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Voltammetric Studies of Ion Transfer Across Model Biological Membranes

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1 Introduction

In recent years there has been a renewed interest in electrochemistry and the application of electroanalytical techniques to problems of biological interest. Biochemists are now enjoying success in the electrochemical study of biomolecules (including some biopolymers) using polarography and related methods,[1-6] while technologists are exploiting the properties of membranes and interfaces for the production of fuel.[7-9] In their naturally occurring state many biomolecules of interest from the electrochemical standpoint are either associated with, or bound within, a membrane. It is an attractive notion that a combination of techniques from membrane technology and electroanalytical chemistry be applied to the study of biopolymers in their native state. Also of interest is the examination of the extent to which electroanalytical methods may be applied to questions concerning the physical chemistry of ion transport across membranes. Buck[10] has published a very detailed review of the general aspects of electroanalytical methods as they may be applied to membranes, although his review considers membranes which differ from biological membranes in both dimensions and molecular organization. There are also several recent monographs devoted to biological electrochemistry.[1-4,11] This paper will consider the application of one electroanalytical technique, namely, voltammetry, as a means of studying the physical chemistry of charge-transfer processes occurring at a membrane/electrolyte interface, where the membrane under consideration is a planar lipid bilayer formed in a controlled manner.

The bilayer lipid membrane is a model of biological membranes and is well suited to the study of electrical properties of membrane-bound components and

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[1] J. Kuta and E. Palacek, in 'Topics in Bioelectrochemistry and Bioenergetics', Vol. 5, ed. G. Milazzo, Interscience, New York, 1983, p. 1.

[2] E. Palacek, ref. 1, p. 65.

[3] F. A. Armstrong, H. A. Hill and N. J. Walton, *Quart. Rev. Biophys.*, 1986, **18**, 261.

[4] 'Bioelectrochemistry I', ed. G. Milazzo and M. Blank, Plenum Press, New York, 1983.

[5] 'Biological Electrochemistry', Vol. 1, ed. G. Dryhurst, K. M. Kadish, F. Scheller, and R. Renneberg, Academic Press, New York, 1982.

[6] 'Comprehensive Treatise of Electrochemistry', Vol. 10, ed. S. Srinivanasan, Yu. A. Chizmadzhev, J. O'M. Bockris, B. E. Conway, and E. Yeager, Plenum Press, New York, 1985.

[7] M. Gratzel, in 'Modern Aspects of Electrochemistry', Vol. 15, ed. R. E. White, J. O'M. Bockris, and B. E. Conway, Plenum Press, New York, 1983, p. 83.

[8] 'Comprehensive Treatise of Electrochemistry', Vol. 3, ed. J. O'M. Bockris, B. E. Conway, E. Yeager, and R. E. White, Plenum Press, New York, 1981.

[9] J. Koryta, 'Ion Selective Electrodes', Cambridge University Press, Cambridge, 1975.

[10] R. P. Buck, *CRC Crit. Rev. Anal. Chem.*, 1975, **5**, 323.

[11] 'Modern Bioelectrochemistry', ed. F. Gutmann and H. Keyser, Plenum Press, New York, 1986.

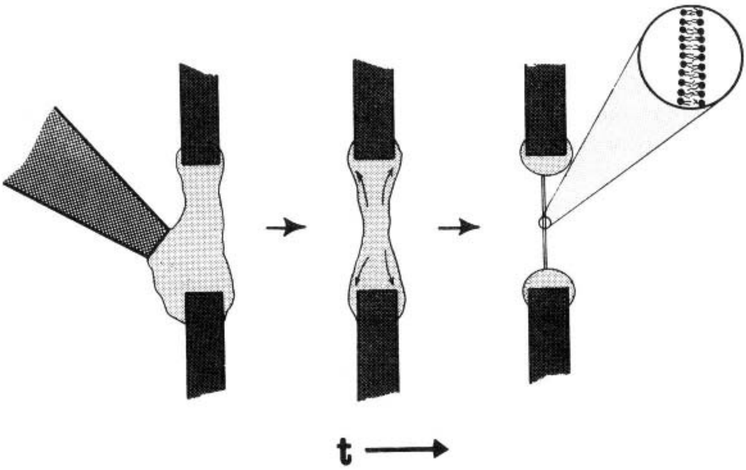

Figure 1 *Formation of bilayer membrane within aperture in a hydrophobic support*

biological transport. Lipids are dissolved in organic solvents and painted (or injected) onto a hydrophobic support which is immersed in an aqueous electrolyte solution. The lipid solution within the orifice of the support (*e.g.* a hole in a Teflon beaker) thins towards the hydrophobic material, leaving behind a bimolecular leaflet (Figure 1) of amphiphatic lipid molecules. There is usually some incorporation of solvent between the layers, and this solvent affects physical parameters which influence membrane permeability.[12–15] Solvent-free membranes may also be prepared, although doing so often requires an alternative method of bilayer formation[16,17] or the use of some lipid combination (*e.g.* squalene/phosphatidyl choline) in which one component is liquid.

The principal selling point of this particular model system is that the membrane which is formed in this manner is planar, and, by choosing an appropriate support, the membrane can function as the sole conduit of charge (or material) between two compartments. This particular feature makes the planar bilayer membrane ideally suited for the study of electrical and electrochemical phenomena associated with membrane processes by using an electrode arrangement such as that illustrated in Figure 2. A more comprehensive treatment of the methods of membrane research may be found in several texts,[18–21] and the reader is referred to these for

[12] A. D. Pickar and R. Benz, *J. Membrane Biol.*, 1978, **44**, 353.

[13] R. Benz, O. Frohlich, and P. Lauger, *Biochim. Biophys. Acta*, 1977, **464**, 465.

[14] B. M. Hendry, B. W. Urban, and D. A. Haydon, *Biochim. Biophys. Acta*, 1978, **513**, 106.

[15] D. C. Peterson, *Biochim. Biophys. Acta*, 1983, **734**, 201.

[16] S. H. White, *Biophys. J.*, 1978, **23**, 337.

[17] V. Vodyanoy and R. B. Murphy, *Biochim. Biophys. Acta*, 1982, **687**, 189.

[18] M. K. Jain, 'The Bimolecular Lipid Membrane', Van Nostrand Reinhold Company, New York, 1972.

[19] R. Fettiplace, L. G. Gordon, S. B. Hladky, J. Requena, H. P. Zingsheim, and D. A. Haydon, in 'Methods of Membrane Biology), Vol. 4, ed. E. D. Korn, Plenum Press, New York, 1975, p. 1.

details regarding membrane formation solutions and other general aspects of experimental procedure.

Electroanalytical techniques such as polarography or voltammetry generally measure a response to a charge-transfer event which occurs across an interface. The most familiar example of such a process is electron transfer from a metal electrode to an electroactive molecule in solution (or *vice versa*). Very simply, the potentiodynamic methods,[22] a category to which voltammetry and polarography belong, involve the application of a potential across an electrode/electrolyte interface and the measurement of the current response to variations of the potential. The current response of processes occurring at a metal electrode are indicative of the nature of the charge-transfer reaction (*e.g.* redox) at the interface. Experimental data derived from measurements of this kind permit the elucidation of mechanism and the thermodynamic parameters of the process. The method is not restricted to electron transfer at electrodes however; the methods of electroanalytical chemistry are now being applied to charge transfer (usually ionic) across an interface separating two immiscible liquids, known by the acronym ITIES, for Interface between Two Immiscible Electrolyte Solutions.[23-27]

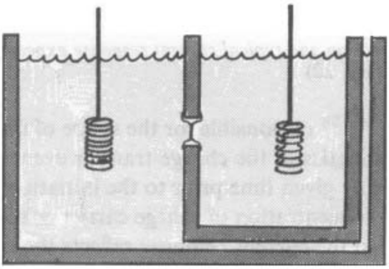

Figure 2 *Simple experimental arrangement for electrical and electrochemical measurements on membrane-partitioned cells*

Figure 3 depicts the time-dependent modulation of potential and typical current response for an interfacial charge-transfer process, as observed in a cyclic voltammetry experiment. This 'duck-shaped' current–voltage (i–V) profile is obtained for both interfacial electronic and ionic processes. The underlying

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[20] H. T. Tien and R. E. Howard, in 'Techniques of Surface and Colloid Science', Vol. 1, ed. R. J. Good, R. R. Stromberg, and R. L. Patrick, Dekker, New York, 1972, p. 110.

[21] H. T. Tien, 'Bilayer Lipid Membranes', Dekker, New York, 1974.

[22] D. D. MacDonald, 'Transient Techniques in Electrochemistry', Plenum Press, New York, 1977.

[23] J. Koryta, *Electrochim. Acta*, 1979, **24**, 293.

[24] D. Homolka, Le Quoc Hung, A. Hofmanova, M. W. Khalil, J. Koryta, V. Marecek, Z. Samec, S. K. Sen, P. Vanysek, J. Weber, and M. Brezina, *Anal. Chem.*, 1980, **52**, 1606.

[25] J. Koryta and P. Vanysek, in 'Advances in Electrochemistry and Electrochemical Engineering', Vol. 12, ed. H. Gerischer and W. Tobias, Wiley-Interscience, New York, 1981, p. 113.

[26] P. Vanysek, 'Electrochemistry of Liquid/Liquid Interfaces', Springer-Verlag, Berlin, 1985.

[27] 'The Interface Structure and Electrochemical Processes at the Boundary between Two Immiscible Liquids', ed. V. E. Kazarinov, Springer-Verlag, Berlin, 1987.

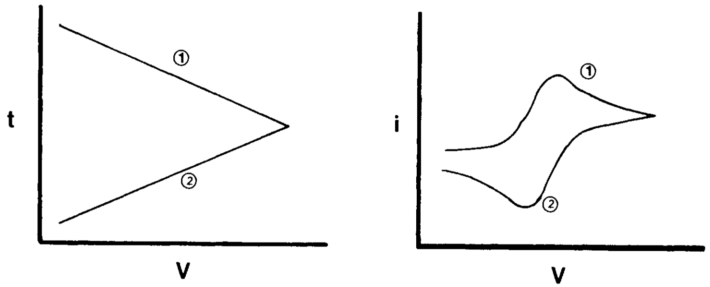

Figure 3 *Potential modulation and typical current response expected in a cyclic voltammetry experiment (adapted from ref.* 22)

physical mechanism[22,26-28] responsible for the shape of the curve is based on the interplay between the kinetics of the charge-transfer event and mass transport of the charge carriers. At any given time prior to the initiation of the potential sweep there exists some local concentration of charge carrier at the interfacial boundary. As the potential is varied the current response reflects the conductance of the cell; at a potential corresponding to the energy of the charge-transfer reaction a rapid increase in the measured current is observed as the molecules in the interfacial region partake of the charge-transfer reaction. Provided that the charge-transfer event is faster than the diffusion of carrier to the interface there occurs a depletion of the carrier in a layer adjacent to the interfacial boundary; at this stage there is observed a maximum and decay in the i–V profile, *i.e.* a 'peak'. Analysis of these profiles, or voltammograms as they are more often called, provides information concerning the nature of the charge-transfer process.

