temperature (Table 2). Depending on the probe design, the response time can be further decreased without affecting the condition of membrane-controlled diffusion: for example, the ring-shaped cathode of Kimmich and Kreuzer[75] had a 95% response time of only 0.4 s. Microprobes have very fast response time (0.1 s for 95% response) due to a very thin membrane. Davies[37] showed that 100% response time of the DO probe can be as fast as 0.5 ms. Slow response can be made faster or compensated for electronically[22, 84].

When using a DO probe for measuring unsteady concentration change, it is important to check the probe response before and after use to prevent measurement errors. The response check has to be made in the same liquid as the test medium under similar hydrodynamic conditions. This is also a good way of checking bad or aged probes.

5.3 Effect of Temperature

A number of authors[21, 104, 106, 131] reported 1-5% increase in probe sensitivity per °C for various DO probes. Evidently, the measurement error would be large if the temperature of the medium is not well controlled. In addition to the temperature coefficient of the probe, the oxygen solubility changes with temperature. For example, in water, the oxygen solubility changes approximately 2%/°C at 25 °C. If the temperature coefficient of the probe is assumed 3%/°C, the combined error in calculating oxygen concentration will be greater than 5%/°C in the worst case. Therefore, good temperature control of the medium (±0.1 °C or better) is essential for accurate measurements.

The effect of temperature on probe sensitivity can be estimated by using the Arrhenius relationship for the diffusivity, D_m [Eq. (43)] and the solubility, S_m, of the membrane[16]:

$$S_m = S_m^* \exp(-\Delta H/RT) \quad , \tag{44}$$

where ΔH is the heat of solution. Since the permeability, P_m is a product of solubility and diffusivity, Eqs. (43) and (44) are combined as follows:

$$P_m = P_m^* \exp(-E/RT) \quad , \tag{45}$$

where

$$E = \Delta H + E_D \quad .$$

The steady-state current output of the probe given by Eq. (10) can be rewritten as follows:

$$I_T = NFA \frac{p_o}{d_m} P_m^* \exp(-E/RT) \quad . \tag{46}$$

The activation energy, E, was 8.8 kcal (g-mole)$^{-1}$ for polyethylene[16] and 7.8–9.6 kcal (g-mole)$^{-1}$ for polypropylene membranes[91].

Under a given oxygen partial pressure, Eq. (46) can be rearranged as follows:

$$I_T = A_1 \exp(-A_2/T) \quad , \tag{47}$$

where

$$A_1 = NFA(P_m^*/d_m)p_0 \quad ,$$

and

$$A_2 = E/R \quad .$$

Thus, when the logarithm of the current output is plotted against T^{-1}, a straight line with a slope of $-A_2$ is obtained. According to Eq. (47), the sensitivity increases with increase in temperature but the trend is not linear but exponential. Therefore, the practice of giving temperature coefficients of sensitivity per °C is basically incorrect, although it may be applicable for a narrow temperature range[16]. In Eq. (47), A_1 is a function of cathode area, membrane thickness and membrane permeability whereas A_2 depends only on the property of the membrane.

Temperature compensation of the probe sensitivity can be accomplished by several methods. The simplest and most accurate method is the use of calibration curves. The dissolved oxygen level can be directly read from the calibration curve corresponding to the temperature of the medium. This method is somewhat cumbersome and may not be applicable for continuous measurements.

Another method is an automatic compensation using a thermistor. The resistance of a thermistor R_T is given by the following equation[23]:

$$R_T = B_1 \exp(B_2/T) \quad , \tag{48}$$

where B_1 and B_2 are constants for a given thermistor. If a thermistor is used as the cell load resistance and the voltage developed across it is measured using a voltmeter or a recorder with a very high input impedence (Fig. 16a), then the voltage measured, V_T, is:

$$V_T = I_T R_T \quad . \tag{49}$$

From Eqs. (47)-(49), the voltage V_T, which is a measure of oxygen tension becomes as follows:

$$V_T = A_1 B_1 \exp(B_2 - A_2)/T \quad . \tag{50}$$

If $B_2 = A_2$, then the measured voltage is independent of T. Thus, the temperature dependency of the probe sensitivity can be completely compensated.

In practice, the selection of a thermistor poses some problem because R_T is usually high: on the order of 5-500 K. A high value of R_T not only requires a recorder with very high input impedence but also adversely affects probe performance as described in Sect. 4.5. Morisi and Gualandi[112] circumvented this problem by using a compensating voltage source in the circuit (Fig. 16b), which resulted in a temperature compensa-

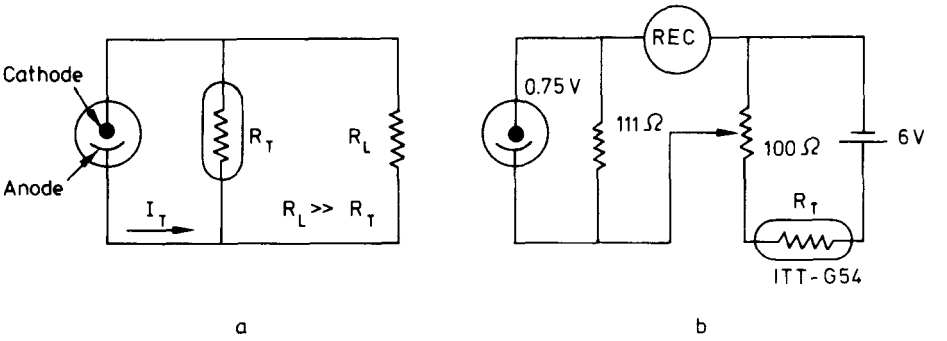

Fig. 16. (a) Principle of temperature compensation, (b) temperature compensation circuit (Morrisi and Gualandi[112])

