The Estimation of the Diffusion Constant and Solubility of \( \text{O}_2 \) in Tissue Using Kinetics

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The diffusion of a gas through a substance in which it is soluble is analogous to the passage of electric current through a circuit with both capacitance and resistance. We model steady-state diffusion employing this analogy, and extend the model to include a description of the kinetics of systems under circumstances of changing partial pressure, applying two physical constants from electrical circuitry to gas diffusion: capacitance (\( C \)) and resistance (\( R \)). We represent the substrate of the diffusion as a capacitor being charged through a resistor after the rapid imposition of a voltage change. Using the insight derived from this model we have devised an experimental system that allows us to approximate both \( D \), the diffusion coefficient, and \( \alpha \), solubility, directly from the kinetic data. We do this by recording the exponential change in \( P_{\text{O}_2} \) on one side of a sheet of material both with and without the addition of a purely resistive barrier of known resistivity. The method was used to estimate \( D \) and \( \alpha \) for distilled water at a number of temperatures, olive oil, and the belly skin of \( \text{Rana catesbeiana} \).

Introduction

The rate at which oxygen diffuses through biological materials is a critical factor in the analysis and understanding of processes in respiration physiology. In higher animals, oxygen carried to the tissues by the convective system of blood transport completes the remainder of this journey to the mitochondria via diffusive mechanisms (Weibel, 1984). In addition, many animals acquire a significant fraction of oxygen from the environment through cutaneous permeance of the gas. For example, many amphibia (especially during larval stages) are highly dependent upon cutaneous uptake of oxygen (Feder & Burggren, 1985). Despite their well-developed gills, Antarctic icefishes obtain up to 30% of their respiratory needs for oxygen via cutaneous route (Hemingsen, 1991). Thus, it is of considerable interest to know the rate of diffusion of a gas, e.g. oxygen, across biological materials such as membranes and tissues. Understanding the factors that facilitate or impede this movement is of equal importance.

Laws of Gas Diffusion

Quantification of diffusion was historically begun by formulating a mathematical model for the process. The steady-state movement of a gas through a sheet of constant thickness consisting of a substance in which that gas is soluble can be
described by a simple differential equation

\[ \frac{dM}{dt} = (D \alpha) A \Delta P/l, \]  

(1)

where \( M \) is the quantity (in moles) of the gas, \( D \) the diffusion constant (cm\(^2\) s\(^{-1}\)), \( A \) the cross-sectional area of the material (cm\(^2\)), \( \alpha \) the solubility of the gas in the substance (moles of gas l tissue\(^{-1}\)), \( \Delta P \) (mmHg) the difference in partial pressure of the gas across the sheet, and \( l \) the thickness of that sheet (cm). Note that \( D \alpha = K \), Krogh’s constant (see below). This is a statement of Fick’s first law arranged most appropriately for steady-state diffusion through a flat sheet of material (Riggs, 1963). We employ the symbol \( \alpha \) as opposed to \( \beta \), which normally includes all sinks for the gas within the liquid, including chemical scavenging. We thus confine our treatment to physical solubility. In the steady-state condition, the rate of movement of the gas is directly proportional to the solubility. The diffusion coefficient, \( D \), is a useful descriptor of the behavior of the gas (Riggs, 1963). For the rest of this paper, we shall exclusively consider oxygen. Analysis of oxygen’s movement in biological systems presents unique problems because living material has the potential to act as a sink for the gas owing to its utilization during respiration.

Direct measurement of \( dM/dt \) for \( O_2 \) is feasible if the material being tested is not actively utilizing the gas. Krogh (1919) measured, over a long interval, the amount of \( O_2 \) that passed through a sheet of tissue, across which he maintained a constant partial pressure differential. He was thus able to express a mean rate. Clearly, if the tissue is utilizing the oxygen, the overt rate of passage will be lower. Krogh defined a parameter, \( K \), which he called the diffusion constant (now called Krogh’s constant), which normalized this rate for sample dimensions and the partial pressure differential. Mahler et al. (1978) experimentally demonstrated that \( K \) closely approximates \( D \alpha \) by validating assumptions about the diffusion of gases. Obviously, this method cannot separate \( D \) and \( \alpha \). It is, however, possible to estimate \( D \) and \( \alpha \) directly.