This paper examines the application of voltammetric methods to electrochemical cells of the type illustrated in Figure 2. These cells are similar to those defined by an interface between two immiscible liquids since the membrane acts as a partition between two electrolyte solutions. By posing a barrier to charge transfer between the electrolyte solutions, the membrane behaves as an interface between two 'immiscible' aqueous electrolyte solutions. How one regards the membrane as an ITIES system depends on whether one considers the microscopic or macroscopic features of the membrane. One can either consider the membrane as a very thin barrier between bulk phases or imagine separate ion-transfers across the interface of each membrane face (*cf.* Figure 4A and 4B).

2 Experimental Aspects and the Equivalent Circuit

A simple two-electrode cell as used in the measurement of electrical parameters of membranes (Figure 2) may also be used for cyclic voltammetry experiments with the appropriate experimental arrangement. The original membrane polarography

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[28] A. J. Bard and L. R. Faulkner, 'Electrochemical Methods', Wiley, New York, 1980.

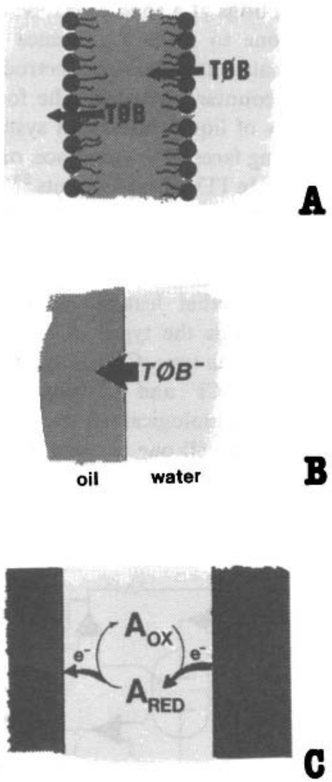

Figure 4 *Ionic charge-transfer event in membranes (A) in comparison to charge-transfer across liquid/liquid interfaces (B) and thin film electrode cells (C)*

experiments[29] were performed using such a cell, although more recently, three-[30] and four-[31]electrode cells have been reported. The purpose of cells in which there are more than two electrodes is the minimization of error in the measurement of potential at the interface of interest. The error in potential measurement is usually attributed to the resistance of the electrolyte solution, and is called '*iR* drop' in the electrochemistry literature.

In conventional electrochemistry experiments one is interested in a reaction at a particular electrode and wishes to measure that electrode potential accurately. One therefore would like to place the reference electrode as close to this surface as possible. For more details on the problem of '*iR* compensation' the reader is referred to texts on electrochemistry (*e.g.* ref. 28), but for our purposes, suffice it to

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[29] E. A. Liberman and V. P. Topaly, *Biochim. Biophys. Acta*, 1968, **163**, 125.
[30] H. T. Tien, *J. Phys. Chem.*, 1984, **88**, 3174.
[31] M. A. Habib and J. O'M. Bockris, *J. Bioelectricity*, 1984, **3**, 247.

say that for cells in which reactions at a solid electrode are under investigation, a three-electrode cell permits one to place a reference probe very close to the electrode of interest (usually called the working electrode) while the current may be carried by an additional counter electrode. The four-electrode cell is more commonly found in the study of liquid interfacial systems, and by placing two reference electrodes on opposing faces of the interface, one can measure the trans-interfacial potential accurately. In ITIES experiments[23–26,32,33] current is carried by a pair of platinum electrodes separated by the interface and the potential measured using the reference electrode pair.

Before discussing which of these electrode arrangements is best suited for membrane voltammetry, a few other features of membrane cells should be addressed. The first point concerns the types of electrodes used in membrane electrical measurements of the static type. Generally, saturated calomel (SCE) or Ag/AgCl electrodes are used since Cl^- and K^+ ions constitute an excellent tonic medium for model membranes and biological tissues. In a voltammetry experiment conducted with this two-electrode cell one is using reference electrodes to carry current, and therefore caution must be exercised that the current level be kept low.

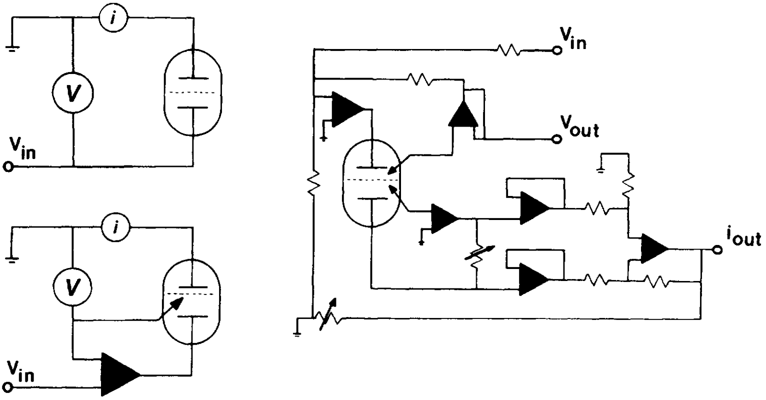

Figure 5 *Membrane-partitioned cell design. Two-, three-, and four-electrode arrangements (four-electrode circuit from refs.* 25 & 33)

According to two sources,[34,35] 'safe' currents are of the magnitude 1 μA or less, else the electrodes will polarize. The other point to consider is the equivalent circuit of the membrane-partitioned cell. Both the solution and membrane possess some electrical resistance, the sum of these in part being responsible for the total measured potential. In many experimental situations the membrane resistance is

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[32] Z. Samec, V. Marecek, and J. Weber, *J. Electroanal. Chem.*, 1979, **100**, 841.
[33] Z. Figaszewski, Z. Koczorowski, and G. Geblewicz, *J. Electroanalytical Chem.*, 1979, **100**, 841.
[34] N. D. Green, 'Experimental Electrode Kinetics', Rensselaer Polytechnic Institute, Troy, 1965.
[35] H. H. Willard, L. L. Merritt, and J. A. Dean, 'Instrumental Methods of Analysis', Van Nostrand, New York, 1974, Ch. 19.

orders of magnitude greater than the solution resistance and one can neglect the solution resistance.

Selection of a cell for membrane voltammetry should be made on consideration of the above comments. The two-electrode cell using SCE or Ag/AgCl reference probes is convenient for both electrical measurements and voltammetry since, in general, the membrane resistance puts a stringent limit on the amount of current which will flow in a given cell. This, plus the fact that the solution resistance may be neglected, makes concern over *iR* drop and the use of multiple electrode cells superfluous. Despite this fact, a four-electrode arrangement, when feasible, is the preferred cell since it grants more flexibility in the range of measurable currents (and hence concentration ranges). The four-electrode cell also has the added advantage of removing the calomel half-cell and associated salt bridges from the current-carrying process. In these latter cells platinum (or some other inert electrode) carries the current (*cf.* refs. 23—26,32,33).

The equivalent circuit of a membrane is often given as a parallel arrangement of resistor and capacitor (neglecting the electrical properties of the solution) since the membrane is often regarded as a thin dielectric sandwiched between electrolyte conductors. This simple representation yields a linearized loop for a characteristic i–V curve. As would be expected, the slope of the i–V trace depends on the resistance, whereas the thickness of the loop obtained (owing to the dynamical nature or the directional traces) depends on the capacitance, and therefore the rate of polarization. The model may be further elaborated by the addition of a voltage source in parallel with the rest of the circuit, and this addition permits one to model a Nernstian membrane potential gradient.

A more realistic equivalent circuit has been used to describe the i–V behaviour of ion transport across membranes, and this model improves on the simple resistor/capacitor representation by the incorporation of a term which mimics the effects of mass transfer in ion flux.[36–39] A circuit element known as Warburg Impedance[40] is put in series with the resistive element of the simple model described above (capacitance still in parallel with the resistance/impedance pair). This Warburg Impedance represents a resistance to mass transfer and follows from a division of total impedance into charge transfer and mass transfer terms. The model now begins to resemble that of a conventional electrode and has been shown[36–39] to predict the qualitative behaviour of i–V curves resulting from ion transport across membranes. These results will be discussed further in the following section which examines the dynamic i–V profiles (*i.e.* voltammograms) of ion transport.

In concluding this section it should be remarked that the modification of membranes (and presumably reactions occurring at the membrane surface) will affect all the properties of the equivalent circuit. This complicates the interpretation

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[36] R. deLevie and H. Moreira, *J. Membrane Biol.*, 1972, **9**, 241.
[37] R. deLevie, N. G. Seidah, and H. Moreira, *J. Membrane Biol.*, 1972, **10**, 171.
[38] R. deLevie and N. G. Seidah, *J. Membrane Biol.*, 1974, **16**, 1.
[39] R. deLevie, N. G. Seidah, and H. Moreira, *J. Membrane Biol.*, 1974, **16**, 17.
[40] E. Warburg, *Weid. Ann.*, 1899, **67**, 493.

of a given voltammogram, yet makes them very informative with regard to the dynamic nature of the membrane. In addition to kinetic information derived from the classical interpretation of the voltammetric peaks, it is possible to glean transient changes in membrane conductance, capacitance, and open circuit (Nernstian gradient) potential from a family of voltammograms separated in time or some other reaction coordinate.

3 Current and Ion Flux

The simplest example of a conducting membrane is one across which ions may readily diffuse under the influence of an electric field. In such a system the ion is the mobile charge-carrier, and the current response is indicative of the kinetics of the phase-transfer characteristics of a given ion; the phase-transfer process being the passage of ions between aqueous phases *via* an organic interstitial layer. The energetics may be envisaged as a typical energy barrier/reaction coordinate profile in which the membrane is depicted as a potential barrier (to ionic diffusion), and the reaction coordinate taken to be the position normal to the plane of the membrane. When polarized, the cell responds with a migration of ions towards one or the other membrane face, while the current is carried by those permeant ions capable of crossing the barrier imposed by the membrane between the electrolyte solutions.