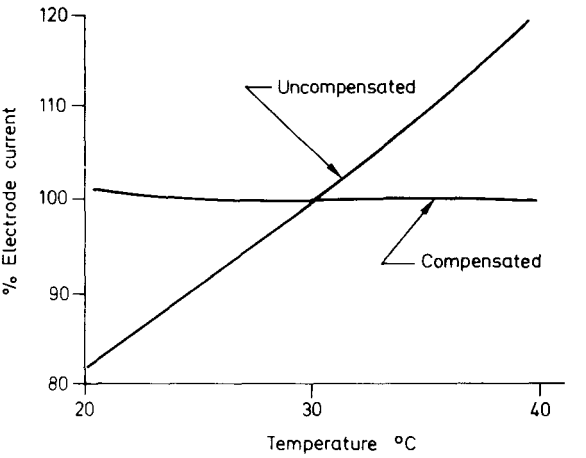

Fig. 17. Result of temperature compensation (Berkenbosch[17])

tion of 0.1%/°C in the range of 17-26 °C. Others[16, 23] gave temperature compensation circuits for polarographic probes. Figure 17 shows a result of temperature compensation.

It has to be noted, however, that the probe response characteristics change as a result of temperature compensation. An over-compensation occurs during the initial stage of transient response because the thermistor responds much faster than the probe[112].

A method commonly employed for temperature compensation in commercial units involves changing the degree of current amplification by using a thermistor in the feed back circuit of the amplifier[142]. Often, a separate knob is available for manual temperature compensation. Most of the commercially available probes incorporate a thermistor in the probe body for temperature measurement and compensation.

5.4 Effect of Liquid Film

The DO probe, when placed in a stagnant liquid, produces a diffusion gradient extending outside the membrane and farther into the liquid. The size of the steady-state diffusion field is proportional to the size of the cathode. When the liquid is stirred, the diffusion gradient can no longer be extended beyond the liquid film around the membrane. Since the diffusion gradient becomes steeper with decreasing liquid film thickness, the current output of the probe increases with liquid velocity (Fig. 18b). This so-called "flow dependency" of the probe sensitivity is higher for a probe with a larger cathode diameter (Fig. 18a) because the size of the stagnant diffusion field is proportionally larger than that of a small cathode.

For proper operation of the probe, the liquid has to be stirred beyond a certain level (v_c in Fig. 18a) in order to maintain membrane-control of oxygen diffusion. The critical velocity, v_c, of the liquid is the velocity where the probe output reaches 95% (95-99% depending on the definition) of the steady value. Of course, v_c depends on liquid viscosity but, given a liquid, v_c is smaller for smaller cathodes. For example, with a 25 μm Teflon membrane, a cathode of 5 mm diameter required v_c of 70 cm s^{-1} in water[3], whereas only 5 cm s^{-1} was required for 25 μm diameter cathodes[45]. Nomograms for assessing the critical velocity corresponding to different size cathode are given by several authors[3, 128]. When the cathode diameter is less than 1 μm, the probe becomes insensitive to liquid flow even without the membrane[136]. In this case, the diffusion field of the cathode is so small that it is always contained inside the minimum liquid boundary layer around the cathode. This is why such probes are capable of measuring local concentration rapidly with a high spacial resolution.

Theoretically, the effect of liquid film on probe performance can be estimated from Eq. (17). Given the same oxygen partial pressure, the current output in the gas phase, I_G and that in the liquid

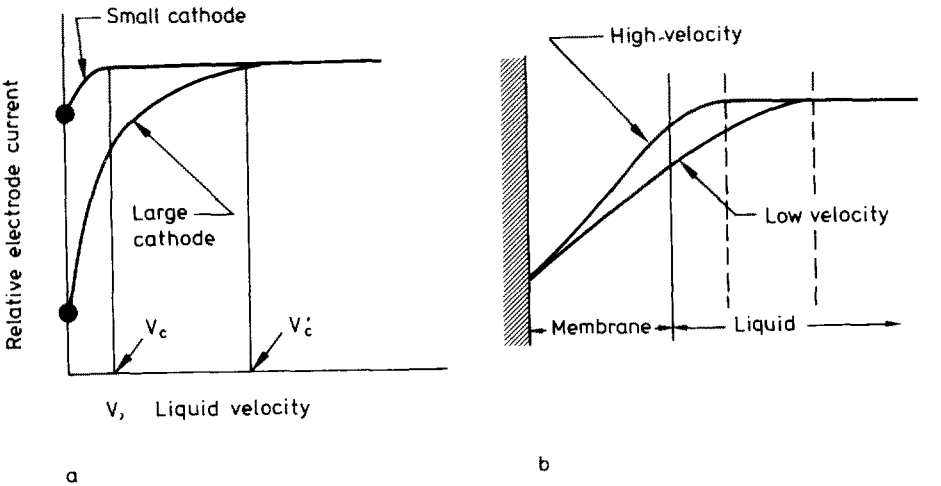

Fig. 18. Flow sensitivity of DO electrodes (a) effect of cathode size; (b) effect of liquid velocity

