A METHOD TO RELATE STEADY-STATE IONIC CURRENTS, CONDUCTANCES, AND MEMBRANE POTENTIAL IN ION EXCHANGE MEMBRANES WITH UNKNOWN THERMODYNAMIC PROPERTIES

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ABSTRACT A method is presented by which the steady-state properties of an homogeneous, permselective membrane at uniform temperature can be predicted without knowledge of its thermodynamic properties other than assuming that they are functions only of local mole fractions in the membrane. By making this assumption, it is shown how the ionic conductances can be calculated at any point in the membrane from two sets of measurements, (*a*) R_{symm}, the steady-state resistance of the membrane measured between identical solutions and (*b*) V_0, the potential difference between nonidentical solutions for zero current. These two parameters are measured at different external solution compositions (e.g. a varying sodium-potassium ratio ranging from zero to infinity). From these measurements it is shown how the flux equations may be integrated without a knowledge of mobilities, activity coefficients, and other interior membrane parameters. The application of the method to fixed site membranes with variable mobilities is described and the theory for this particular case has also been verified experimentally in glass membranes.[1] A possible application to biological membranes is discussed and a comparison is made between the present treatment and previous treatments used to calculate the steady-state properties of cell membranes, notably the theory of Teorell, Meyer, and Sievers and the constant field theory.

In relating various transport phenomena in membranes such as fluxes, currents, and potentials, the usual procedure has been to separate the processes into interior diffusion processes and boundary equilibrium phenomena (Teorell, 1935; Schlögl, 1954; Conti and Eisenman, 1965). The diffusion equations may then be integrated between the interior boundaries of the membrane and combined with the boundary conditions to obtain the fluxes in terms of total membrane potential and external solution conditions. This procedure, however, requires that the mobilities, activity

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[1] Eisenman, G., and J. Sandblom. Data to be published.

coefficients, and pressure be either constant or else known functions of the concentrations in the membrane, which in many cases they are not.

It was found, for example, in the course of testing the membrane theory by Conti and Eisenman (1965) which assumes mobility ratio and difference in standard chemical potentials to be constant and implicitly assumes the individual mobilities to be independent of mole fractions, that the theory was insufficient in accounting for certain electrochemical phenomena in glass membranes.[1] The current-voltage relationships for these glass membranes were more complex than expected due to the variation of the mobilities with local mole fractions.[1]

A strong mole fraction dependence of mobilities has also been observed in membranes made of benzene-saturated polystyrene filters (Ilani, 1966). For these types of membranes the calculation of fluxes and of the current-voltage relationships become difficult and existing theories are inadequate. A more general theory is therefore needed and is presented in this paper.

A different method for solving the flux equations in steady state is used, in which the usual procedure of separating boundary and interior processes is circumvented and in which the thermodynamic variables in the membrane need not be known functions of the concentrations. It is therefore possible to calculate ionic fluxes in membranes of very complex behavior and due to the nonspecific assumptions involved, the present method also offers an advantage over the classical methods used to calculate the steady-state ionic fluxes in cell membranes (e.g. Hodgkin and Katz, 1949; Linderholm, 1952), which assume mobilities to be constant in the membrane.

In the following treatment the only assumption made about the thermodynamic properties of the membrane is that they are functions only of the local mole fractions of the permeable ions (i.e. the fraction of total concentration of cations or anions in the membrane). In addition the membrane is assumed to be homogeneous. The I-V relationships as well as the ionic currents are then defined entirely in terms of the local ionic conductances L_i which can be calculated from two sets of simple measurements [(*a*) R_{symm}, the steady-state resistance of the membrane measured between identical solutions and, (*b*) V_0, the total membrane potential for zero current] as will be explained in the text.

It will be shown that a membrane which is homogeneous and in which the thermodynamic properties depend only on local mole fractions will have a completely linear (ohmic) resistance in symmetrical solutions leading to a simple relationship between the symmetrical resistance R_{symm} and the local ionic conductances. The method is therefore not applicable to membranes which have a nonlinear resistance in symmetrical solutions.