One standard method for estimating \( \alpha \) involves equilibrating a tissue sample of precisely known volume with a solution having a given oxygen tension, and then sealing it in a chamber with an equally precisely known volume of solution with a much higher \( P_{O_2} \) (air and 100% \( O_2 \) are often standards). The tissue will absorb (and perhaps, depending on the condition of the tissue, utilize) oxygen from the surrounding volume of liquid. \( P_{O_2} \) is monitored with a polarographic oxygen electrode until a new equilibrium is reached, and the amount of oxygen absorbed can be estimated from the new equilibrium values. This yields a value for the solubility and utilization of the gas in the sample (Mahler, 1985). This method cannot yield an estimate for \( D \) directly, but if \( K \) is measured by estimating \( dM/dt \) (as above), \( D \) can be calculated subsequently.

Non-steady-state systems require further consideration. A uniformly thick membrane, assumed to be infinite, is the best-studied and most useful experimental system. The variable \( l \) is the displacement along a vector orthogonal to the plane of the membrane. The sheet, consisting of some substance in which oxygen is soluble, is open to gas with a given \( P_{O_2} \) on one side, and backed with a non-permeable substance on the other side (arbitrarily at \( l = 0 \)) where the oxygen tension may be measured. After equilibration, the \( P_{O_2} \) at the electrode is the same as that on the other side. At \( t = 0 \), the \( P_{O_2} \) is rapidly changed to a new value. Fick’s second law describes the kinetics of the resulting change in \( P_{O_2} \) at the measuring electrode (Tinoco et al., 1978):

\[ D \partial^2 P/\partial l^2 - \partial P/\partial t = 0. \]

(2)

The partial pressure will vary both with displacement within the membrane along the \( l \)-axis, and with time. This equation is identical in form to the equation which describes an analogous situation in thermodynamics. Given a slab of uniform thickness and infinite length and width placed against an insulating barrier, and subjected to a sudden change in temperature on the free side, this equation describes the kinetics of the temperature change on the other side. The numerical solution to this thermodynamic equation serves for Fick’s second law as well (Carslaw and Jaeger, 1959). Fick’s second law does not contain a solubility term, as does the first law, making it possible to separate the diffusion coefficient \( D \) and solubility, \( \alpha \).
Using Ficks’ second law, $D$ can be estimated directly from the kinetics of the changes that result when a step in $P_{O_2}$ is imposed on the sample. The essentials of this procedure were developed by Takahashi et al. (1966), who looked at the consumption of $O_2$ in rabbit cornea. In this method, a tissue sample is draped over a polarographic electrode (without a membrane) bathed in aqueous humor and the time course of the polarizing current change is observed. After an initial rapid change owing to the physical shift from aqueous humor to tissue, the current undergoes an exponential decay to a new steady-state value. This curve can be used to estimate $D$ directly by plotting on semi-log paper the difference between the $P_{O_2}$ at time $t$ and its steady-state value. The time for this value to fall one logarithmic unit yields $D$ directly from $D = 0.99332 \frac{l^2}{t_1}$, where $l$ is the thickness of the sample. If a cover slip is then placed over the tissue, thereby cutting exposure to air in the chamber, the current at the electrode drops linearly to near 0 amp as the oxygen is used up, and this drop yields the solubility. This method works only for estimating solubility if the tissue is actively consuming oxygen and is sensitive to drift in the electrode output over long periods.

Mahler (1978) employed this method to validate the theoretical basis of Fick’s diffusion equations for tissue slices (see above). In addition, the method was extended as follows: the tissue was placed over a polarographic $O_2$ electrode in a temperature-controlled chamber in which the gas mixture could be quickly altered. The system was allowed to equilibrate at one $O_2$ tension, and then a rapid change to another tension was effected thus applying a known starting $\Delta P_{O_2}$. Using both the original paradigm of Takahashi et al. (1966) and the modified method, he was able to estimate $D$ from the rate constants derived from fitting exponential curves to the $P_{O_2}$ time courses.