phase, I_L can be expressed as follows:

$$I_G = NFAP_m\left(\frac{1}{d'}\right)p_o \ , \tag{51}$$

$$I_L = NFAP_m\left(\frac{1}{d' + \frac{P_m}{P_L}d_L}\right)p_o \ , \tag{52}$$

where

$$d' = d_m + \frac{P_m}{P_e}d_e \ . \tag{53}$$

Thus,

$$\frac{I_L}{I_G} = \frac{1}{1+L} \ , \tag{54}$$

where,

$$L = \frac{P_m/d'}{P_L/d_L} \ . \tag{55}$$

The significance of the liquid film resistance can be determined by a single parameter L defined by Eq. (55), which is the ratio of mass transfer coefficient of the liquid film and that of the membrane (including electrolyte layer). L can be determined experimentally from the measured values of I_G and I_L:

$$L = (I_G/I_L)_{measured} - 1 \ . \tag{56}$$

Equations (54) and (55) show that the liquid film resistance becomes significant in viscous liquids since the oxygen permeability generally decreases with increasing viscosity.

Often there are situations where sufficient velocity of the medium cannot be provided under actual measurement conditions. Examples are *in vivo* blood oxygen measurements and DO measurements in deep waters such as lakes and rivers. When measuring the oxygen tension inside the microbial slime layers[28] and in tissues[136], the medium cannot be stirred at all. Microprobes have been used for the latter applications. Probes for use in deep water bodies employ small cathodes and thick membranes, and sometimes are equipped with stirrers.

Linek and Vacek[92] presented an electrode model incorporating the liquid film resistance. Dang et al.[36] introduced a simplified method of using the probe in very viscous media. This method is given in Sect. 7.1.

5.5 Handling, Maintenance, and Other Practical Considerations

Proper care and precaution are required in order to use a probe successfully. In general, the manual from the probe manufacturer provides adequate information. Some of the important points are given here.

When *refilling* the electrolyte solution, care has to be taken not to include air bubbles inside the electrolyte chamber, since the trapped bubbles interfere with the measurement. The membrane has to be tightly and securely fit over the cathode so that the electrolyte layer between the membrane and the cathode is minimized and kept constant. As shown by the electrode model in Sect. 3, the membrane and the electrolyte layer are directly related to the probe performance.

The *aging of the probe* can be detected by checking the probe sensitivity frequently. An excessive increase or decrease in current at a given oxygen tension indicates aging. A more convenient way of checking is by the transient response of the probe. Aged probes almost always show slower response. The loss of linearity in calibration and unstable performance are symptoms of aged probes. Aged probes can be rejuvenated by refilling the electrolyte and by cleaning the anode and cathode. The cleaning procedures are given in Sect. 4.2.

Special care has to be exercised in *handling steam sterilizable probes.* The most serious problem is the loss of electrolyte during the cooling cycle of autoclaving. If part of the electrolyte is lost so that the lead anode protrudes above the surface, then, after a few weeks, the anode breaks at the electrolyte surface. It is essential to maintain a slow cooling rate or to pressurize the autoclave above the atmospheric pressure during the cooling cycle in order to prevent the loss of electrolyte by evaporation or boiling[42]. The probe has to be "conditioned" before starting measurements. The purpose of conditioning a probe is to de-oxygenate the electrolyte so that the current generated in the external circuit is a measure of oxygen entering through the membrane. Autoclaving usually conditions the probe but the anode and the cathode have to be shorted during and after steam sterilization to help remove and maintain oxygen-free condition in the electrolyte. Although a probe can normally withstand 15–30 times of autoclaving[26, 42, 141], it was recommended to change the electrolyte after 5 or 6 sterilizations[120]. The above discussions mainly apply to galvanic probes but similar precautions are necessary for polarographic probes.

The position of a DO probe in an air-sparged stirred tank or a bubble column affects measurement accuracy. Evidently, the probe has to be located at a position where the local liquid velocity is above v_c defined in Sect. 5.4. However, locating the probe close to the agitator creates the problem of direct contact between the bubbles and the probe membrane. The readings may be distorted unless the oxygen concentration in bubbles is in equilibrium with liquid. Linek and Vacek[94] have shown that the probe reading varies depending on probe location in the tank. When the probe is used in a large bioreactor, placing the probe in stagnant water regions has to be avoided, not only because of the large liquid film effect, but because in these ranges microbial films are formed on the membrane surface, especially in highly viscous media. Linek and Vacek[94] recommended locating the probe in a bypass loop connected to the stirred tank, to avoid bubbles contacting with the membrane. The probe may be inserted from the bottom[91] or side of the vessel so that the probe membrane faces upwards. Sometimes, putting a suitable shield around the tip of the probe effectively prevents direct contact of bubbles with the membrane. Although the optimum location of the probe has to be determined case by case, the above guidelines have to be taken into consideration.

6 Sources of Error in Measurements

6.1 Errors due to Probe Characteristics

Improper calibration of probe may cause measurement errors. For polarographic probes, LeFevre et al.[89] showed that even a newly cleaned electrode (with 600-grade emery paper and ammonium hydroxide) showed a slight change in calibration after several hours. When the electrode is used for a long time without cleaning, the calibration changes rapidly with time and often becomes non-linear and unpredictable: sometimes the sensitivity increases[88] but sometimes it decreases[89]. Although the galvanic probe is generally more stable than the polarographic type, the calibration changes eventually[61, 107]. Calibrating the probe before and after use is recommended.