A membrane of heterogeneous structure for example will in general show rectification in symmetrical solutions (Patlak et al., 1962). The same is true if the membrane parameters are not only functions of local mole fractions but also depend on external forces. A typical example in which this occurs is a mobile site membrane, where the sites can redistribute under the influence of an applied electric field and where the symmetrical membrane resistance will be highly nonlinear (Conti and Eisenman, 1966). A similar phenomenon may also be observed in the presence of water flow producing salt filtration and a redistribution of ions in the membrane (Teorell, 1961). The present treatment is therefore only applicable to fixed site membranes in the absence of water flow and when the thermodynamic properties depend only on local mole fractions.

The second set of measurements necessary to calculate the ionic conductances is provided by V_0, the total membrane potential at zero current. Although in principle the present treatment is valid for any number of ions of arbitrary valence, the only expression for V_0 which has been derived under the mentioned nonspecific conditions is applicable only to coion-impermeable membranes (Sandblom and Eisenman, 1967). Since furthermore the method has already proved to be successful in the description of transport phenomena in hydrated glass membranes where these conditions are fulfilled and where the ionic mobilities have been found to be functions of the degree of ion exchange,[1] we shall confine the treatment to ideally permselective (i.e. coion-excluding) membranes and later discuss some possible generalizations.

Several of the equations derived by this general method turn out to be similar or identical to the equations derived for fixed site membranes by Teorell (1953) in the limit of large fixed site concentration as well as to the equations obtained using the constant field assumption (Goldman, 1943; Hodgkin and Katz, 1949) in the limit of complete coion exclusion. The present treatment also contains the treatment of Conti and Eisenman (1965) as a special case. *There is however, a fundamental difference between previous and present treatments, namely that whereas the older theories always permit a calculation of concentration profiles and potential profiles within the membrane, the present method, due to the nonspecific assumptions, leaves the profiles undetermined.* We therefore conclude that characterization of transport phenomena alone, such as fluxes and potentials for various solution conditions surrounding the membrane, is not sufficient to specify the internal concentration and potential profiles which also depend on the equilibrium properties of the membrane (e.g. ion exchange isotherms, activity coefficients, etc.).

DESCRIPTION OF THE SYSTEM

The system to be considered consists of an homogeneous membrane surrounded by two external solutions kept at constant concentrations and containing n number of permeating species. We choose a coordinate system with the x-axis perpendicular to the membrane boundaries and assume that all fluxes are directed along the axis. Additional assumptions are (a) local mole fraction dependence of thermodynamic properties; (*b*) uniform temperature; (*c*) no frictional coupling forces between the ions in the membrane; (*d*) complete coion exclusion; and (*e*) steady state.

When assumptions (*b*) and (*c*) are fulfilled, we can use a generalized form of the Nernst-Planck equation:

$$J_i = -u_i c_i \frac{\partial \bar{\mu}_i}{\partial x} \qquad i = 1, 2, \cdots n. \quad (1)$$

where u_i are the mobilities, c_i the concentrations, J_i the fluxes, and $\bar{\mu}_i$ the electrochemical potentials defined as

$$\bar{\mu}_i = RT \ln c_i + RT \ln f_i + \mu_i^0(p) + z_i F \psi.$$

where ψ is the electric potential, p the pressure, f_i the activity coefficients, and μ_i^0 the standard chemical potentials of the ions. If the quantities u_i, f_i, and μ_i^0 are known functions of the concentrations in the membrane, equations (1) can be integrated across the interior of the membrane, which is the classical way of treating the problem and which gives the fluxes and currents in terms of the boundary conditions inside the membrane. In order to express the relationships in terms of the external boundary conditions, some properties of the boundaries must be known or assumed. For this purpose it is necessary to assume only that the continuity of electrochemical potentials is preserved at all phase boundaries which is valid as long as the boundary processes are not rate-limiting. This assumption, together with the assumption that the variables u_i, f_i, μ_i^0, and c_i, defining the thermodynamic properties of the membrane, are functions only of local mole fractions, permits a procedure for calculating the steady-state properties different from the classical one.