Solubility could be estimated in this system by Mahler’s (1978) method owing to the utilization of $O_2$ by the tissue (in this case frog sartorius muscle).

As described above, estimation of the terms contributing to Krogh’s diffusion constant, $K$, requires two independent sets of measurements. In the case of non-respiring tissue, $K$ and either $\alpha$ or $D$ can be measured directly, permitting calculation of the remaining variable. Operationally, kinetic determination of $D$ is relatively easy to perform. Experimental estimation of $\alpha$, however, is more difficult.

Below, we describe a method for directly estimating both $D$ and $\alpha$ from easily obtained kinetic data. The method can be applied to both non-respiring tissues and to those actively utilizing oxygen. The insight for development of this methodology was gained by considering the behavior of an analogous electrical circuit.

**DIFFUSION AND ELECTRICAL CURRENT COMPARED**

**The Steady State**

We shall demonstrate that the flow of electric current in simple circuits containing a source of electromotive force (EMF), resistance, and capacitance, and the diffusion of a gas across the membrane system described above, are analogous, obeying equivalent laws. For the steady state, this may be seen in the rate equations describing the flow of the substances in question. Equation (1) above describes the flow of gas. Ohm’s law serves for charge:

$$\Delta V = IR,$$  \hspace{1cm} (3)

where $\Delta V$ is the voltage differential, $I$ the current in amperes, and $R$ the resistance in $\Omega$. Initially, this does not look like Fick’s first law, but rearranging eqn (3),

$$I = \frac{\Delta V}{R} = \frac{dq}{dt},$$

where $q$ is the charge in Coulombs gives:

$$\frac{dq}{dt} = \frac{\Delta V}{R}.$$  \hspace{1cm} (3b)

Resistance can be represented by the relationship $R = \frac{\rho l}{A}$, where $\rho$ is the specific resistivity, $l$ the length of the conductor, and $A$ is its cross-sectional area. The conductivity of the material, $\sigma$, is related to the resistivity by $\sigma = 1/\rho$, so $R = l/\sigma A$. Substituting this expression into the rearranged eqn (3) yields

$$\frac{dq}{dt} = \sigma A \Delta V/l.$$  \hspace{1cm} (3b)
TABLE 1

A comparison of parameters used in studies of diffusion and electrical circuits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diffusion</th>
<th>Electrical circuitry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Driving force</td>
<td>( P ) (partial pressure)</td>
<td>( V ) (voltage)</td>
</tr>
<tr>
<td>Quantity</td>
<td>( M ) (moles)</td>
<td>( q ) (charge in coulombs)</td>
</tr>
<tr>
<td>Ability to conduct</td>
<td>( K ) (Krogh's constant)</td>
<td>( \sigma ) (conductivity)</td>
</tr>
<tr>
<td>Concentration</td>
<td>( C ) (mole/unit volume, ( M/l^3 ))</td>
<td>—</td>
</tr>
<tr>
<td>Solubility</td>
<td>( \alpha ) (conc./Part. Press.—( C/P ))</td>
<td>—</td>
</tr>
<tr>
<td>Capacity for storage</td>
<td>( \zeta ) (( M/P ), or ( \alpha lA ))</td>
<td>( C ) (( q/V ))</td>
</tr>
<tr>
<td>Resistance to flow</td>
<td>( R ) (( l/D\alpha A ))</td>
<td>( R ) (( l/\sigma A ))</td>
</tr>
<tr>
<td>Coefficient of diffusion</td>
<td>( D ) (( K/\alpha ))</td>
<td>—</td>
</tr>
</tbody>
</table>

Returning to eqn (1), and using the relationship \( K = D\alpha \), it may be rewritten as

\[
\frac{dM}{dt} = K\alpha A\Delta P/l
\]

and parallels are immediately clear. The voltage and partial pressure differential are analogous, as are \( K \) and \( \sigma \), and \( M \) and \( q \). Cross-sectional area \( A \) and length \( l \) are obvious.