The calibration has to be made in the liquid where the actual measurements are to be made. Severinghaus[131] showed that the probe sensitivity decreased as much as 16% in 50% glycerine solution compared with that in water. Even small cathodes with thick membranes show changes in calibration in different media. For example, a Clark-type sensor with a 20 μm diameter cathode and a 20 μm polypropylene membrane, exhibited a 2-6% change in calibration between gas and blood[1]. This trend will be more significant with larger cathodes. When the liquid changes its property during measurement such as in batch cultivation, the calibration change has to be taken into account, especially when there is a marked difference in liquid viscosity between the initial and the final medium.

The temperature of the medium has to be controlled within ±0.1 °C both for the calibration and the actual measurement in order to achieve maximum accuracy. In calculating the actual dissolved oxygen concentration, two types of errors are involved: one is the temperature dependency of the probe sensitivity and the other is the change in oxygen solubility with temperature. With a poor temperature control, for example, ±0.5 °C, the measurement error could be as high as 10%[130]. Probes with automatic temperature compensation would be better for steady-state measurements but the transient behavior of the probe may not be good if the time response of the probe and that of the compensating circuit are not well matched. As mentioned earlier, over-compensation occurs for short times when a thermistor is used as a compensator[112]. When a different thickness membrane is used, the temperature coefficient of the probe has to be reestablished. The temperature compensation set for one membrane thickness may not hold for membranes of different thickness[91].

Liquid hydrodynamics is also a source of error in DO measurements. A thick liquid film around the membrane decreases the probe sensitivity and increases the response time of the probe. Linek and Vacek[92] showed that substantial error could be introduced in measuring the probe constant if the liquid film resistance is neglected. Both the calibration and the actual measurement have to be made under similar hydrodynamic conditions to avoid possible measurement errors. Whenever possible, the stirring speed or the liquid velocity has to exceed the minimum value beyond which the probe sensitivity is not affected. Methods of obtaining the probe constant in the presence of liquid film were given by Linek and Vacek[92] and Dang et al.[36].

As shown in Sect. 4.6, the correct selection and the stability of the polarization voltage are essential for good performance of the probe. The polarization or bias voltage has to be maintained constant. This is especially true when the polarogram has a narrow plateau. When the plateau is not well defined, which is the case for very small cathodes, a slight change in polarization voltage causes a considerable change in calibration. Carey and Teal[29] showed that the shape of the polarogram changed depending on the electrolyte concentration, membrane material and membrane thickness. The loss of water from the electrolyte, the aging of the probe and the change in membrane property due to repeated autoclaving are likely to result in polarization voltage change and thus the instability of the probe. Sometimes, the polarization voltage is changed due to the parallel resistor used for measurement, especially when a large cathode is involved. As outlined in Sect. 4.5, the resistor or the input impedence of the amplifier has to be selected not to affect polarization voltage.

6.2 Errors due to Measurement Medium

When the probe is used in *polluted environments,* the cathode surface can be poisoned and this results in the deterioration of probe performance. Sulfurous gases such as H_2S, SO_2, and thio-organic materials are reported to be poisonous to cathodes[64]: Ag is most sensitive and less so with Pt and Au. Pretreating the sample solution before using the probe is one solution. Another approach is to remove H_2S by a special membrane. If a lense tissue soaked in cadmium nitrite is sandwiched between the membrane and the cathode, H_2S can be effectively removed[64]. Other gases that interfere with dissolved oxygen measurement include Cl_2, Br_2, I_2, and oxides of nitrogen.

When oxygen is consumed by reaction in the liquid film around the DO probe, measurement errors occur depending on reaction kinetics, bulk oxygen concentration and the thickness of the liquid film. Lundsgaard et al.[103] studied this reaction effect by employing a steady-state mass balance inside the liquid film. They showed that, for a zeroth order reaction, the oxygen tension at the membrane surface, p'_m is given as follows:

$$p'_m = \frac{p_o - \frac{1}{2}(k_o/P_L)\, d_L^2}{1 + (P_m/P_L)(d_L/d')} , \tag{57}$$

where k_0 is the zeroth-order rate constant. When no reaction occurs in the liquid film, p_m becomes:

$$p_m = \frac{p_o}{1 + (P_M/P_L)(d_L/d')} . \tag{58}$$

Equation (57) shows that if there is a reaction inside the liquid film, the partial pressure at the membrane surface will become lower than that without reaction [Eq. (58)]. The

relative error can be found by dividing Eq. (57) by Eq. (58):

$$\frac{p'_m}{p_m} = 1 - \frac{1\, d_L^2\, k_0}{2\, P_L\, p_0} \quad . \tag{59}$$

For fixed values of liquid film thickness, d_L, and liquid oxygen permeability, P_L, the percentage error will become high at low p_0 and at high k_0. Given $k_0 = 20\ \mu M\ s^{-1}$, $d_L = 30\ \mu m$[103], and $P_L = 7.075\,(10^{-7})\ ccO_2\ cm^{-1}s^{-1}$ at 760 mm Hg[128], the oxygen partial pressure of the bulk liquid has to exceed 6 mm Hg in order to have an error less than 5%. This corresponds to approximately 4% saturation of oxygen at room temperature. Lundsgaard et al.[103] calculated errors for Michaelis-Menten kinetics and concluded that the oxygen probe can not work in dense microbial cultures with K_M (Michaelis-Menten constant) lower than 1 μM, when bulk concentration is in the order of K_M.