Previous experimental determination of i–V profiles for ion transport has been largely achieved through static (*i.e.* steady state) means rather than by dynamic means. Two types of curves are reported for i–V profiles of membranes. The type of i–V curve one gets has been shown to depend on the concentration of the lipophilic ion,[41] with A and B in Figure 6 corresponding to the lower and upper concentration limits, respectively. This concentration dependence was attributed[41] to a change in the rate-limiting process of the current flux; at lower concentrations the current is limited by the rate of diffusion of the lipophilic ion to the membrane surface and/or diffusion into the membrane. At higher concentrations the membrane itself becomes rate limiting, and therefore the cell is characterized by different kinetics. In his original explanation of this concentration effect, LeBlanc[41] claimed that the behaviour at high concentrations was due to the onset of 'Space Charge Limitation'. About a year later a theory of Space Charge Limited ion-transport was published.[42]

It was reasoned that since a lipophilic ion generally diffuses into the membrane leaving behind its counterion (*e.g.* tetraphenylborate and its counterion Na^+), a space-charge region is developed in which negative charge accumulates within the membrane. As the overall concentration of the lipophilic ion increases, the membrane concentration of the ion also increases until the point of saturation is reached. At this point the current becomes limited by the membrane and the kinetics of the ion flux is dictated by space-charge effects, or more accurately, an analogue of the effect known in solid-state physics (*cf.* refs. 43—45).

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[41] O. H. LeBlanc, *Biochim. Biophys. Acta*, 1969, **193**, 350.

[42] B. Neumcke and P. Läuger, *J. Membrane Biol.*, 1970, **3**, 54.

The notion of space-charge limitation is best understood by regarding the membrane as a capacitor between 'ionic electrodes'. In general, conductivity is defined as $\sigma = ezn_{\text{ion}}u_{\text{ion}}$, where e and z denote the unit of electric charge and charge of the ion, respectively; n and u represent the concentration and mobility of the ion. Therefore, since the conductivity varies with concentration, it is possible that at low ion concentrations the conductivity of the membrane may be higher due to the low volume of the membrane and a high affinity of the lipophilic ion for the organic layer. At higher concentrations of ion the membrane ultimately saturates, and further increases only raise the conductivity of the solution phase. At this latter stage the membrane can be described as an ionic capacitor, and the current response to a given applied voltage is a function raised to some power,[43–45] a form of the Child–Langmuir Law.

The basic tenets of this model were elaborated upon in a more detailed mathematical description by deLevie and co-workers.[36–39] Rate laws and *i–V* characteristic curves were derived from the Nernst–Planck and Poisson equations, both of which are used in the theoretical analysis of space-charge effects.[43] In their model deLevie *et al.* resolve the transport process into several conceptual steps, namely: (1) movement across the unstirred boundary layer adjacent to the

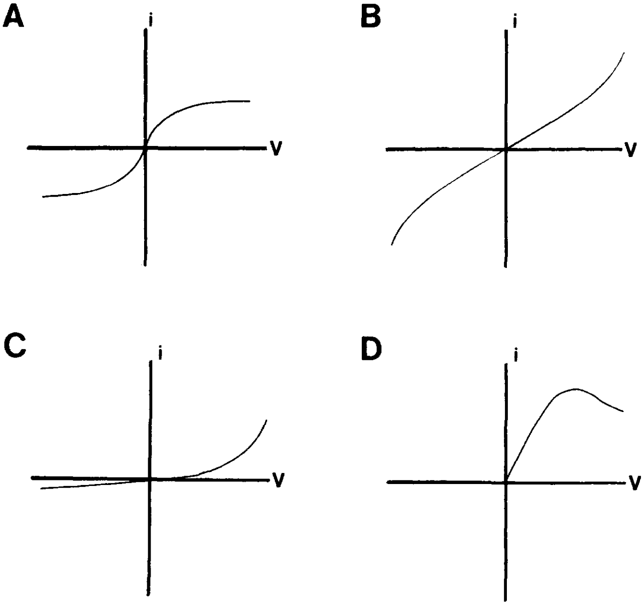

Figure 6 *Current–potential profiles of ion-conducting membranes. (A) Diffusion-limited* i–v *curve (low ionic concentration). (B) Space-charge limited (high ion concentration). (C) Fully rectified curve* ($C_\alpha \gg C_\beta$). *(D) Polarographic response (after ref.* 29)

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[43] M. A. Lampert and P. Mark, 'Current Injection in Solids', Academic Press, New York, 1970.
[44] R. H. Tredgold, 'Space Charge Controlled Conduction in Solids', Elsevier, Amsterdam, 1966.
[45] F. Gutmann and L. E. Lyons, 'Organic Semiconductors', Wiley-Interscience, New York, 1967.

membrane surface, (2) movement through the space-charge layer at the surface, (3) movement into the hydrophobic region, and (4) translocation across the hydrophobic region. The analysis of the total process is facilitated by this breakdown which permits an assessment of various contributing factors when an *i–V* profile is simulated from the equations.

The numerical simulation and analysis of ion transport[38,39] through the aqueous layer substantiates the explanation of *i–V* behaviour proposed on the basis of empirical data.[41] Variation of a parameter which is proportional to the membrane/water partition yields a series of curves illustrating a transition from the *i–V* profile of Figure 6A to that depicted in Figure 6B. This transition is governed by the relative concentration of ions in the two phases, and the trend is such that at high excess of ion in the aqueous phase the conductance is limited by the membrane. At concentration levels where the membrane concentration exceeds that of the aqueous phase the constant field approximation determines the shape of the profile.[39] The space-charge layer also affects the shape of the *i–V* curve in the sense that an asymmetric distribution of charge density between the two faces of the membrane will drastically affect the symmetry of the *i–V* curve; in the extreme case, totally rectifying the curve (see Figure 6C).

So far our description has been limited to steady-state descriptions of *i–V* behaviour. The original application of a dynamic electrochemical technique to a membrane (bilayer) partitioned cell was reported by Liberman and Topaly[29] who obtained their *i–V* curves of ion transport by polarographic means. In these experiments regions of 'negative resistance',[29] similar to linear sweep voltammetry peaks, were reported. These peaks (as illustrated in Figure 6D) were found to vary with ion concentration and polarization rate; there was also noted an effect attributable to the presence of other ions in the aqueous phase (*e.g.* Cu^{2+}).[29] LeBlanc[41] took note of these experiments and he attributed these peaks to the relaxation processes following the attainment of the limiting current. The specific mode of relaxation is not treated in this paper[41] although it may be presumed that relaxation is meant by LeBlanc to be the current decay which follows the system's

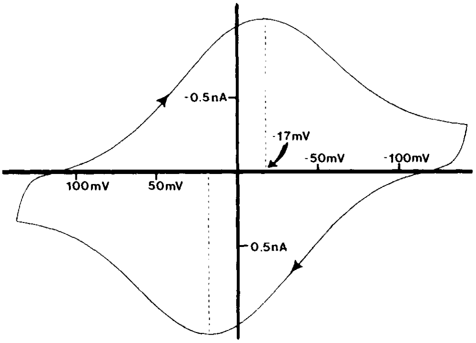

Figure 7 *Voltammogram of membrane-partitioned cell containing* 10 μM *tetraphenylborate. Experimental details as in ref.* 47, *excepting the three-electrode configuration which was used here*

attainment of diffusion-limited migration of charge carriers. Other authors in more recent kinetic studies of ion migration express the kinetic equations in the form of a biphasic exponential decay, and similarly use the term relaxation when referring to the kinetics of ion transport.[12,46] However, the application of polarography to problems of the electrochemistry of model membrane processes did not progress much beyond its inception.

Figure 7 illustrates a voltammogram obtained for a bilayer membrane-partitioned cell to which tetraphenyl borate ($T\varphi B^-$) has been added.[47] As indicated, there occurs a peak in association with each sweep of potential corresponding to a cyclic change in the polarity of the membrane. The voltammetric peak behaves in a manner similar to that observed in an electrodic process;[47] there is a linear dependence of peak current ($i_{\text{max.}}$) upon both the square root of the polarization rate and $T\varphi B^-$ concentration. One can also observe small effects in the shape of the profile if other specific ions are added (Bender, unpublished), much as was first demonstrated by Liberman and Topaly.[29] In fact, the original polarography data[29] for bilayers can be reproduced by voltammetric means. In the light of what is now known about the electrochemistry of liquid/liquid interfaces, and the apparent analogy between these processes and those occurring at the metal/electrolyte interface (*cf.* refs. 25—27), it seems worthwhile to re-examine the dynamic *i*–*V* curves of membranes with the hopes of acquiring a deeper understanding of membrane physical chemistry.

In a voltammetry experiment the important measured parameters are the peak potentials and the corresponding currents. The midpoint potential, defined as the average of the peak potentials obtained on the forward and reverse scans, can be related to thermodynamic quantities, for example, the midpoint potential of a redox couple for an electrode process,[28] or the equilibrium phase partition in an ITIES cell.[26] For a membrane-partitioned cell in which the ionic distribution is symmetric the midpoint potential is invariably zero. When ionic conditions are asymmetric the midpoint potential is equal to the open circuit potential $\frac{59}{n} \ln (C_\alpha/C_\beta)$. This fact is consistent with the notion of a partition of ions between two phases and permits one to define a membrane overpotential,[47] but it is not at all informative with regard to the energetics of the membrane-transport process. One must instead look to other parameters for thermodynamic information.

The peak separation of a given voltammogram is often taken as an indicator of reversibility (in the Nernstian sense) of the charge-transfer process. For a reversible electrode process, peak separation is given by the relation $\frac{RT}{nF}$, or $\frac{59}{n}$ mV at room temperature.[48] Another indicator of a reversible process is a linear relationship between peak current and the square root of the polarization rate, concurrent with an independence of peak potential and polarization rate.[48] Although there is no reason *a*

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[46] J. P. Dilger and R. Benz, *J. Membrane Biol.*, 1985, **85**, 181.

[47] C. J. Bender and H. T. Tien, *Anal. Chim. Acta*, 1987, **198**, 259.