Table 1 summarizes these relationships, and gives two new ones not defined up to this point. In order to understand and utilize the parallels, it is necessary to have units of resistance and capacitance. These circuit elements are useful in describing the behavior of current and charge, and as will be demonstrated, are equally important in understanding the kinetics of gas diffusing through tissue. Pursuing the analogy further, capacitance in an electrical circuit is defined by

\[
C = \frac{q}{\Delta V}
\]

or the amount of charge that can be stored by a capacitor for a given voltage differential. We define capacitance for a diffusion system as

\[
\zeta = \frac{M}{P},
\]

where \( M \) is the number of moles of the gas, and \( P \) the partial pressure that results in this level of saturation. We do not use the letter \( C \) since it has been taken to mean concentration in the diffusion system. This is justifiable solely on the obvious mathematical relationship, but also physically. Both versions of capacitance describe the ability to store something, either electrons or gas molecules, based on externally imposed conditions and the physical characteristics of the reactor.

With regard to solubility of a gas, \( \alpha \), we note that

\[
\zeta = \frac{C}{P},
\]

where \( C \) is the molar concentration of the gas produced by a given partial pressure \( P \) (in atmospheres) (Riggs, 1963). Since we are dealing with a slice of tissue of uniform thickness, the volume can be expressed as

\[
\text{Volume} = lA,
\]

where \( l \) is the thickness of the slice, and \( A \) is its area. Thus by simple substitution,

\[
\zeta = \frac{\alpha lA}.\]

Resistance in the diffusion system follows by similar arguments. For electricity,

\[
R = \frac{l}{\sigma A}
\]

and since \( \sigma \) is equivalent to Krogh's constant \( K \)

\[
K = D\alpha
\]

and

\[
R = \frac{l}{D\alpha A}.
\]

We assign the name “Krogh” (abbreviated Kr) to the unit of capacitance, and “Fick” (abbreviated F) to resistance for units in the diffusion system.
The Non-Steady State

As noted above, it has been shown that first-order kinetics adequately describe the behavior of a system consisting of a membrane exposed on one side towards a gas in which the \( P_{O_2} \) is rapidly changed (Mahler, 1978; Mahler et al., 1985). This is formally described by Fick’s second law in theory. During the first minutes of these tests, there appears a pronounced sigmoid shape to the curve. Although Fick’s second law yields a sigmoid curve, the lag in the curve also results from the finite time needed to purge the chamber of the first gas mix by the second (Mahler, 1978; Mahler et al., 1985). Both in theory and in practice, the curve quickly resolves to a single exponential, and exponents were derived from an equation of the form \( ae^{(-kt)} \) rather than the exact solution (Mahler et al., 1985). Thus, the following equation adequately describes the relevant portion of the kinetics when determining \( D \):

\[
P_{O_2}(t) = P_{O_2}^{data} + D\frac{\partial P_{O_2}}{\partial t} \exp(-kt),
\]

(11)

where \( t \) is the time, \( P_{O_2}^{data} \) the starting value for the partial pressure in the chamber, \( D\frac{\partial P_{O_2}}{\partial t} \) the partial pressure differential, and \( k \) the exponent describing the reaction (Mahler et al., 1985). (For experimental convenience, the partial pressures are given in mmHg.) Based on governing equations and behavior, the analogous electric process is the charging of a capacitor through a resistor, described by the relationship

\[
V(t) = V_0 + AV\left(1 - \exp\left(-\frac{t}{R}\right)\right),
\]

(12)

where \( V \) is the voltage, \( V_0 \) the initial voltage at \( t = 0 \), \( R \) the resistance and \( C \) the capacitance (Richards et al., 1960). Using this simplified model for insight, it is obvious that given a device with unknown capacitance and resistance, it is impossible to determine either \( R \) or \( C \) from a kinetic experiment wherein the device is charged with a known voltage. The same is true of the tissue system.

Setting \( G = 1/R\zeta \):

\[
P_{O_2}(t) = P_{O_2}^{data} + C\frac{\partial P_{O_2}}{\partial t} \exp\left(-\frac{t}{R\zeta}\right).
\]

(13)

An important insight inherent in Fick’s second law is contained in this analogy, namely that solubility will not affect the rate of the kinetic reaction, while \( D \) is critical as is obvious from the following:

\[
R\zeta = \left(\frac{1}{D}\right)\\Delta x = l^2/D.
\]

Using either eqn (12) or (13) as a basis for nonlinear regression, it is possible to extract an exponent, \( G_1 = 1/RC \) or \( 1/R\zeta \) that characterizes the behavior of both the circuit and the sheet of tissue with equal precision.