When an oxygen microelectrode is used for measuring the oxygen concentration gradient inside the *microbial slime layers*[28] and the stagnant liquid layers[86], the following questions arise: "How local is this measurement?" and "Does the oxygen consumption by the probe disturb the existing concentration gradient?". According to Grünewald[58], the microprobe measures local concentration without affecting the existing concentration gradient outside the membrane only when the diffusion gradient due to cathode reaction is entirely confined inside the membrane (Fig. 19a).

When the diffusion gradient of the cathode extends outside the membrane, the existing concentration gradient in the medium changes. In this case, the probe measures the concentration not at the surface of the membrane but at some distance away from

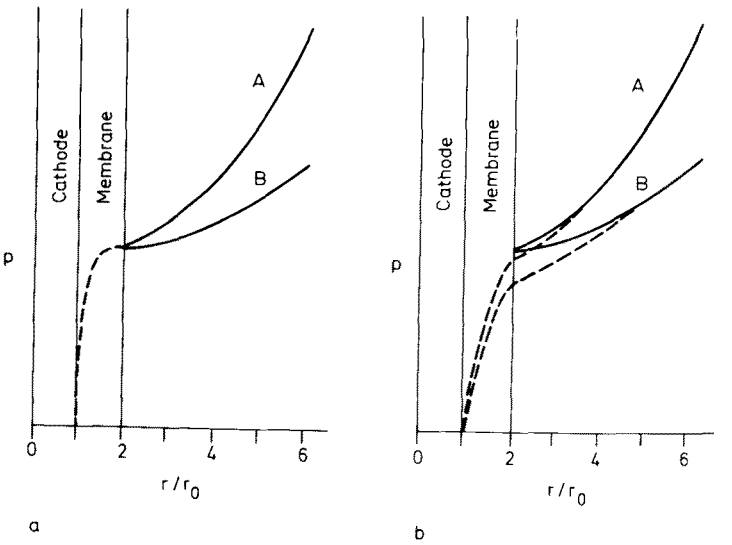

Fig. 19. Spacial resolution of microelectrode: (a) probe with membrane-controlled diffusion (b) measurement error due to loss of membrane-controlled diffusion (Grünewald[58])

the membrane. As shown in Fig. 19b, the measurement error becomes smaller when there is a steeper gradient (A in Fig. 19b) outside the membrane. A steeper gradient means a large flux of mass and the fraction consumed by the probe will become smaller compared with that of a less steep gradient (B in Fig. 19b). Therefore, the maximum error will occur when there is no oxygen concentration gradient outside the membrane. This case was dealt with in Sect. 3.3. The condition for an accurate measurement of local concentration was given by Eq. (31):

$$\frac{P_0\, d_m}{P_m\, r_0} \geqslant 99 \ .$$

When $P_0 = P_m$ (this is the worst case), or in media with very low oxygen permeability, the cathode diameter has to be less than 0.02 μm in order to measure oxygen tension at 1 μm in front of the cathode. The situation becomes better when P_0 is considerably larger than P_m. When a polystyrene-covered microcathode is used in water at 25 °C, the cathode diameter can be increased to 0.4 μm in order to have the same spacial resolution[86]. Silver[135] showed experimentally that the spacial resolution of microelectrodes was better than those predicted by mathematical models[6, 58]. An excellent guideline for selection and calibration of microelectrodes was given by Silver[135].

7 Applications

7.1 Measurement of k_La and Respiration Rate

Since the introduction of dynamic measurement technique by Bandyopadhay and Humphrey[8], DO probes have been widely applied in measuring the aeration capacity of bioreactors and wastewater treatment units[15, 36, 62, 63, 90–96, 115, 124, 147]. The aeration capacity of a given vessel is characterized by the *volumetric mass transfer coefficient*, k_La, which is the most important parameter in scale-up of aeration devices. Although other methods[40] have been used in measuring k_La, the DO probe method is simpler and more convenient. However, some precautions are required in applying this method as will be discussed below.

The method involves measuring the DO concentration in the liquid phase to a step change in gas concentration. The dissolved oxygen is first removed, usually by sparging with nitrogen, and the change in DO is monitored with the probe after the resumption of oxygen supply. In this case, the oxygen mass balance in the liquid phase gives the following expression:

$$\frac{dc}{dt} = k_La(c^* - c) \ , \tag{60}$$

where c^* is the saturation value of DO at the gas-liquid interface and c is the concentration in the liquid bulk. If the DO probe can follow the concentration change instantaneously, then k_La is simply the negative slope of a plot, $\ln(c^* - c)$ *vs* t. But, since the probe has a finite response time, the above method leads to large errors.

Recently, a number of authors[15, 62, 90, 147] used electrode models for evaluating k_La. The mathematical treatment was essentially the same as that for obtaining the step response of the probe [Eq. (40)] except that an exponential rather than a step change in concentration was used as the boundary condition. The normalized probe response to the resumption of aeration was derived as follows[90]:

$$\Gamma = 1 - \frac{\pi\sqrt{B}}{\sin(\pi\sqrt{B})}\exp(-k_La t) - 2\sum_{n=1}^{\infty}(-1)^n\frac{\exp(-n^2kt)}{n^2/B-1}, \quad (61)$$

where

$$B = (k_La)/k ,$$

and k is the probe constant defined by Eq. (11).