To show this, we shall use a slightly different form of equations (1), namely:

$$J_i = -\frac{L_i}{F^2 z_i^2} \frac{\partial \bar{\mu}_i}{\partial x} \qquad i = 1, 2 \cdots, \cdots, n. \quad (2)$$

where L_i are called the partial specific ionic conductances (Fitts, 1962), and are seen by comparison with equations (1), to be proportional to the product of mobility and concentration. The ionic conductances L_i are related to the integral conductances, g_i, often used in biological applications, by the following equation (Finkelstein and Mauro, 1963):

$$\frac{1}{g_i} = \int_0^d \frac{dx}{L_i} \quad (3)$$

In order to present the method we shall first show how the concept of corresponding solutions introduced by Scatchard (1953) can be used to integrate the flux equations, once the ionic conductances L_i are known. We shall then explain how the ionic conductances may be calculated from measurements of V_0 and R_{symm}.

PROPERTIES OF A CORRESPONDING SOLVENT MEMBRANE

In order to visualize how the current-voltage relationships of the membrane may be deduced we shall construct the following hypothetical membrane diagrammed in Fig. 1 and based on the concept of corresponding solutions (Scatchard, 1953). The membrane is divided into a number of compartments, each filled with the same solvent that surrounds the membrane. These compartments also contain the same coions as the surrounding solutions and since complete coion exclusion has been assumed, each little solvent compartment will retain its original *total* concentration throughout the experiment. We shall also reduce the size of the solvent compartments to such an extent that their influence on the diffusion rate can be neglected. The diffusion rate is then determined purely by the membrane parts (shaded areas in Fig. 1). For each solvent compartment, we can also write the condition of electroneutrality:

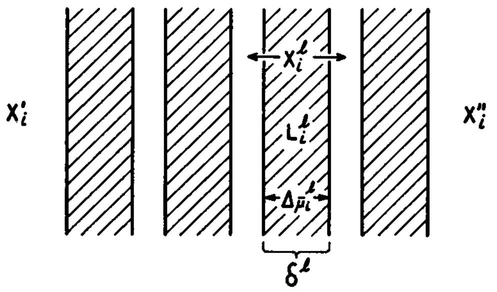

FIGURE 1 Diagram of the membrane and corresponding "solvent membrane." The membrane is divided into a number of small compartments surrounded by solutions containing the same solvent as the external solutions. The parameters of the *l*th membrane compartment are shown in the figure and the arrows indicate that the equivalent fraction X_i^l refers to the surrounding solution compartments which are in equilibrium with the membrane compartments.

$$\sum_{i=1} X_i = 1, \tag{4}$$

where the summation is carried out over the counterions and where X_i is the equivalent fraction of ionic species *i*.

Suppose now, that we make the number of solvent compartments vere large and make them very close, and that we perform a diffusion experiment in whch the outer solutions are kept constant. The ionic composition in the small solvent compartments will then change during the course of the experiment, but will constantly be in equilibrium with the composition in the membrane compartments.

Provided that the compartments are sufficiently close, the flux of ion species i between two neighboring solvent compartments can be expressed as:

$$J_i = -\frac{L_i^l}{F^2 z_i^2} \frac{\Delta \bar{\mu}_i^l}{\delta^l}, \tag{5}$$

where l denotes a particular membrane compartment and δ^l the distance between the neighboring solvent compartments. The difference in electrochemical potential refers to the two inner boundaries of the membrane compartment l, but due to the continuity condition this difference is also the same for the two neighboring solvent compartments. L_i^l is the ionic conductance in the membrane compartment and if we assume the two neighboring solvent compartments to be sufficiently close to have approximately the same composition (i.e. membrane compartment l is submersed in symmetrical solutions), we can write L_i^l as a function of the local surrounding solution composition $(X_1, \cdots, X_n)$. That L_i^l can be obtained in this way is a result of our assumption that the thermodynamic properties depend only on local mole fractions, in which case each set of mole fractions in the solvent compartments completely determines the mole fractions in the adjacent membrane compartments and vice versa. Since we are also dealing with steady-state conditions, there is no concentration change anywhere in the membrane, and J_i is consequently the same throughout the whole system.