It is at this point that the practical usefulness of treating the diffusion system in this manner becomes apparent. The behavior of circuit elements is well known, and the analytical need for describing both capacitance and resistance in the diffusion system makes analogous manipulations much simpler. The insight provided permits the advance in technique we report here. In a simple \( RC \) circuit in which neither the resistance \( R \) nor the capacitance \( C \) is known, deriving a rate constant \( G \) from the curve that results when a known voltage is rapidly applied cannot separate out the two elements. However, if a known resistance, \( R_m \), is added in series with the unknown resistance \( R \), giving a total resistance (still unknown) of \( R_T = R_m + R \), and the voltage applied once again, it becomes possible to calculate values for \( C \) and \( R \) from the resulting rate constant \( G \) in the following manner.

A set of simultaneous linear equations describes the system before and after addition of the new resistance:

\[
G = 1/R_c,
\]

(16)

\[
G = 1/(R_m + R_c)C.
\]

(17)

Solution of this system of equations yields

\[
R = R_mG/(G - G_m),
\]

(18)

and

\[
C = 1/(R_mG_mG/(G - G_m)).
\]

(19)

Equations (18) and (19) hold for diffusion as well, and if a material of known resistance and negligible capacitance is placed over the sample, the two runs, one with and one without \( R_m \) will
yield $G_s$ and $G_m$ such that the resistance of the sample can be estimated. When this is done, using

$$z = \frac{\zeta}{IA}$$

and

$$D = \frac{1}{R_s z A},$$

the diffusion coefficient and the solubility can be extracted as well. $R_m$ must first be estimated by extracting exponents $G_e$ and $G_m$ from a substance of known $D$ and $z$ and back-calculated from the relationship

$$R_m = \left( R G_e / G_m \right) - R_s.$$

If the tissue is actively respiring, there is a sink for $O_2$ other than its solubility in the medium, namely its chemical utilization. We represent the chemical uptake of $O_2$ by a resistor $R_s$, which shunts current past the capacitor at a rate proportional to the voltage on the charged side of the capacitor. This is reasonable, as the utilization of the $O_2$ by the tissue is also proportional to $P_{O_2}$. The voltage in the capacitor never reaches the voltage imposed on the circuit in the steady state, but is charged to the value predicted by Ohm’s law for the network. The time course of the buildup of voltage on the capacitor follows this relationship:

$$V_i = \Delta V - (R \Delta V/(R + R_s))(1 + R_s/R \exp(-t/\tau)),$$

(20)

where $\tau = (CRR_s)/(R + R_s)$ (Rogers, 1957).

Assume that the current cannot be measured directly in this system, thus paralleling the case of diffusion. The procedure to estimate values for resistance and capacitance is to record the time course of the capacitor’s charging when a known voltage is applied without the shunt resistor ($R_s$) in parallel, with and without a known resistance of appropriate value in series with this network, precisely as has been done above. This will yield the values of $C$ and $R$. Repeating the sequence with the shunt resistor $R_s$ in place, the kinetics follow eqn (19), yielding exponents from which $R_s$ may be extracted, as above. By extension, the same may be done in the diffusion system by using respiring tissue, and tissue in which respiration has been eliminated by appropriate treatment (as below with sodium azide).

One problem with this system from the start has been the lag period at the beginning just after the $P_{O_2}$ in the chamber changes. This was first noticed by Takahashi et al. (1966), and further discussed by Mahler (1978) and Mahler et al. (1985). In the case of the latter work, it was expedient to ignore the early data points. In the work reported here, we find that ignoring the deviation and fitting a single exponential curve to the data gives quite a good result. However, we have gotten some improvement in fit by statistically removing the lag. We proceed as follows: when the $O_2$ electrode alone is exposed to the gas in the chamber with no tissue covering it, it does not register immediately when a rapid change in $P_{O_2}$ is initiated, but displays first-order kinetics as it comes to equilibrium with the new oxygen tension. These data yield a rate constant, $G_e$, obtained by nonlinear regression that quantifies both the delay in response in the electrode and the time for purging the chamber. A dual exponential model may be applied to the kinetics when both the tissue and the resistance membrane are tested subsequently using the observed value for $G_e$:

$$P_{O_2} = P_{O_2} + AP_{O_2}$$

$$\times (1 - \exp(-G_e t))(1 - \exp(-t/R_s \zeta)).$$

(21)