The value of k_La can be obtained from the experimental data by the nonlinear least squares fitting method[62, 147]. Linek[90] showed a simplified method of obtaining k_La: the infinite series terms in Eq. (61) becomes negligible beyond a certain value of kt, and k_La can be determined from the slope of a plot, $\ln(1-\Gamma)$ vs time. In estimating k_La, Wernau and Wilke[147] used a generalized graph prepared from the probe response equation. Their method required only the slope at the inflection point of a plot, Γ vs t.

The above methods are applicable when the liquid film resistance around the probe membrane is negligible. However, the methods lead to errors when there is a large film resistance. Such is the case when the liquid velocity around the probe is insufficient or when the liquid is viscous. Linek and Vacek[92] incorporated the liquid film resistance in the electrode model and presented a probe response equation for calculating k_La. The parameter L defined by Eq. (55) was used to represent the liquid film effect. Linek and Benes[93] showed that probes exhibiting tailing response were inconvenient for k_La measurement since they require additional parameters for describing the probe dynamics. The mathematical expression for the probe response became progressively complicated with increase in the number of parameters.

The methods of evaluating k_La based on electrode models are somewhat complicated mathematically and often require numerical calculations by the computer. A simpler method was introduced recently[36, 115] which enabled estimating k_La directly from the probe response curves. Nikolaev et al.[115] showed that the area between the normalized step response curve and the normalized aeration response curve (Fig. 20) is equal to $1/k_La$:

$$\int_0^{\infty}(1-\Gamma)_{\text{aeration}}\,dt - \int_0^{\infty}(1-\Gamma)_{\text{step}}\,dt = \frac{1}{k_La} . \quad (62)$$

They reported a good agreement between this method and the Winkler method. This type of parameter estimation is called the "moment method". Linek and Benes[96] showed that this method gave correct estimation of k_La even with probes showing tailing response. The other advantage is that the method can be used in viscous media because the liquid film effect is cancelled out in estimating k_La when both the step response and the aeration response are obtained under the same hydrodynamic conditions.

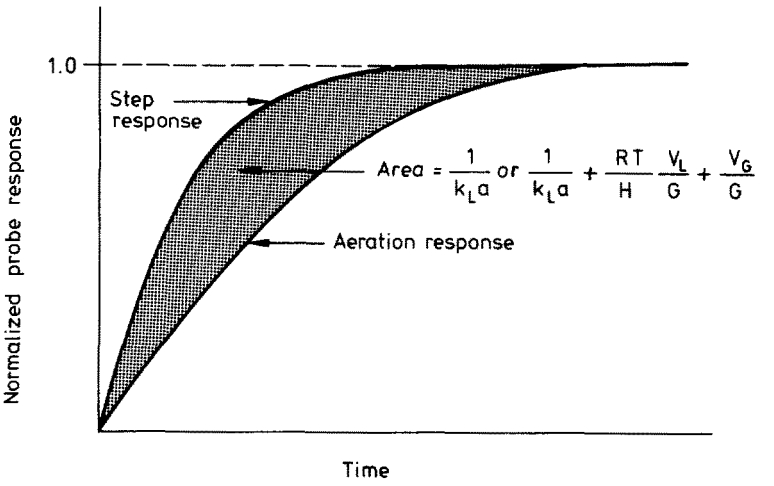

Fig. 20. Graphical method of determining k_La (Dang et al.[36], Nikolaev et al.[115])

Another possible source of error in estimating k_La, *the gas dynamics,* was recently identified by Dunn and Einsele[40]. When the dissolved oxygen is removed by sparging with nitrogen, the dispersed gas phase is pure nitrogen before the resumption of oxygen supply. During the initial stage of aeration, the nitrogen dilutes the gaseous oxygen and is displaced at a rate depending on the mean gas residence time. The measurement error due to this gas dynamics is especially significant in the case of intensely agitated tank reactors, which exhibit a well-mixed gas phase. In order to take the gas dynamics into account, Dang et al.[36] employed an oxygen balance in the gas phase assuming well-mixed dispersed gas phase:

$$\frac{dc_g}{dt} = \frac{c_g' - c_g}{V_G/G} - k_La\,(c^* - c)\,\frac{V_L}{V_G}\ , \tag{63}$$

where c_g, c_g', V_G, V_L, and G are oxygen concentration in the dispersed gas phase, inlet gaseous oxygen concentration, the volume of the dispersed gas phase, liquid volume and the gas flow rate, respectively. By solving Eq. (63) simultaneously with Eq. (60) and two equations describing probe dynamics, they derived the following relationship:

$$\int_0^\infty (1-\Gamma)_{\text{aeration}}\,dt - \int_0^\infty (1-\Gamma)_{\text{step}}\,dt = \frac{1}{k_La} + \frac{RT}{H}\frac{V_L}{G} + \frac{V_G}{G}\ , \tag{64}$$

where R and T are gas law constant and absolute temperature, respectively. As shown in Fig. 20, the shaded area corresponds to $1/k_La$ plus the correction terms given in Eq. (64). The method was successfully applied in evaluating k_La in very viscous media.

Linek and Vacek[94] also showed that a substantial error could be introduced in the estimation of k_La when the effect of start-up period (the period of time that will elapse

before the new concentration level is established and/or before the k_La regains its steady-state value after the resumption of oxygen supply) was neglected. Expressions for the probe response were derived by assuming linear change of the pertinent characteristics during the start-up period.