We have consequently, in making equation (5) apply identically to both sets of compartments, made use of three assumptions: steady state, continuity of electrochemical potentials, and the thermodynamic properties depending on local mole fractions only.

Clearly, if we let the number of solvent compartments tend to infinity, and the distance between them approach zero, equation (5) will approach and become identical to equation (2). We can then visualize two membranes, a hypothetical one containing the same solvent as the surrounding solutions and having a given fixed charge distribution determined by the coion concentration in the corresponding solvent compartments (the solvent membrane is by definition also coion-excluding). If the conductance function $L_i(X_1, \cdots, X_n)$ is a known function of the equivalent fractions at any point in this membrane, the profiles, potentials, and fluxes for this membrane are completely determined by equations (4) and (5).

The second membrane is the real membrane, whose behavior is not affected by the presence of the corresponding solvent membrane, which is purely hypothetical. The hypothetical and real membranes have the same properties as far as the fluxes and total membrane potentials are concerned, since at steady state the same constant amount is flowing through both membranes. We have therefore obtained a means to relate the fluxes, currents, and total membrane potential of the real membrane without knowing the actual profiles within it. It should be emphasized that the corresponding solvent profiles or hypothetical profiles which are obtained as a result of solving equations (4) and (5) are not the same as the membrane profiles or real profiles. In fact, we know nothing about the real profiles as long as the equilibrium properties of the membranes are unknown.

However, if the real profiles are determined by measuring the concentrations in each little membrane compartment, then the equilibrium properties can be calculated. They could also be calculated from the conductance functions, if some known functional relationship existed for mobilities or equilbrium properties such as constant mobility ratio, constant pressure, etc.

We have therefore, by this method, circumvented the procedure of solving for concentrations and the potential at the boundaries inside the membrane which with the classical method is necessary in order to arrive at the fluxes and current-voltage relationships. It amounts to introducing the boundary conditions (continuity of electrochemical potentials) before integrating the flux equations instead of after the integrations. It also illustrates a fact pointed out by Guggenheim (1950), namely, that the splitting of the electrochemical potential in an electric and chemical component is without physical significance. We can for example, conceive of filling our solvent compartments with any arbitrary solvent and consequently achieving any splitting of ψ and μ_i in equation (2) that we like.

For convenience, however, we shall choose the solvent in the hypothetical membrane to be the same as in the external solutions. We can therefore write equation (5) explicitly as:

$$J_i = -\frac{L_i(X_1, \cdots, X_n)}{F^2 z_i^2} \frac{d}{dx} (RT \ln \gamma_i X_i + F z_i \phi) \tag{6}$$

All the quantities in equation (6) refer to the corresponding solvent membrane and the notation ϕ is used for the potential to indicate that it is not the same as the interior membrane potential ψ. Similarly the notation γ_i refers to the activity coefficients in the corresponding solvent membrane to distinguish them from f_i, the activity coefficients in the real membrane. Any variations in the activity coefficients f_i and standard chemical potential μ_i^0 in the membrane will appear in the evaluation of the conductances L_i as functions of the equivalent fractions $X_1, \cdots, X_n$.

EVALUATION OF THE IONIC CONDUCTANCES, L_i

In evaluating the conductances L_i as functions of equivalent fractions in the external solutions we shall restrict the treatment to the case of two counterions with equal valence z.