[48] R. S. Nicholson and I. Shain, *Anal. Chem.*, 1964, **36**, 706.

priori for not applying these criteria to the membrane-partitioned cell, it is apparent from the voltammogram obtained from the $\mathrm{T\varphi B^-}$-containing cell that a problem arises in the interpretation of peak separation. The separation of 34 mV (see Figure 7) is inconsistent with an expected result of 59 mV due to the unitary charge of $\mathrm{T\varphi B^-}$.

One way to interpret this discrepancy is to conclude that the unit of charge transferred is actually 2. Suggestions have been made that lipophilic ions act as carriers of their counterions.[49] If this is the case, one might then imagine a pool of $\mathrm{T\varphi B}$–Na within the membrane that discharges $\mathrm{Na^+}$ and $\mathrm{T\varphi B^-}$ from opposite sides of the membrane when a voltage is imposed. Such a mechanism would be characterized by an $n = 2$ charge-transfer process. No direct test (*e.g.* labelled Na) has been attempted, however concentration gradients of Na–$\mathrm{T\varphi B}$ across the membrane yield Nernst potentials implying a unitary charge. Replacement of $\mathrm{Na^+}$ with a lipophilic cation (*e.g.* tetrabutylammonium) had no effect on the voltammetric response (Bender, unpublished); it had been reasoned that since tetrabutyl ammonium can get into the membrane phase of its own accord, what may be a concerted $n = 2$ charge-transfer step might be replaced by two $n = 1$ steps with one being rate limiting. The results remain inconclusive.

In practice, examples in which a lipophilic ion carrier delivers another ion into a 'hostile' environment for reaction are common.[50,51] Despite this, however, no ITIES experiments report any discrepancies of the type where anomalies in charge transferred are observed. The problem does not reside with the ITIES experiment; it has been clearly demonstrated that ionic charge-transfer across a liquid/liquid interface is subject to the same criteria as the common electrodic processes at metal/electrolyte interfaces.[23–26]

There do exist electrochemical cells for which the $\frac{59}{n}$ mV does not apply. In the thin layer voltammetry cell[52,53] peak separation is zero for a reversible process and deviates from zero for irreversible processes as the rate of the charge-transfer process decreases or the activation barrier of the process becomes asymmetric. The cell containing $\mathrm{T\varphi B^-}$ exhibits the onset of irreversible behaviour (variation of peak potential as polarization rate is changed) at scan rates above 500 $\mathrm{mV.s^{-1}}$,[47] and deLevie[54] has reported on the adsorption of $\mathrm{T\varphi B^-}$ onto the surface of membranes *via* admittance measurements. These experiments suggest that there exists some degree of irreversibility associated with $\mathrm{T\varphi B^-}$ transport. If the membrane can in fact be regarded as a thin layer ITIES voltammetry cell, these data might be consistent with our expectations.

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[49] P. Läuger, R. Benz, G. Stark, E. Bamberg, P. C. Jordan, A. Fahr, and W. Brock, *Quart. Rev. Biophys.*, 1981, **15**, 513.

[50] E. V. Dehmlow and S. S. Dehmlow, 'Phase Transfer Catalysis', Verlag Chemie, Weinheim, 1980.

[51] C. M. Starks, *J. Am. Chem. Soc.*, 1971, **93**, 195.

[52] A. T. Hubbard and F. C. Anson, in 'Electroanalytical Chemistry', Vol. 4, ed. A. J. Bard, Dekker, New York, 1970, p. 129.

[53] F. E. Woodard and C. N. Reilley, in 'Comprehensive Treatise of Electrochemistry', Vol. 9, ed. E. Yeager, J. O'M. Bockris, B. E. Conway, and S. Sarangapani, Plenum Press, New York, 1984, p. 353.

[54] R. deLevie, N. G. Seidah, and G. Larkin, *J. Electroanal. Chem.*, 1974, **49**, 153.

In terms of its dimensions the membrane is certainly a thin layer system provided one considers the bilayer and its incorporated solvent as the thin layer, and the bathing solution as 'electrodes' capable of supplying ionic charge into the thin layer (compare Figures 4A and 4C). One problem associated with taking this simplistic approach is whether one can adopt the unique mass transport characteristics of a conventional thin layer electrochemical cell. The membrane is composed of lipids whose behaviour is typically considered to be liquid crystalline; the geometry of the cell allows one to disregard anisotropy in conductivity, but there are questions as to how the kinetic equations might be interpreted. With only a few exceptions, molecular aspects of diffusion in membranes are dropped to a first approximation, and the membrane is treated as a continuum. In this case, the system should closely resemble that of a thin layer electrochemical cell. The following sections examine several theories of membrane transport and attempt to reconcile voltammetric behaviour with predicted trends.

4 The Hole-fluctuation Theory of Ion Flux

The representative *i–V* curves of Figure 6 and Figure 7 may have already suggested to the reader that dielectric breakdown (*cf.* texts by Fröhlich,[55] Gallagher,[56] or Adamczewski[57] of the membrane might in part govern the electrical behaviour of the membrane-partitioned cell. Due to the extremely thin nature of the bilayer, application of even a moderately small potential across the membrane induces a great electric-field gradient. When comparable electric fields (10^5 V.cm^{-1}) are imposed across electrolyte solutions, a behaviour similar to that illustrated in Figure 8A (see p. 334) is observed and is known as the Wien Effect.[58] The rapid rise in conductance at high applied fields is attributed to what amounts to a breakdown of the solution dielectric as ions are stripped of their 'ionic atmosphere'.[58,59]

Direct experimental evidence implicates such a mechanism in the case of membranes under a large applied field. For example, all *i–V* curves are bounded by a large rise in conductivity which is associated with a voltage at which the membrane ruptures. Furthermore, if one observes the membrane through a low-power microscope during the cyclic modulation of voltage there are seen electromechanical effects (see Section 7 below) which suggest varying degrees of stress imposed upon the thin film. The fact that these phenomena are observed for all membranes and emulsions has led to the development of a model which has recently appeared in a review.[60] The model has as its basis the statistical mechanics of evaporation/condensation and represents an attempt towards a unified theory of membrane/thin film properties.

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[55] H. Fröhlich, 'Theory of Dielectrics: Dielectric Constant and Dielectric Loss', Clarendon Press, Oxford, 1949.

[56] T. J. Gallagher, 'Simple Dielectric Liquids: Mobility, Conduction, and Breakdown', Clarendon Press, Oxford, 1975.

[57] I. Adamczewski, 'Ionization, Conductivity, and Breakdown in Dielectric Liquids', Taylor and Francis, London, 1969, Part IV.

[58] H. Falkenhagen, 'Electrolytes', Clarendon Press, Oxford, 1934, pp. 82—9; 225—39.

[59] S. I. Smedley, 'The Interpretation of Ionic Conductivity in Liquids', Plenum Press, New York, 1980, Ch. 2.

[60] D. Exerowa and D. Kashchiev, *Contemp. Phys.*, 1986, **27**, 429.

The hole-fluctuation theory of Exerowa and Kashchiev[60] is based on a mechanistic construct, that is to say it is not founded directly upon an observable, but rather inferred from phenomenological truths: (1) membranes (emulsions and films) have finite lifetimes which vary; (2) membranes (emulsions and films) are also permeable (to some degree) to ions and solvent. The authors of this model introduce the notion of holes as a dynamic appurtenance of thin films and subject the rates of appearance and growth of these holes to conditions outlined by the statistical thermodynamics of condensation (Exerowa and Kashchiev[60] cite the text of Pound[61] as a source). The membrane is regarded as '... a two-dimensional system populated by microscopic holes formed by the fluctuation of vacancies (*i.e.* molecule-free sites) in the bilayer.'[60] In this sense holes in the membrane may be treated in a manner somewhat akin to a two-dimensional Ising model. The random appearance and growth (*i.e.* aggregation) of holes governs the permeability and ultimate stability of a thin film by granting access to molecules or wreaking sufficient havoc upon the film's structure that it ruptures. This latter process is considered to be that which determines the lifetime of the membrane.

It is the work of hole formation which determines the extent to which holes sufficiently large to accommodate small molecules are created. The work function is composed of two terms which are summed, one having statistical origins, and the other related to the surface tension of the hole's boundary. Expressed as

$$W_i = ikT \ln (C_e/C) + P_i \qquad (1)$$

for an i-sized hole, the logarithmic term represents a supersaturation energy which depends on the ratio of vacancy densities (defined as that density '... at which an infinitely large hole in the bilayer remains in equilibrium with the surrounding vacancy "gas"'[60] and the total density of vacancies.[62,63] This term is, therefore, designated as a thermodynamic driving force for the condensation of holes.[60] The variable P_i is called the process work and represents a product of the area of the hole's rim and an energetic term which the authors call the 'specific free-energy of the hole edge'.[60] The energy of the hole edge is equivalent to the surface tension of the lipid in the region surrounding a hole.

The model is inspired by the authors' desire to unify under a single theoretical mechanism the phenomena of membrane stability and permeability.[60] These phenomena are linked by the observation that under an applied potential there are observed an increase in membrane permeability and a decrease in membrane stability (as measured by a membrane's lifetime). These observations are attributed to a reduction of the effective work necessary to create a hole of a given size, *i.e.*

$$W_{\mathrm{eff}} = W_i - iqV^2 \qquad (2)$$

where W_i is the work term in the absence of an applied field, q is equal to

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[61] J. P. Hirth and G. M. Pound, 'Condensation and Evaporation', Pergamon Press, Oxford, 1963.

[62] D. Kashchiev and D. Exerowa, *J. Colloid Interface Sci.*, 1980, **77**, 501.

[63] D. Kashchiev and D. Exerowa, *Biochim. Biophys. Acta*, 1983, **732**, 133.

$(\varepsilon_m/\varepsilon_s - 1)AC_m/4$ (where ε_m, ε_s, C_m, and A are the dielectric constants of membrane and solvent, membrane capacitance, and area of the hole), and V the applied potential. It is by facilitating the formation and growth of holes that membrane permeability and stability are affected in the observed manner.