Votruba et al.[143, 144] studied the *effect of air bubbles* on k_La determination by the dynamic method. They stated that when the bubbles hit the probe membrane frequently, the probe measures not the bulk concentration but a concentration between the equilibrium value and the bulk value. They used a concept of local gas hold-up in estimating the actual concentration from the probe reading, and presented an equation for calculating k_La. However, since the standard DO probes are hit mainly by large bubbles, the estimation of local gas hold-up by the probe may not be adequate and thus the use of their method requires caution. There have been some arguments on the application of this method[95, 145].

The moment method of obtaining k_La (Fig. 20) is in general more convenient and accurate compared with the methods based on electrode models. An added advantage is that the method can be applied even in very viscous media such as in dense cultures. However, in order to apply this method successfully, the experimental conditions such as aeration rate, stirring rate, liquid phase composition and the electrode location, have to be the same for both the step response and the aeration response experiments[36]. Also, the probe location has to be selected to avoid direct contact of the probe membrane with the bubbles. In obtaining the step response, Dang et al.[36] recommended moving the probe rapidly from a nitrogen stream into oxygen saturated liquid.

The *respiration rate*, R, in microbial suspensions has been measured with DO probes. The method involves monitoring the probe response after a sudden interruption of oxygen supply[8]. The rate of change in dissolved oxygen is then equal to the respiration rate:

$$\frac{dc}{dt} = -R \quad . \tag{65}$$

When applying this method, it is important not to let the dissolved oxygen concentration go down beyond the critical value, below which the respiration of microorganisms is damaged[63, 91]. Also, the oxygen supply through the free surface has to be negligible.

7.2 Other Applications

Aiba et al.[2, 4] and Berkenbosch[17] used DO probes for measuring oxygen permeability and diffusivity through *polymer membranes*. The method involved measuring the probe covered with a test membrane to a known oxygen partial pressure. P_m was measured from Eq. (10) with known values of N, F, A, d_m, and p_0. Equation (8) was used for calculating D_m via three different methods: the direct estimation by Eq. (8), the moment method and the slope method. The moment method involved using the area under the normalized response curve, which is similar to that used for k_La measurement. For these applications, the cathode diameter of the probe has to be large compared with the

thickness of the membrane because Eq. (10) was derived under the assumption of one-dimensional diffusion.

Oxygen diffusivities of liquids have been estimated by monitoring the response of the microprobe after a step change in oxygen partial pressure in the gas phase[46, 55, 86]. In this case, the depth of liquid has to be small to prevent convenction. The analysis is similar to that given by Eq. (1) and its boundary conditions. This method was used for estimating the oxygen diffusivity in *bovine serum albumin solutions* and in electrolyte solutions[55].

The oxygen microprobes have been used in measuring the local concentration profiles in *microbial slime layers*, from which the apparent oxygen diffusivity was calculated[28]. The surface renewal rate in an agitated liquid was measured with the microprobe by Bungay et al.[27] and Tsao et al.[139]. The oxygen microprobe was also shown to be able to measure the length and the velocity scales of liquid movement at the liquid surface region[86]. This method was applied in showing the changes in hydrodynamic parameters at the gas-liquid interfacial zone with the added surface active materials such as surfactants and proteins.

DO probes measure oxygen partial pressure in the liquid. Therefore, solubility data are needed for conversion to concentrations. Several methods are available for measuring oxygen solubility. One method involves stripping of dissolved oxygen in an air-saturated liquid by sparging with inert gas (H_2 or CO_2) and monitoring gas phase oxygen[47]. The procedure can be used for determining oxygen solubility in as little as 0.1 ml water. Fatt[47] used a simpler approach by monitoring oxygen tension change after adding a small sample of test liquid in a closed flask which was previously filled with water of known oxygen solubility. For small changes in oxygen tension in the flask, the Henry's Law coefficient for the contents of the flask can be considered to remain constant. Therefore, the change in oxygen tension, p, can be taken to be proportional to concentration change:

$$S_L = S_w \frac{p_2 - p_1}{p_1 - p_0} , \tag{66}$$

where S_w = oxygen solubility in water, p_0 = initial oxygen tension in flask, p_1 = oxygen tension after adding 0.25 ml of air-saturated water, p_2 = oxygen tension after adding 0.25 ml of air-saturated sample.

Since the ratio of sample volume to flask volume used was 1×10^{-3}, the solubility of the water in the flask could be assumed to remain constant even after adding the sample. The accuracy of this method was comparable with other methods.

Often, data on *oxygen solubilities of cultivation media* is needed to convert partial pressures into actual concentrations by Henry's Law. Although a correlation is available for estimating oxygen solubility in an electrolyte solution[35], the method gives an estimation errors of -8% to +12%[98]. Besides, the culture medium contains non-electrolytes such as glucose, alcohols, hydrocarbons, etc. Liu et al.[98] used a DO probe for measuring oxygen solubility in actual medium by using a microorganism of known resperation rate, R, which was separately determined by the Warburg manometric method. The

method involved measuring the change in oxygen partial pressure in a flask filled with actual medium sampled from the bioreactor. The solubility S_0 is calculated as follows:

$$S_0 = R \Big/ \left(\frac{dp_0}{dt}\right) , \tag{67}$$

where R = respiration rate measured from Warburg method, $\left(\frac{dp_0}{dt}\right)$ = measured change in partial pressure.