The fit of this equation to the sigmoid curve is reasonable over the single exponential portion of the curve, and the constants extracted for calculating $D$ and $z$ are improved. A second benefit, is that the naked electrode may be run a second time after the two tissue runs. Comparison of the exponents before and after the main runs give a measure of the stability of the system over the course of the experiment. We have also tried allowing the nonlinear modeling program fit both exponents freely. This yields a better fit of the model curve to the data, but we prefer the use of the exponent deterministically derived from the chamber characteristics to maximize objectivity.
Test of the Model

To test the efficacy of the model in the actual practice of estimating values of $D$ and $x$, we elected to measure these parameters in a test material for which values have been measured by other means. The procedure first requires that the resistance of the membrane be estimated, as above. To do this, we used known values for $D$ and $x$ for distilled water in a glass fiber matrix, and then used the methodology outlined above to estimate these parameters in olive oil. We have also measured frog belly skin for which no data of this nature are available.

Experiments were conducted in a domed acrylic chamber machined for the purpose (Fig. 1). Similar equipment has been described and employed by Ellsworth & Pittman (1984), and Desaulnier et al. (1996). The chamber consisted of a volume closed by a lid with a tightly fitted O-ring to seal it hermetically. Provision was made for the insertion of a polarographic oxygen electrode (Radiometer E5046-0) flush through the surface of the lid. The electrode registered firmly, and could thus be returned to the same position reliably. A gas inlet was let into the side of the domed portion, and an outlet was provided at the apex of the dome. To ensure that materials are held in place over the membrane of the electrode, the region of the lid containing the electrode was covered by a stainless-steel washer with an O ring. This sat firmly over the electrode surface and was held in place and registered by thumb screws. The chamber could be almost completely immersed in water from a refrigerated circulating water bath (Neslab RTE8) to control internal temperature. Bath temperature was monitored continually. Gases introduced into the chamber first passed via aquarium “air stones” through cylinders also immersed in the water bath to hydrate them and adjust them to the proper temperature.

The electrode was polarized to its optimal voltage (706 mV) with a Hewlett-Packard 6214C power supply, and the resulting current was monitored with a Kiethley 485 picoammeter. The output voltage of this ammeter was digitized with a Metrabyte DAS8 A/D converter in a Zenith micro-computer and the resultant data stored on disk for further analysis. Data were reformatted to convert the raw A/D numbers to $P_{O_2}$, compensating for the saturation of the gas with water vapor. The data were normalized to a $P_{O_2}$ of 159 mmHg for air, and 760 mmHg for pure $O_2$. Where the data approached the asymptote, both starting and ending values were used. If the curve was clearly still rising (see, for example, the curves for olive oil in Fig. 5), only the starting value was used for scaling. Reformatted data were uploaded into an IBM mainframe for nonlinear regression analysis to extract exponents (NLIN procedure, Statistical Analysis System).

First to estimate the resistance ($R_m$) of the dialysis membrane, it is necessary to use a sheet of material for which characteristics of solubility and diffusion rate are known and then back-calculate. We used distilled water in a Whatman glass fiber filter. Hydration of this medium, as measured by wet weight/dry weight ratios is about 95%. Two layers of the filter material were used throughout. Two hydrated filters are approximately 500 mm thick as measured by a vernier caliper depth gauge applied to the retaining washer before and after the installation of the filter. The accuracy of this method of measurement was verified with a microscope by focusing

![Diagram of the acrylic chamber used to conduct the tests.](image-url)
on the washer with and without the material present and estimating the thickness by the movement of the calibrated fine focus knob. The dependence of the final result on thickness is critical, and samples were measured each time. The cross-sectional area of the O-ring is 0.2042 cm². Since only 95% of the test material is actually water, we employed this as a correction factor on the cross-sectional area, and used 0.194 cm² for A throughout. Solubility of O₂ in distilled water at 25°C is 2.831 × 10⁻² ml O₂ ml⁻¹ (Altman and Dittmer, 1971), and D is 2.57 × 10⁻⁵ cm² s⁻¹ (Battino et al., 1968). After extracting the exponent for the filters with the membrane covering them, Rₘ can be derived as above.