As appertains to observed *i–V* curves, the hole-fluctuation theory is attractive because it rationalizes the tendency of all *i–V* curves to exhibit behaviour often associated with dielectric breakdown of conductors. The modified work function yields a voltage-dependent permeability,

$$P(V) = P_0 \exp(iqV^2/kT) \tag{3}$$

Where P_0 is the permeability coefficient in the absence of an applied field.[60] This expression for a variable permeability can be incorporated into flux equations. For example, the Goldman equation expresses the current flux as[64]

$$J = -P\frac{zFV}{RT}\frac{C_\alpha - C_\beta \exp(zFV/RT)}{1 - \exp(zFV/RT)} \tag{4}$$

where P is ordinarily taken as a constant. Heinz[65] points out the inadequacy of the simple Goldman equation in describing 'real' systems as being its inherent tendency towards predicting ohmic i–V curves unless $C_\alpha \neq C_\beta$. Replacement of P in equation (4) with the voltage dependent $P(V)$ of equation (3) corrects this deficiency, although this is not the solution Heinz and others adopt.

The hole-fluctuation theory succeeds in that it interrelates membrane stability and permeability. When used to modify the Goldman Equation, the *i–V* behaviour illustrated in Figure 6A is reproduced, and the permeability factor P_0 can be used to adjust for differences among different ions. As might be reasoned, the P_0 term must incorporate chemical factors such as phase partition or reaction of electrolyte with lipid, which follows from conventional definition (*cf.* ref. 66). There are no means by which current-limiting behaviour (Figure 6B) might be described by the modified form of equation (4), but such behaviour might be described adequately by modifying the current expressions with terms accounting for the ratio of hole formation and delivery of ions to the membrane surface. How one would use this theory to predict *i–V* behaviour of carrier-mediated transport is unclear; in all likelihood one would have to turn to an entirely different description.

5 Continuum Theories of Ion Flux

Other theoretical approaches to the problem of membrane permeability disregard the microscopic dynamics of the membrane structure in the sense that heterogeneous defects such as holes and channels are relegated to the category of a special case (now becoming a rapidly growing subfield of membrane biophysics). Instead, passive and voltage-induced diffusion of ions is regarded as a continuum problem; a charge of infinitesimal dimensions must cross the interface between

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[64] D. E. Goldman, *J. Gen. Physiol.*, 1944, **27**, 37.

[65] E. Heinz, 'Electrical Potentials in Biological Membrane Transport', Springer-Verlag, Berlin, 1981, Ch. 2.

phases of different dielectric constant, generally without regard for the dimensions of the layers.[65,66] Some variations of the continuum model have been proposed to account for collisional encounters between migration ion and lipids,[67,49] but deLevie counters this contention as to the importance of microscopic collisions, indicating that the continuum model fails only at high concentrations of ions in a space-charge layer approaching molecular dimensions.[38]

Common to both the dielectric continuum and liquid solution theoretical approaches to membrane permeation is the notion that an energy barrier determines the kinetics of transport. This barrier may be introduced as an energetic requirement due to either the injection of a charge into a low-dielectric medium (*cf.* ref. 65) or displacement of position in the partially organized array of the membrane.[67,68] The shape of the barrier varies (Figure 8); for the most part, the dielectric continuum barrier is represented by a triangular hyperbolic function,[65] whereas the molecular liquid model employs a more complicated barrier function representative of multiple encounters.[67,68] A more recent paper[69] dealing with barriers resulting from the dielectric model examines the effect of introducing rhombic functions (Figure 8C) upon the kinetics of ion transport, and in so doing blurs the distinction between the barriers of dielectric and liquid solution models. Incidentally, the shapes of these barriers resemble those which one would predict on the basis of Overbeek's principle of summing double layers which overlap.[70]

Kinetic parameters are determined by the shape of the energy barrier which in turn is affected by forces. In the specific case of a membrane-partitioned electrochemical cell, this force is electromotive. With this rationale, it is a relatively simple matter to obtain a set of equations which describe the experimentally observed electrodic behaviour of voltage-induced ion transport across membranes. These equations may be adopted with suitable modification from the familiar derivations of equations describing electrode processes, such as those derivations which appear in standard texts (*e.g.* ref. 28). The modification consists of the re-definition of the equilibrium potential from which the overpotential is defined. Ordinarily, when considering a redox couple in equilibrium with a metal electrode, a potential defined by $E_0 = \frac{RT}{nF} \ln (C_{ox}/C_{red})$ is attained. Overpotential is defined as the difference between the applied potential and the equilibrium potential.[28] In the case of a membrane partitioning solutions of an electrolyte of concentrations C_α and C_β, a potential defined as $E_{0,m} = \frac{RT}{nF} \ln (C_\alpha/C_\beta)$ is measured, the absolute polarity being determined by the orientation of the cell. Experiments in which voltammograms are obtained from membrane-partitioned cells have demonstrated that such voltammograms have $E_{0,m}$ as their midpoint poten-

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[66] E. L. Cussler, 'Diffusion, Mass Transfer in Fluid Systems', Cambridge University Press, Cambridge, 1984, Ch. 15.

[67] R. Parlin and H. Eyring, in 'Ion Transport Across Membranes', ed. H. T. Clark, Wiley, New York, 1954, p. 103.

[68] N. E. Starzak, 'The Physical Chemistry of Membranes', Academic Press, Orlando, 1984, Ch. 11.

[69] J. E. Hall, C. A. Mead, and G. Szabo, *J. Membrane Biol.*, 1973, **11**, 75.

[70] J. T. Overbeek, in 'Colloid Science', Vol. 1, ed. H. R. Kruyt, Elsevier, New York, 1952, p. 247.

tial[47,71,72] For a membrane system one can therefore define an overpotential in a fashion analogous to that described above:

$$\eta = V - \frac{RT}{nF}\ln(C_\alpha/C_\beta) \qquad (5)$$

although $E_{0,m}$ is not a true equilibrium potential in a rigorous sense.

With this definition and the theoretical formalism of standard texts,[28] one arrives at equations for diffusion-limited and kinetically-limited rates of charge transfer,

$$i = i_0\left\{\frac{C_\alpha(0,t)}{C_\alpha}\exp(-anF\eta/RT) - \frac{C_\beta(0,t)}{C_\beta}\exp[(1-a)nF\eta/RT]\right\} \qquad (6)$$

and

$$i = i_0\{\exp(-anF\eta/RT) - \exp[(1-a)nF\eta/RT]\} \qquad (7)$$

respectively. Here the notation of Bard and Faulkner[28] has been used; the parameter a represents a measure of the symmetry of the energy barrier, and $C_i(0, t)$ is a time-dependent function expressing the concentration of the ion at the membrane surface ($x = 0$). The C in the denominator corresponds to the bulk solution ion concentration. The problem in applying these equations to membrane processes lies in the interpretation of the various parameters. As mentioned previously, peak potentials, separation of peaks, and voltammogram shape are ordinarily indicative of the kinetic aspects of the charge-transfer process (*cf.* refs. 28 and 73). However, it is the fact that membrane science is accursed with a plethora of models of ion transport which deranges a simple interpretation of voltammetric parameters. At the present level of understanding one is resigned to the collection of experimental data and comparing trends with expectations based on sundry models.

It is known that the voltammogram of membrane systems exhibit variations which depend upon the ion acting as the charge carrier,[29,47] although as yet no careful study has been attempted in order to compare observed peak potentials and the ion's affinity for the membrane phase. There is also the lipid composition of the membrane, organic solvent incorporated during membrane formation, *etc.* to consider. Some preliminary evidence of a qualitative nature is presented in the paper of Bender and Tien,[47] where voltammograms obtained from cells containing $T\varphi B^-$ and some biological stains clearly differ. It was found that peak potentials of Gentian Violet and Janus Green B were much greater than that of $T\varphi B^-$ and it might be inferred that, stains tending to bind materials, the energetic demands of ionic dye transport are greater than that of $T\varphi B^-$.[47]

Peak potentials have also been found to vary with membrane thickness, based on rather simple experiments in which the voltage is cycled during membrane

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[71] C. J. Bender and H. T. Tien, *J. Electroanal. Chem.*, submitted.

[72] C. J. Bender and H. T. Tien, *Anal. Chim. Acta*, 1987, **201**, 51.

[73] K. J. Vetter, 'Electrochemical Kinetics: Theoretical and Experimental Aspects', Academic Press, New York, 1967.

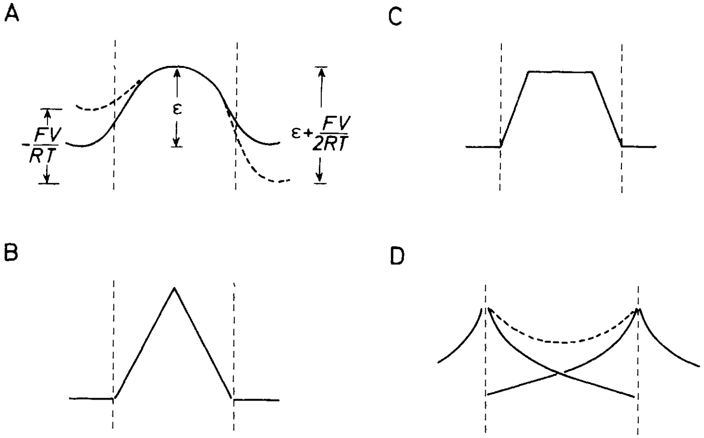

Figure 8 *Barrier models used to derive trans-membrane ion transport kinetics. Panel D illustrates the principle of summation for overlapping double layers, which gives a result identical to the empirical models*

formation (Bender and Tien;[47] P. Krysinski, personal communication). During formation the membrane thickness decreases until it finally attains the bilayer or 'black' state. In the experiments cited, peak potentials in excess of 150 mV are obtained in $T\varphi B^-$-containing cells shortly after membrane formation, and the potential steadily decreases with time as the membrane achieves its 'black' state. Once reaching the 'black' or bilayer state the peak potential remains invariant. Again, these results suggest the increased energetic requirements or kinetic effects of a thicker membrane, and not a retardation due to the increasing organization of the constituent lipids (P. Krysinski, personal communication.) This result is a potent argument against the need to consider microscopic or collisional properties of the membrane.