Membrane Potential at Zero Current

Tht total membrane potential at zero current is measured for various solution compositions (the solution on one side of the membrane is kept constant and varied on the other side) and from these measurements it is often possible to calculate a set of permeability ratios P_i/P_j defined by the following equation:

$$V_0 = \frac{RT}{zF} \ln \frac{\sum_i a_i' \frac{P_i}{P_j}}{\sum_i a_i'' \frac{P_i}{P_j}} \tag{7}$$

where a_i are the activities in external solutions and the superscripts refer to the two sides of the membrane.[2] The permeability ratios are known to be constant (i.e. independent of external solution conditions) in many ion exchange membranes, including also some biological situations (Eisenman, 1965).

The Meaning of Permeability Coefficients

Depending on the transport mechanism, or on the assumptions used in deriving equation (7), the permeability ratio has been given such various physical meanings as mobility ratio (Planck, 1890), ion exchange equilibrium constant (Nicolsky, 1937), the product of the mobility ratio and the Donnan ratio (Teorell, 1935; Meyer and Sievers, 1936), the product of the mobility ratio and the distribution coefficient ratio (Hodgkin and Katz, 1949), or the product of the mobility ratio and the ion exchange equilibrium constant (Helfferich, 1962; Karreman and Eisenman, 1962). Equation (7) has also been derived under the nonspecific conditions of the present treatment (Sandblom and Eisenman, 1967) and the existence of a constant permeability ratio therefore does not involve any assumptions about either the mobility ratio or the ion exchange equilibrium constant separately, and consequently does not restrict the generality of this treatment. If the permeability ratio is constant, equation (7) can be used to calculate the ratio P_1/P_2 directly from V_0.

When use is made of the continuity of electrochemical potentials at each of the membrane boundaries, the permeability ratio is also related to the ionic conductances at all points in the membrane by the following simple equation (Sandblom and Eisenman, 1967, equation 24):

$$\frac{L_1 \gamma_2 X_2}{L_2 \gamma_1 X_1} = \frac{L_1 a_2}{L_2 a_1} = \frac{P_1}{P_2} \tag{8}$$

The activities a_1 and a_2 refer to the corresponding solvent membrane.

In order to see this more clearly, the permeability ratio could have alternatively been defined from equation (8) and equation (7) then obtained as a result of this

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[2] Equation (7) describes the potential of a wide variety of glass electrodes in mixtures of any two univalent cations as well as in certain three-ion mixtures in which one ion is held at constant activity (e. g., Na-K mixtures at constant pH, Eisenman et al., 1966).

definition. This may be done by dividing two of equations (6) and rearranging:

$$\frac{J_1}{J_2} \cdot \frac{L_2\,\gamma_1\,X_1}{L_1\,\gamma_2\,X_2} = \frac{d\gamma_1\,X_1\,e^{Fz\phi/RT}}{d\gamma_2\,X_2\,e^{Fz\phi/RT}} \tag{9}$$

Using the definition (8) and assuming the permeability ratio to be constant, equation (9) can be integrated to give:

$$\frac{J_1}{J_2} \cdot \frac{P_2}{P_1} = \frac{a_1''e^{FzV/RT} - a_1'}{a_2''e^{FzV/RT} - a_2'} \tag{10}$$

where V is the total membrane potential. This formula is equivalent to the extended (fixed site) Behn formula given by Teorell (1951). It has also been derived by Conti and Eisenman (1965) under more general conditions, valid for permselective fixed site membranes. For two counterions and zero current:

$$J_1 + J_2 = 0$$

and equation (10) can be rearranged to give equation (7).

From equation (8) and a measurement of the permeability ratio it is possible to calculate the ionic conductance ratio L_1/L_2 as a function of equivalent fractions in the corresponding solvent membrane.

Symmetrical Membrane Resistance

In order to obtain a second relation between the ionic conductances, we shall use the expression for the theoretical membrane resistance (Teorell, 1953):

$$R = \int_0^d \frac{dx}{\sum_i L_i} \tag{11}$$

This resistance is measured at high frequencies (in the biological literature the inverse of the theoretical resistance is often referred to as the chord conductance).