The electrode was fitted with a fresh membrane and solution and allowed to equilibrate and stabilize in air-saturated distilled water at the temperature at which the test run was to be conducted. The electrode was then affixed to the test chamber and the chamber purged with air at 70 ml s⁻¹ until a stable current reading was obtained. At this point, O₂ was quickly substituted for the air at the same flow rate and data acquisition was begun. Two minutes (120 s) of data were collected as described above. The chamber was disassembled and the test material, equilibrated with ambient air, was placed over the electrode. The reassembled chamber was returned to the water bath and allowed to stabilize under the same flow of air. Immediately upon switching to O₂, 10 min (600 s) of data were taken. This procedure was repeated and a piece of dialysis membrane (Spectra-Por #1, Molecular Weight Cutoff 6–8000), hydrated in distilled water, was placed over the test material. Ten minutes (1200 s) of data were taken in this final run. In practice, if tissue samples were run, it was desirable to run the tissue with the membrane first, since often the tissue would stick to the O-ring. The dialysis membrane did not stick to the O-ring, and could be easily removed from the sample. A run without the membrane was done last.

We tested olive oil at 25°C, for which published values for solubility and the diffusion constant are available. As it takes longer for the system to equilibrate with the oil, we extended the length of the runs as follows: electrode alone, 3 min, filter alone, 15 min, filter with dialysis membrane, 30 min. Even with these longer times, the asymptote was not approached closely, and we scaled the data to the starting P₀₂ only. We also tested belly skin of Rana catesbeiana. The animal was euthanized according to standard practice, and the belly skin was immediately dissected away and placed in 0.02% NaN₃ for a minimum of 1 h to stop oxygen usage. Tissue samples were treated in the same manner as the filter, times were the same as for the experiment with water.

**Results**

Figure 2 shows a typical family of curves for a protocol with glass fiber filters saturated with distilled water. Raw data from the filter over the electrode and the filter covered with the dialysis membrane are shown. Note the characteristic sigmoid shape of the time course. The theoretical curves from the model, employing the derived exponents, are superimposed. Figure 3 shows each of the individual data plots from Fig. 2 expanded, with the theoretical curve from the model, employing the derived exponents, superimposed. The resistance of the membrane (Rₘ) at 20°C was 538 H. B. DOWSE ET AL.

![Fig. 2. Family of curves that results when filter paper is used as a test substrate over the O₂ electrode. Solid line: electrode alone; long dashed line: double thickness of filter material saturated with distilled water; short dashed line: filter overlain with a single layer of dialysis membrane hydrated in distilled water. Temp. = 20°C, thickness = 500 μm, Pₜₐₐₐ = 17.5 mmHg, ambient barometric pressure = 758 mmHg. Values were corrected to actual P₀₂ in the test chamber.](image)
FIG. 3. Individual curves from Fig. 2 expanded and plotted individually. The data are shown as dots connected by a solid line. The superimposed dashed line represents the theoretical line predicted by the model using the exponent derived from nonlinear regression of the equation on the raw data. In the case of the filter and the filter with the membrane, the effect of the delay in response owing to the electrode itself has been mitigated by a double-exponential statistical manipulation (see text).

(a) Electrode alone; (b) electrode covered with a double thickness of hydrated filter; (c) filter covered by hydrated dialysis membrane (see Fig. 2).

calculated to be $3.273 \times 10^5$ and at 25°C $3.169 \times 10^5$ F. (Recall that the letter F stands for “Fick”, the unit of resistance defined above.) At 20°C, belly skin of *Rana catesbeiana* yielded values of $D = 5.74 \times 10^{-6} \pm 7.1 \times 10^{-7} \text{cm}^2 \text{s}^{-1}$ (S.E.M.) and $\alpha = 6.30 \times 10^{-2} \pm 1.08 \times 10^{-3} \text{ml gas ml tissue}^{-1}$ (S.E.M.) $N = 2$ (Fig. 4). Olive oil at 25°C yielded $D = 4.24 \times 10^{-6} \pm 2.63 \times 10^{-7} \text{cm}^2 \text{s}^{-1}$ (S.E.M.) and $\alpha = 0.1267 \pm 8.8 \times 10^{-3} \text{ml gas ml tissue}^{-1}$ (S.E.M.) $N = 4$ (Fig. 5).