When combined with enzymes, a DO probe becomes a sensor for specific substrates such as glucose, galactose, ethanol, methanol, etc. A number of enzymes, known as oxygen oxidoreductases, oxidizes substrates by utilizing molecular oxygen to form a product and H_2O_2. The formation of H_2O_2 can be monitored by the DO electrode, which is directly proportional to the substrate concentration[32]. Usually, an enzyme is entrapped or immobilized inside the membrane of the DO probe. Glucose electrodes commercially available are based on this principle. A family of polarographic enzyme electrodes for measuring alcohols and other substrates are described by Clark[32, 33].

8 Conclusions

Although it seems to be possible to design and construct DO probes to meet most of the requirements for specific applications, several problems still remain to be solved. Among them are: long term stability, calibration problems, and reliable measurement of low oxygen tension in dense microbial cultures. Some of the current trends and possible future developments are given below.

Better materials for the electrode components need to be continuously searched. A membrane material with antifouling feature is desirable to prevent growth of microorganisms on the surface. Heat resistance of the membrane need to be improved. The use of an ion-exchange membrane as the electrolyte[114] can certainly improve the probe performance. There has to be a solution to the aging of the cathode surface. Metal oxide may be an alternative. Zirconia cells used for oxygen measurement at high temperatures do not require calibration. Recently, β-alumina was shown to measure oxygen at room temperature[102]. The integrated circuit fabrication technique[137] can be further applied in making probes having uniform characteristics. The use of disposable sensing tips is currently employed by some manufacturers.

The method of operating DO probes can be improved. For example, in a long-term continuous measurement in bioreactors, the probe life can be extended if the probe is operated on a pulse mode under a microprocessor control. Since the probe will be on only for a fraction of time, the probe life can be extended and the probe malfunction can be found from the transient response each time when the probe is switched on. By improving the probe design and the instrumentation, it would be possible to have a long-lasting and self-calibrating probe system.

9 Acknowledgement

The authors wish to thank Mr. Alan Abel of Drexel University for his assistance in preparing this manuscript.

10 Nomenclature

a	gas-liquid interfacial area
A	surface area of cathode
A_1, A_2	quantities defined in Eq. (47)
B	quantity defined in Eq. (61)
B_1, B_2	thermistor constants defined in Eq. (48)
c	concentration of dissolved oxygen
c_g	inlet gaseous oxygen concentration
c'_g	oxygen concentration in dispersed gas phase
c_0	bulk concentration
c_i	initial concentration
c^*	equilibrium concentration
d_e	thickness of electrolyte layer
d_L	thickness of liquid film
d_m	thickness of membrane
$\bar{d}_t$	equivalent thickness defined by Eq. (20)
$\bar{d}$	equivalent thickness defined by Eq. (18)
d'	equivalent thickness defined by Eq. (53)
D_e	oxygen diffusivity of electrolyte layer
D_L	oxygen diffusivity of liquid film
D_m	oxygen diffusivity of membrane
D^*_m	oxygen diffusivity of membrane at base temperature
D_o	oxygen diffusivity of medium
D_1, D_2	oxygen diffusivities
E	activation energy for oxygen permeation
E_D	activation energy for oxygen diffusion
F	Faraday's constant (96,500 coulombs/g-equivalent)
G	gas flow rate
H	Henry's Law constant
H	heat of solution
I	oxygen current
I_G	probe current in gas phase
I_L	probe current in liquid phase
I_s	steady-state current
I_t	transient current
I_T	oxygen current as function of temperature
J	oxygen flux
k	probe constant defined by Eqs. (11) or (19)
k_e	mass transfer coefficient of electrolyte layer
k_L	liquid phase mass transfer coefficient
k_{LM}	mass transfer coefficient of liquid film around membrane
K_M	Michaelis-Menten constant
k_m	mass transfer coefficient of membrane
k_o	zeroth order reaction constant
K_o	overall mass transfer coefficient
L	quantity defined by Eq. (55)
N	number of electrons per mole of oxygen reduced
p	oxygen partial pressure
p_e	oxygen partial pressure at electrolyte/membrane interface
p_m	oxygen partial pressure at membrane/liquid interface
p'_m	oxygen partial pressure defined by Eq. (57)

p_0	oxygen partial pressure of bulk medium
P_e	oxygen permeability of electrolyte layer
P_L	oxygen permeability of liquid film
P_m	oxygen permeability of membrane
r	distance from center of cathode
r_0	radius of cathode
R	gas law constant or reaction rate
R_L	load resistance
R_T	resistance of thermistor
s	quantity defined by Eq. (29)
S_m	oxygen solubility of membrane
S_m^*	oxygen solubility of membrane at base temperature
S_0	oxygen solubility of medium
S_w	oxygen solubility of water
t	time
T	absolute temperature
v	liquid velocity
v_c	critical liquid velocity
V	voltage drop
V_g	volume of gas absorbed
V_G	volume of dispersed gas phase
V_L	volume of liquid
V_s	volume of absorbing solvent
V_T	voltage drop across thermistor
x	distance from the surface of cathode
α	Bunsen coefficient defined by Eq. (36)
μ_1, μ_2	liquid viscosities
$\Gamma_{95\%}$	95% response time
Γ	normalized probe response defined by Eq. (39)

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