In symmetrical solutions we shall show that under the assumptions made, the resistance will be linear and equation (11) takes a very simple form. Using equation (9) we obtain the following:

$$\frac{J_1}{J_2} \cdot \frac{P_2}{P_1} = \frac{d\gamma_1\,X_1\,e^{Fz\phi/RT}}{d\gamma_2\,X_2\,e^{Fz\phi/RT}} = \frac{\gamma_1\,X_1\,e^{Fz\phi/RT} - \gamma_1'X_1'}{\gamma_2\,X_2\,e^{Fz\phi/RT} - \gamma_2'X_2'} = \frac{\gamma_1''X_1''e^{FzV/RT} - \gamma_1'X_1'}{\gamma_2''X_2''e^{FzV/RT} - \gamma_2'X_2'} \tag{12}$$

If the solutions on either side of the membrane are equal, it follows that $\gamma_1''X_1'' = \gamma_1'X_1'$ and $\gamma_2''X_2'' = \gamma_2'X_2'$ and inserting this in equation (12), we get:

$$\frac{J_1}{J_2} \cdot \frac{P_2}{P_1} = \frac{\gamma_1'X_1'}{\gamma_2'X_2'} = \frac{\gamma_1\,X_1\,e^{Fz\phi/RT} - \gamma_1'X_1'}{\gamma_2\,X_2\,e^{Fz\phi/RT} - \gamma_2'X_2'} = \frac{\gamma_1\,X_1}{\gamma_2\,X_2} \tag{13}$$

Combining this with the condition of electroneutrality it is evident that X_1 and X_2 are independent of x, the position in the membrane, when the external solutions are identical. This is also true for the case when the permeability ratio is not constant although the proof is omitted here.

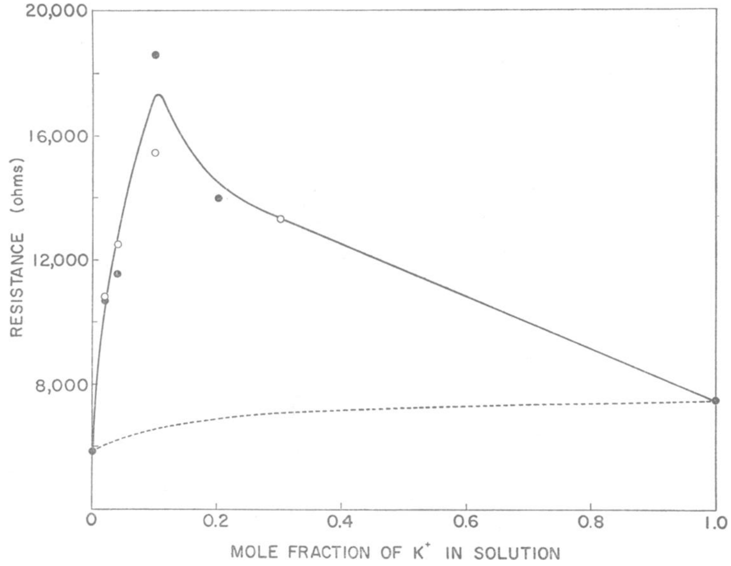

Figure 2 Resistance of a thin (ca. 0.1 μ) hydrated NAS 27-4 glass membrane (interposed between identical solutions of varying Na^+-K^+ compositions) as a function of the mole fraction of K^+ in solution (constant ionic strength of 1.0 M). The filled and open circles refer to two series of measurements of the resistance, which was found to be time-independent and voltage-independent under these experimental conditions. The dashed curve illustrates the resistance expected if mobilities were constant, and the observed maximum in resistance indicates that mobilities are mole fraction–dependent. The data are to be published.[1]