**Discussion**

Previous estimates for olive oil at 25°C, based on accepted methodologies, are $D = 7.46 \times 10^{-6} \pm 8.0 \times 10^{-7}$ (Davidson *et al.*, 1952) and $\alpha = 0.116$ (no error given) (Battino *et al.*, 1968) and
FIG. 4. Data from frog (*Rana catesbeiana*) belly skin. Raw data are shown with solid lines, and the curves fitted by the model are dashed. The left pair of curves come from the skin alone, and the right pair from skin plus hydrated dialysis membrane to add resistance to the system.

FIG. 5. Data as above, from olive oil. The time course was long and the oxygen levels did not approach the asymptotic value for pure O$_2$ closely, but the extracted exponents yielded a good estimate for $D$ and $\alpha$.

$\alpha = 0.102$ (Davidson *et al.*, 1952). Our estimate for $D$ is slightly lower than that of Davidson *et al.* (1952), but the agreement is acceptable. Solubility values are in very close agreement with previous work. Using the known values for water to estimate $R_m$ for establishing the corresponding values for olive oil represents a rigorous test for the system given that both $D$ and $\alpha$ differ by an order of magnitude between the two substances. Isolated perfused *Rana catesbeiana* belly skin has been used to measure gas exchange (Pinder *et al.*, 1991; Clemens & Feder, 1992), however data such as we present here for solubility and the diffusion coefficient have not yet been published (A.W. Pinder, pers. comm.).

We have shown that the method described here can be used effectively to estimate the diffusion coefficient, $D$ and solubility, $\alpha$. The method is straightforward conceptually and easy to implement.

As noted above, estimating $D$ by the older techniques is relatively simple, but $\alpha$ is substantially more difficult. Outputs from a polarographic oxygen electrode that are in the nanoamp range must be monitored for substantial intervals of time, while the system must remain stable for hours (see, for example, Desaulnier *et al.*, 1996). This work was done in the laboratory of one of us (B.S.) and serves as a good comparison of the two techniques. To obtain $\alpha$, muscle tissue in a controlled chamber of known volume was exposed to a step increase in $P_{O_2}$. The $P_{O_2}$ was monitored for 2 hr after the change, and the decrease was used to estimate the amount of O$_2$ that dissolved into the specimen (Desaulnier *et al.*, 1996). For fish muscle tissue acclimated to 25°C, the $\alpha_{O_2}$ value was $3.59 \pm 0.02$ (S.E.M.), not far from our value for frog belly skin, and with a similar variability. While ultimate precision may best be attained using the more painstaking methods, this technique provides a technically simplified way of getting acceptable estimates of both constants, especially under field conditions. We have used this method effectively in a comparison of muscle tissue of three species of Antarctic fish with differing strategies of cold adaptation (S. Norton *et al.*, unpublished data). The technique may only be employed with tissue such as muscle or skin, which may be prepared easily to provide thin uniform slices.

Dialysis membrane was chosen for the known resistive element in our system. This material is thin, and a large fraction of its surface is filled with open pores very large with respect to an O$_2$ molecule. Any movement across the membrane will be largely accomplished by way of diffusion through these open pores, which are of a size to allow macromolecules up to 6–8 kD to pass easily. This is the path of least resistance, and will dwarf any passage through the membrane.
material itself. The substance of the membrane will, of course, have its own resistance and capacitance, but passage of gas by diffusion through this substance will be very small in comparison to passage through the oriﬁces, and any capacitance can be safely ignored. The thickness of the dialysis membrane is typically more than two orders of magnitude less than that of the hydrated filter paper.

REFERENCES