The continuum models of ion transport readily accommodate the relationship between membrane thickness and changes in peak potential. The ion in a membrane is treated as a charge between plates of a capacitor, and the energy of the charge is determined by the dielectric constant of the solvent (which may be incorporated between the bilayers) and the thickness of the membrane. The Born charging equation was applied to this problem by Pickar and Benz[12] who calculated the energy difference of charge in membrane capacitors of varying thickness, *i.e.*

$$\Delta W(d) = h\left[\frac{1}{d'} - \frac{1}{d}\right] \tag{8}$$

where $\Delta W(d)$ is the energy difference, d and d' the two membrane thicknesses being compared, and h a constant dependent upon the properties of the solvent (*e.g.* dielectric constant, *etc.*). The rate constant for a process depends exponentially upon the activation energy, therefore this energy difference affects the relative rates of ion translocation across membranes of differing thickness, yielding

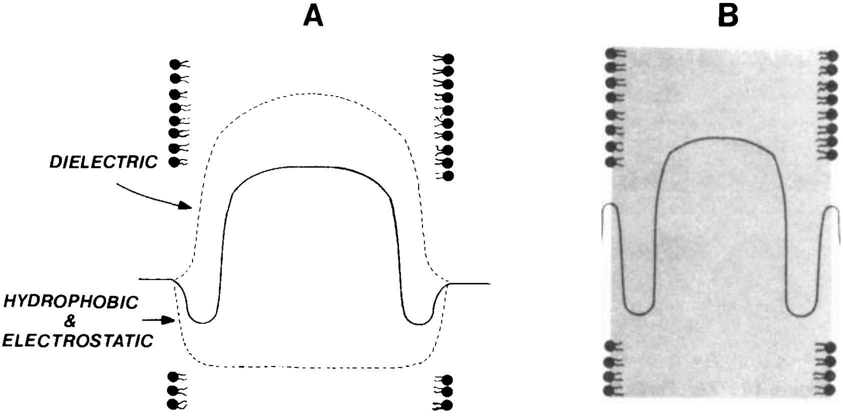

Figure 9 *Elaboration of the barrier models. Decomposition of the potential energy profiles without* (*A*) *an interfacial collisional component, and the same with collisional encounters* (*B*). (*After ref.* 49)

$$k/k' = \exp\left[-\Delta W(d)\right] \tag{9}$$

again citing the results of Pickar and Benz.[12] Relaxation experiments [12,46,49] have provided the data which best support this model, and the rate/thickness interrelationship of equation (9) concurs with the interpretation of voltammetry experiments in which peak separation diminishes as the membrane thins.[47]

Refinements in the nature of the potential barrier are introduced in a review by Läuger *et al.*[49] The major repulsive force which determines the height of the barrier remains the dielectric properties of the thin organic layer, but electrostatic and hydrophobic attractive forces are proposed to modify the resultant energy barrier in a manner outlined in Figure 9. The authors also state[49] that the single energy barrier representing the repulsive dielectric contribution may be a composite of smaller energy barriers which span the membrane. This notion of the composite barrier is actually an idea whose origin is the application of the absolute rate theory to membrane transport, as introduced by Parlin and Eyring.[67]

In the Parlin and Eyring model movement of ions is regarded as a site-to-site transfer of particles over a finite macroscopic distance, therefore the single barrier of Figure 8 is replaced by the more elaborate multiple well potential barrier illustrated in Figure 10. Formulation of the mathematical models consists of equating n consecutive rate equations parameterized as in Figure 10, with λ_i, c_i, and k_i representing distance, concentration, and rate of a single transition between two wells,[67] the flux is then

$$J_i = \lambda_{i-1}c_{i-1}k_{i-1} - \lambda_i c_i k_i \tag{10}$$

for a single well-to-well transition. Each flux for a given transition is a component

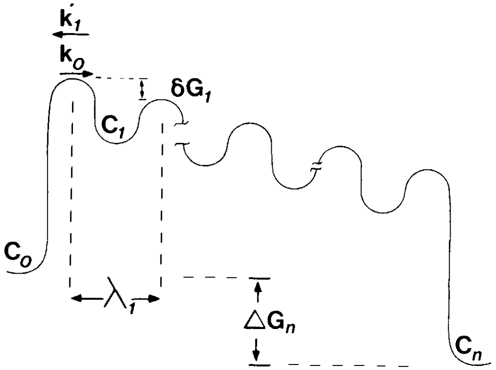

Figure 10 *The Parlin/Eyring model of membrane transport.* (*After ref.* 67)

of a set designated $\{J_i\}_{j=0}^{n-1}$. Under steady-state conditions the rate of ionic flux J is equal over all barriers and equal to the net flux across the membrane. Multiplying each element J_i (for i $\neq$ 0) by $\prod_{j=1}^{i} \frac{k'_j}{k_j}$ and summing terms gives (recalling that $J = J_i = J_j$, *etc.*)

$$J = \frac{\lambda_0 k_0 \left[c_0 - \frac{\lambda_n}{\lambda_0} \prod_{i=0}^{n-1} \frac{k'_{i+1}}{k_i} c_n \right]}{1 + \sum_{i=1}^{n} \prod_{i=1}^{i} \frac{k^j}{k^j}} \tag{11}$$

The rates are related to the heights of the individual barriers by

$$k_i = k_i \frac{kT}{n} \exp\left(-\Delta G_i^{\neq}/RT\right)$$

which permits equation (11) to be rewritten as

$$J = \frac{\lambda_0 k_0 \left[c_0 - \frac{\lambda_n}{\lambda_0} \exp\left(\Delta G_n/RT\right) c_n \right]}{1 + \sum_{i=1}^{n-1} \exp\left(\delta G_i^{\neq}/RT\right)} \tag{12}$$

where ΔG_n is the energetic difference between the zeroth and the nth minimum, and $\delta G_i^{\neq}$ is the peak-to-peak energy difference between site i $-$ 1 and i. For a membrane across which there is imposed a potential V, the difference in energy between the zeroth and nth minimum corresponds to $\Delta G = zFV$ and the problem becomes one of simplifying equation (12) by rearrangement into a form which permits an approximate solution.

Noting that the rate of entry into the membrane is altered from k_0 to $k_0^0 \exp\left(zFV/RT\right)$ when the potential is applied, and that $\delta G_i^{\neq} = \frac{izFV}{n}$, the authors[67] define the variable $x = \exp\left(zFE/nRT\right)$ which allows the rate equation to be written

$$J = \frac{\lambda_0 k_0^0 x^{-\frac{1}{2}}\left(c_o \frac{\lambda_n}{\lambda_0} x^n c_n\right)}{\sum\limits_{j=0}^{n-1} x^j} \tag{13}$$

which, strictly speaking, is not rigorous since the term $x^{-\frac{1}{2}}$ does not arise naturally from the change of variables. The $x^{-\frac{1}{2}}$, however, does permit (again, with certain approximations) the reduction of equation (13) to

$$J = P\left[c_0 \exp\left(-zFV/RT\right) - \frac{\lambda_n}{\lambda_0} c_n \exp\left(zFV/2RT\right)\right] \tag{14}$$

where $P = \lambda_0 k_0^0/n$ is a permeability function. Further simplification results from the expansion of the exponential and retention of the first terms only, leaving

$$J = P\left[\left(c_0 - \frac{\lambda_n}{\lambda_0} c_n\right) - \frac{1}{2}\left(c_0 - \frac{\lambda_n}{\lambda_0} c_n\right)\frac{zFV}{RT}\right] \tag{15}$$

in which diffusion and voltage-induced transport rates contribute.[67]

The Parlin/Eyring model likewise predicts the qualitative reduction in transport rates as membrane thickness increases (taken as either an increase in the number of sites, n, or an increase in the magnitude of λ_i), however despite the arguments of its advocates,[68] there seems to be no particular advantage in formulating a model on the basis of multiple site-to-site transitions. The approximations are certainly no better than those of assuming a continuum from the onset; in fact, equation (15) can be used to derive the expression describing the liquid junction potential of Planck, upon which the continuum models are based. Since this is true it is possible to use the Parlin/Eyring model to examine intramembrane charge distributions and energetics in terms of a theory familiar to all physical chemists. The model also permits a comparison of membrane transport and particle motion in homogeneous fluids.

Parameters such as well depth and number can be varied with interesting results (see refs. 67 and 68), but the model provides little information as to the manifestation of solute–solvent interactions in membrane transport. Currently popular modifications of similar theories, such as those describing ionic conductance in solids,[74,75] include the Langevin model (*cf.* ref. 76). This model holds that particle motion is akin to a Brownian dynamical description in which collisions are inelastic.[76] Such modifications to the Parlin/Eyring theory might permit a more definitive parameterization of transport rates (and *i*–*V* curves) in terms of molecular properties.

The most thorough mathematical model of membrane transport is that of deLevie and co-workers[36–39] which, like the theory of Pickar and Benz,[12,49] treats the membrane as a continuum. After making this basic assumption, the Nernst–

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[74] S. A. Adelman and J. D. Doll, *Acc. Chem. Res.*, 1977, **10**, 378.

[75] S. A. Adelman and J. D. Doll, *J. Chem. Phys.*, 1976, **64**, 2375.

[76] N. G. van Kampen, 'Stochastic Processes in Physics and Chemistry', North-Holland, Amsterdam, 1981.

Planck and Poisson equations (*cf.* ref. 28) are solved for the one-dimensional motion of the charge carrier taken as normal to the membrane plane. These equations are

$$J = zFD\left\{\frac{dC}{dx} + \frac{zFC}{RT}\frac{d\varphi}{dx}\right\} \tag{16a}$$

and

$$\frac{d^2\varphi}{dx^2} = -\frac{4\pi zFC}{\varepsilon} \tag{16b}$$

where J is the current density, z the valency, φ the local potential, ε the dielectric constant, and D the diffusion constant.[36] It should be apparent that (16a) is equivalent to the result obtained from the Parlin/Eyring derivation, which is expressed as a difference equation. Solution of these equations yields hyperbolic and trigonometric functions of real variables which describe the potential, field, and concentration variation across the membrane.[36,77,78]

The numerical results obtained following selective variation of the parameters in the solutions to equations 16 indicate that the distribution of ions within the membrane varies from homogeneous to space-charge layered as the bulk ionic concentration increases.[36] This behaviour compares with Gouy–Chapman diffuse double-layer theory in which there exist two opposing double layers capable of overlap, depending on the dimensions of the system. In this regard, deLevie's model derives behaviour intuitively predicted from Overbeek's theory[70] of opposing/overlapping double layers. In fact, the extent to which this model is parameterized permits one to make a complete analogy between Gouy–Chapman double layer theory and this model of ion distribution within the membrane. This latter point is stressed by the authors of the model.[36]

When subjected to an applied electrochemical potential gradient the solutions to the differential equations are altered in ways that affect their symmetry,[37] and for proper treatment of voltage-induced ion transport the model must be augmented by the contributions of the aqueous layers adjacent to the membrane surface.[38] With this adaptation the assumptions are made that there exists a Nernst diffusion layer of dimension x_w in the aqueous interface, and that a partition coefficient can be defined, $K = \frac{C_w}{C_m}$. With the Nernst diffusion layer the current density is modified to yield[38]

$$i = \frac{zFD_w[C_\alpha - C_\alpha(0, t)]}{x_w} = \frac{zFD_w[C_\beta - C_\beta(0, t)]}{x_w} \tag{17}$$

in which D_w is the aqueous diffusion coefficient of the ion, and other terms are as

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[77] R. deLevie, in 'Advances in Chemical Physics', Vol. 37, ed. I. Prigogine and S. A. Rice, Wiley, New York, 1978, p. 99.

[78] R. deLevie, *J. Electroanal. Chem.*, 1976, **69**, 265.

defined in equation 7. For convenience of manipulation and expression in numerical simulations deLevie uses dimensionless variables, and, in keeping with the original derivation, defines

$$\gamma = \frac{4\pi z^2 F^2 x_m K C_w}{\varepsilon_m RT}$$

as the dimensionless aqueous phase ion concentration.[38] This change of variables allows equation (17) to be written

$$i = \frac{D_w x_m[\gamma_\alpha(0,t) - \gamma_\alpha]}{D_m x_w K} = \frac{D_w x_m[\gamma_\beta(0,t) - \gamma_\beta]}{D_m x_w K} \tag{18}$$

and the coefficients to be incorporated into one constant, $K' = \frac{D_w x_m}{D_m X_w K}$. Setting $\gamma_i = 0$ yields the limiting current, defined as

$$i_{l\alpha} = K'\gamma_\alpha(0,t) \tag{19a}$$

and

$$i_{l\beta} = K'\gamma_\beta(0,t) \tag{19b}$$

The form of a polarographic wave or *i–V* curve may be obtained if one holds that the membrane behaves in a Nernstian manner despite the non-equilibrium condition. Substituting equations (19) into the Nernst relation, $\exp(v) = \gamma_\beta/\gamma_\alpha$, yields[38]

$$v = \ln\frac{i - i_{l\beta}}{i_{l\alpha} - i} \tag{20}$$

Equation (20) can be obtained in a more satisfactory manner from the constant field (*i.e.* Goldman) equation

$$i/v = \frac{\gamma_\alpha \exp(v) - \gamma_\beta}{\exp(v) - 1}$$

Substitution of (19) into this dimensionless form of equation 4 (derived in ref. 37) gives

$$i = \frac{K'[i_{l\alpha}\exp(v) + i_{l\beta}]}{\exp(v) - 1 + K'v[\exp(v) + 1]} \tag{21}$$

from which are obtained equation 20 and the limiting cases described by LeBlanc,[41] with the imposition of suitable conditions.[38]

This continuum model not only describes the variation of *i–V* behaviour as a function of ion concentration, but also explicitly demonstrates the contribution of membrane thickness and membrane/water partition of the ion. These latter factors are shown *via* numerical simulation to influence the slope of the *i–V* curve,[38] and therefore the location of the voltammetric 'peak'. In this regard the *i–V* behaviour of membranes differs from that of a metal/electrolyte (*cf.* refs. 78, and 79 Chapter 1). The *i–V* behaviour of membranes is ohmic, and deviates only as a result of kinetic factors. In electrode reactions, the applied potential must exceed a minimum determined by the thermodynamics of the charge-transfer reaction before the *i–V* curve begins to change markedly. Effects such as dielectric breakdown are not included in the mathematical formalism of deLevie's theory in the sense that simulated *i–V* curves possess the superposition of current-limited and breakdown profiles, however breakdown may be inferred from the charge density and potential profiles which may be simulated.[36]

It is ordinarily frustrating when each proposed model seems to agree with experiment, but this fact perhaps underscores the fundamental equivalence of these theories. The Parlin/Erying adaptation of the absolute rate theory can be used to derive equations which form the basis of continuum models. Furthermore, the transient behaviour as obtained in relaxation experiments is incorporated within the extensive mathematical formalism of deLevie (*cf.* refs. 39 and 78). The analysis of deLevie *et al.* is exhaustive with regard to its complete treatment of the electrical properties of the membrane interface, and readily meshes with analogous theoretical formulations of the nature of interfaces (see also refs. 10,80,81). Although the deLevie model does not establish a temporal description of membrane stability, it does provide a means by which to examine the driving force behind all phenomena. For example, conditions such as ion concentration gradients and imposed voltages, which Exerowa and Kashchiev cite as the driving force for enhanced hole formation and growth, give demonstrable changes in the potential profile within the membrane.[36,37] Potentials approaching dielectric breakdown voltages are predicted as concentration gradients are imposed,[36] thereby establishing the causal basis of stability.

6 Membranes and the Liquid/Liquid Interface

The ITIES system is generally construed to mean those liquid/liquid interfaces which include systems such as unsupported liquid phases, liquid phases soaked in a porous material, and polymeric films.[82] Bilayer membranes are not typically included in this list. Figures 4A and 4B illustrate the basic difference between membrane and ITIES systems. However, it is clear that ions which are to be carriers in either system must be 'semihydrophobic). The definition of a semihydrophobic ion is taken to be the general classification of ions which are soluble to some extent in both water and non-aqueous solvents.[25] Tetra-alkylammonium and tetraphenyl-borate are typical examples by virtue of the hydrophobic organic moiety which shields the charge of each ion. The Gibbs free-energy of phase transfer may

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[79] G. Charlot, J. Badoz-Lambling, and B. Tremillon, 'Electrochemical Reactions', Elsevier, Amsterdam, 1962, Chs. 1&2.

[80] J. D. Reid, O. R. Melroy, and R. P. Buck, *J. Electroanal. Chem.*, 1983, **147**, 71.

[81] O. R. Melroy and R. P. Buck, *J. Electroanal. Chem.*, 1983, **151**, 1.

[82] J. Koryta, *Electrochim. Acta*, 1987, **32**, 419.

be determined from the partition coefficient and used to establish criteria for the observation of a voltammetric response for a given ITIES cell.[24,25]

When one does obtain a voltammetric peak, energetically it may be related to the Gibbs free-energy of transfer, and its behaviour likened to that of electron transfer at the metal/electrolyte interface.[25] Although the analogy between the metal/electrolyte and liquid/liquid interface is often made, this is not altogether valid since there exists no Stern or 'inner' layer to drive charge transfer in the ITIES system.[83,84] Girault advocates the use of the more accurate term 'mixed solvent layer' to describe the liquid/liquid interfacial region.[84]

Koryta and co-workers report results of ITIES experiments relevant to membrane transport.[85] In these experiments, a 'half bilayer' was formed by addition of amphiphile to the cell, and its presumed orientation at the interface (Figure 11). Interestingly, there is observed an inhibition of both semihydrohobic and facilitated ion transport in such a system.[25] The extent to which the current is inhibited (*i.e.* total or partial loss) is not reported.[25] However, a partial inhibition would be expected since the added amphiphile puts at the interface an array of oriented molecules. In addition to the physical barrier, there also exist charges on the hydrophilic side of these molecules, a factor which is bound to affect the charge density at the interface.

These results may reflect fundamental differences in the nature of the double layer possessed by the liquid/liquid interface and membrane/electrolyte interface. It is traditionally held[18,68,86] that the membrane double layer includes a Stern layer, in contrast to the ITIES system. Justification for this assumption that a Stern Layer exists is based on the tendency of materials to adsorb onto, or complex with, the polar surface of the membrane.[68] ^{31}P-n.m.r. has been used extensively in providing evidence for lipid interactions.

The explanation offered by Koryta[25] for the observed inhibition is that the hydration sheath of the ion must be stripped prior to penetration of the phospholipid layer. These same ions, however, readily penetrate bilayer membranes (although a direct comparison of conductivities is lacking). In support of his suggestion, Koryta[25] cites results which demonstrate an increase in conductance following the addition of alkali-earth metals to the monolayer system. It is suggested further that the alkali-earth metals form ion pairs in the hydrophobic region of the amphiphatic monolayer, thus enabling ions to penetrate further.[25] In any case, the effect of alkali metals is significant since it may reveal much about the role of Ca^{2+} in so-called channel phenomena.[87]

It is uncertain whether a hydration sphere of an ion can remain intact during translocation across a membrane, which leads us to consider one more complication peculiar to the double layers of membranes. A phenomenon known as 'flip-flop', in which lipid on one face of the bilayer rotates down into the opposing face,

[83] H. H. Girault and J. D. Schiffren, *J. Electroanal. Chem.*, 1984, **170**, 127.

[84] H. H. Girault, *Electrochim. Acta*, 1987, **32**, 383.

[85] J. Koryta, L. Q. Hung, and A. Hofmanova, unpublished results reported in ref. 25, p. 163—6.

[86] S. McLaughlin, in 'Current Topics in Membranes and Transport', Vol. 9, ed. F. Bronner and A. Kleinzeller, Academic Press, New York, 1977, p. 71.

[87] S. Hagiwara and L. Byerly, *Trends Neurosci.*, 1983, **6**, 189.

has been demonstrated experimentally[88-90] and proposed as a possible mechanism of diffusion past Tight Junctions between cells.[90] It might be imagined that ion transport across membranes could proceed by association of a lipid with an ion, followed by a 'flip-flop', and dissociation of the ion on the other side. Under equilibrium conditions rates of flip-flop are too slow to compare favourably with voltage-induced current fluxes. Flip-flop rates vary, however, as disequilibrium is imposed across the membrane (*e.g.* disparate ion concentration, pH, *etc.*).[91] In Koryta's system inversion of amphiphiles into the organic bulk phase is unlikely, but whether such differences could be responsible for the difference between ordinary ITIES and the monolayer coated ITIES experiments is a matter of speculation.

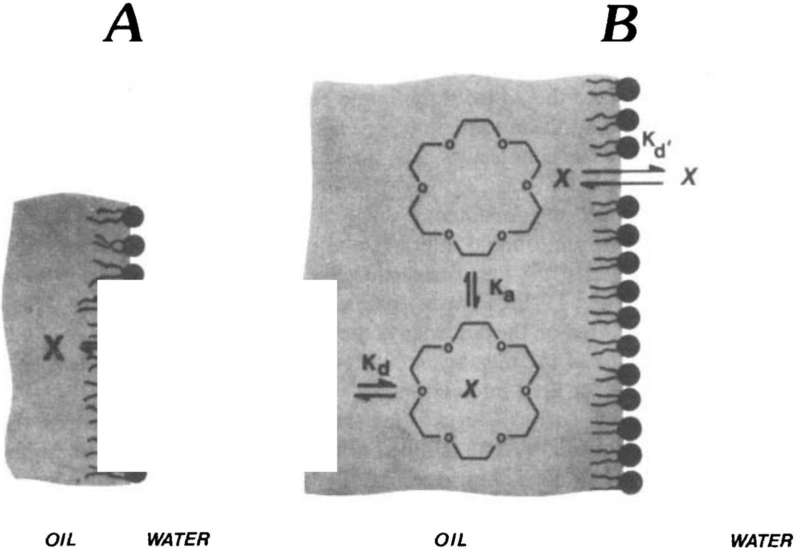

Figure 11 *Kinetic models of simple diffusional (A) and carrier-mediated (B) ion transport, based on the data reported in ref.* 25

7 Electromechanical Effects

The imposition of a potential across a membrane generates a large electric field in the direction perpendicular to the plane of the membrane. Such a field is capable of altering the non-rigid structure of the membrane. In many cases the amphiphatic lipids of which the membrane is composed possess permanent dipoles, and will therefore orient in an electric field. It might be expected that as the field

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[88] J. E. Rothman and E. A. Dawidowicz, *Biochemistry*, 1975, **14**, 2809.

[89] J. E. Roseman, B. J. Litman, and T. E. Thompson, *Biochemistry*, 1975, **14**, 4826.

[90] P. R. Dragsten, R. Blumenthal, and J. S. Handler, *Nature* (*London*), 1981, **294**, 719.

[91] L. D. Bergelson and L. I. Barsukov, in 'Structure and Properties of Cell Membranes', Vol. 1, ed. G. Benga, CRC Press, Boca Raton, 1985, p. 77.

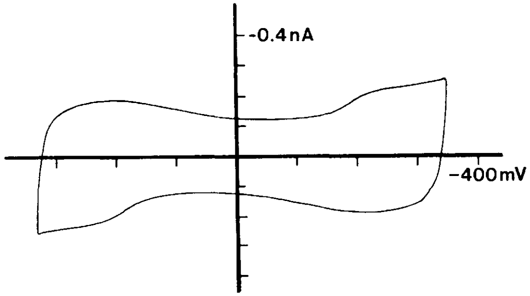

Figure 12 *Current–voltage profile of membrane-partitioned cell responding to electromechanical effect. Polarization rate is* 200 mV.s^{-1}

intensifies the dipoles will tend towards a more parallel alignment. A more general example of such ordering effects is known from studies of liquid crystals and is known as the 'flexoelectric effect',[92,93] and the so-called 'flexoelectricity'.

In the specific case of bilayer membranes, structural changes may be inferred from direct observation of the membrane during voltage modulation. Certainly optical methods (*e.g.* specular reflectance) are also suitable for the assessment of structural changes. Capacitance measurements might also be applicable, but changes in membrane structure might not be easily discerned from ionic surface charge accumulation. Qualitatively, it is possible to assess membrane thickness based on whether the membrane appears coloured or not (the 'black' state corresponding to the bilayer condition). The extent of the Plateau–Gibbs border annulus is also something of an indication of membrane structure, for as the annulus retreats from the membrane towards the hydrophobic support the membrane seems to become increasingly rigid or taut. The black planar surface also seems to become reticulated. Intuitive rationalization might be justified on the basis of surface tension and capillarity arguments.

During simple experiments one can observe cyclic advance and retreat of the Plateau–Gibbs border or coloured fringes thereof. These fringes are largest at potentials near zero and decrease (retreat) as one increases the magnitude of the applied potential (increasing field strength). The rate of physical change parallels the polarization rate, and concurrent electrical measurements at high sensitivity indicate small changes in the *i–V* curve (Figure 12). The changes in the *i–V* curve may reflect a change in the capacitance. Fortunately this effect is very small (less than 50 pA) and does not interfere with other measurements.

Electromechanical effects are mentioned here because they add a complication of which one must be aware. Additional information as to the physics of this phenomenon in membranes may be obtained from several papers and reviews.[94–96]

[92] P. G. DeGennes, 'The Physics of Liquid Crystals', Clarendon Press, Oxford, 1974.

[93] N. March and M. Tosi, 'Polymers, Liquid Crystals, and Low Dimensional Solids', Plenum Press, New York, 1984.

[94] A. G. Petrov and V. S. Sokolov, *Eur. Biophys. J.*, 1986, **13**, 139.

[95] A. G. Petrov, *Nouvo Cim.*, 1984, **3D**, 174.

[96] A. G. Petrov and I. Bivas, *Prog. Surf. Sci.*, 1984, **16**, 389.

8 Conclusions and Outlook

The intention of this paper has been to discuss the recent introduction of membrane voltammetry as a natural extension of the applications of voltammetry to liquid/liquid interfaces. Emphasis has been placed with the types of processes and underlying mechanisms from which a voltammetric response might originate. Unfortunately, as may be inferred from the previous sections, there exist several theoretical descriptions of membrane transport which may be applicable to the experimental results observed. Mention has been made of the similarity between the charge-transfer processes at membranes and their electrodic counterparts, and interpretation based on the theories of membrane transport has been offered in the spirit of stimulating further work into the study of membrane physical chemistry.

The effort expended in acquiring a physical interpretation of membrane voltammetric parameters is justified by the potential analytical applications of the methodology. It is known that many chemical agents such as pharmaceuticals, herbicides, and pesticides are administered in a non-specific manner, and that they reach their site of interaction by diffusion across the membrane. It would seem plausible that a voltammetric method could be useful to assay the kinetics and mechanism of the assimilation of these materials. Preliminary studies have elicited a voltammetric response for a membrane-partitioned cell containing the herbicide diuron, and the voltammogram is similar in form and behaviour to that obtained for tetraphenyl borate (Figure 13). Since membranes may be formed *in vitro* from extracts of living tissues, it should be possible to assess permeability of a given material in a membrane of given composition.

As yet untried is application of the method to whole tissue membranes. Numerous organ tissues (*e.g.* toad urinary bladder) possess ion-transport capabilities and are often examined by clamping within a membrane chamber in a manner analogous to the bilayer membrane. It would be a worthwhile experiment

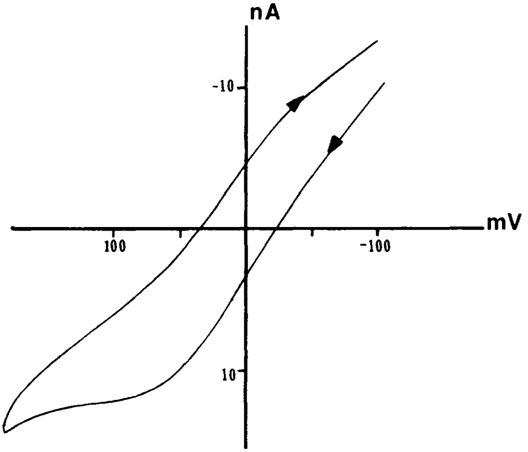

Figure 13 *Voltammogram of membrane-partitioned cell containing* 10 μM *diuron,* 0.1M KCl *supporting electrolyte. Bilayer membrane prepared from phosphatidyl choline*

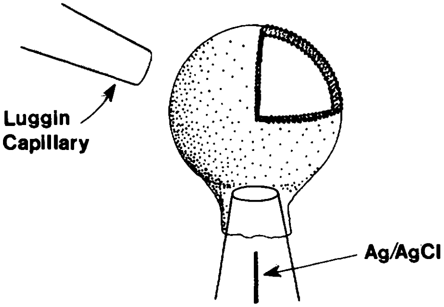

Figure 14 *Proposed arrangement for a membrane drop electrode to be developed from patch clamp apparatus*

to attempt such a study, especially since the voltammetric technique may permit otherwise unattainable kinetic information concerning ion transport and effects of substances such as oubain, an inhibitor of Na^+ and K^+ transport. Of particular interest would be an analysis of coupled transport systems: ion–ion, electron–ion, *etc.*

Besides ion transport, one might speculate as to whether receptor interactions at membranes might be subject to analysis by electrochemical methods, including voltammetry. Specific adsorption at electrodes is a process which may be studied by electrochemical methods, although similarities between electrode processes and membrane-based phenomena are at best tenuous. Makrlik has outlined an analysis of the voltammetric method applied to solution reactions involving a liquid/liquid interface,[97] and a similar analysis for membranes may be feasible. An indirect type of membrane interaction study that would most likely yield to voltammetric analysis is the assessment of membrane permeabilization agents and their effects. Kinetic information might be obtained for transient and reversible permeabilization agents, and the data used to assess cell-direct stimuli.

As for the technological development of the method itself, it is possible that a 'membrane drop electrode' analogous to the electrolyte drop[25,26] method of ITIES could be arranged. Patch clamp techniques (*cf.* refs. 98—100) use an electrode which is a hollow pipette tip onto which a spherical membrane 'patch' is affixed (Figure 14). The membrane patch may be artificially produced, extracted from tissue, or constitute an entire biological cell. The advantage of this approach is its proven performance in cellular transport studies, and, perhaps more importantly, the removal of the hydrophobic support of planar systems. In planar arrangements the membrane actually resides in a recess from the support's outer surface, a less than desirable feature in kinetic studies. The spherical 'electrode' surface also facilitates the mathematical analysis. This latter type of membrane electrochemical

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[97] E. Makrlik, *Z. Phys. Chem. (Leipzig)*, 1987, **268**, 200.

[98] 'Single-Channel Recording', ed. B. Sakmann and E. Neher, Plenum Press, New York, 1983.

[99] B. Sakmann and E. Neher, *Ann. Rev. Physiol.*, 1984, **46**, 455.

[100] 'Voltage and Patch Clamping with Microelectrodes', ed. T. G. Smith, H. Lecar, S. J. Redman, and P. W. Gage, American Physiological Society, Bethesda, 1985.

cell is expected to be most useful in the determination of the physical parameters of experimental significance. It cannot be stressed too strongly, however, that when a given system is under scrutiny, care must be taken to cross correlate data by disparate methods. Enthusiasm for electrochemical studies of the membrane systems should be tempered with this realization.

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