Since X_1 and X_2 are constant in the symmetrical case and the conductances are functions only of the mole fractions, equation (11) can be written:

$$R_{\text{symm}}(X_1, X_2) = \frac{d}{L_1(X_1, X_2) + L_2(X_1, X_2)}, \qquad (14)$$

and from equation (14) it is seen that the resistance in symmetrical solutions R_{symm} is independent of the electric field and is therefore linear. It may then either be measured by an AC measurement or a DC measurement. From equations (4), (8), and (14), it is now possible to calculate L_1 and L_2 as functions of mole fraction X_1 in external solution (equivalent to the corresponding solvent membrane). It is therefore necessary to measure the symmetrical membrane resistance R_{symm} in a series of solutions in which the equivalent fraction X_1 is varied from 0 to 1. Such measurements have been carried out elsewhere[1] with the typical results shown in Fig. 2.

Notice that the dashed curve illustrates the resistance expected (for example, from the treatment of Conti and Eisenman, 1965) if mobilities were constant; while the observed maximum of the resistance indicates that the mobilities are in fact mole fraction–dependent.

Nonhomogeneous Membrane

The theory can also be extended to the case in which the thermodynamic properties are not the same functions of local mole fractions throughout the membrane, but also depend on distance in such a way, that the conductance relationships are the same throughout the membrane apart from a proportionality factor which is different for different parts of the membrane (e.g. a fixed charge membrane with a nonuniform site distribution and where the thermodynamic properties depend on site distribution as well as on local mole fractions (Conti and Eisenman, 1965)). This means that we can write L_i as:

$$L_i(X_1, X_2, x) = f(x) \cdot L_i(X_1, X_2),$$

where X_1 and X_2 are the equivalent fractions in the corresponding solvent membrane. $f(x)$ is a function of distance which is independent of the electric field. Equations (7)–(13) are unaltered by this generalization, and equation (11) takes the following form:

$$R_{\text{symm}} = \frac{S}{L_1(X_1, X_2) + L_2(X_1, X_2)} \tag{15}$$

where S is defined as

$$S = \int_0^d \frac{dx}{f(x)}$$

METHODS OF SOLUTION

1. *Analogue Computer*

When the ionic conductances L_1 and L_2 have been obtained as functions of X_1, the flux equations (6) will contain two empirical functions. The problem must therefore be solved numerically, an endeavor for which the analogue computer is well suited since the empirically obtained functions may be stored in function generators. The functions, $\gamma_1(X_1)$ and $\gamma_2(X_2)$, which in general are empirically determined functions, must also be fed to function generators in order to solve equations (6). It is not unreasonable, however, to neglect variations in activity coefficients throughout the corresponding solvent membrane in which we choose the ionic strength to be constant and the same as that in which R_{symm} is measured.

If the total ionic strength is different on the two sides of the membrane, the boundaries between the corresponding solvent membrane and the external solution may be treated as classical fixed site membrane boundaries (see Teorell, 1953). In the following, however, we shall assume the total ionic strength to be equal on the two sides of the membrane in which case the boundary potentials between the corresponding solvent membrane and the external solutions cancel. If gradients of activity coefficients in the corresponding solvent membrane are neglected, the flux equations (6) can be written:

$$J_1 = -\frac{L_1}{F^2z^2}\frac{d}{dx}\left(RT\ln X_1 + zF\phi\right), \tag{16 a}$$

$$J_2 = -\frac{L_2}{F^2z^2}\frac{d}{dx}\left(RT\ln X_2 + zF\phi\right). \tag{16 b}$$

Multiplying equation (16 *a*) by X_1/L_1 and equation (16 *b*) by X_2 L_2 and adding the two equations we get, using the condition of electroneutrality:

$$\frac{FzJ_1X_1}{L_1} + \frac{FzJ_2X_2}{L_2} = -\frac{d\phi}{dx} \tag{17}$$

If, instead, equation (16 *a*) is multiplied by $1/L_1$ and equation (16 *b*) by $1/L_2$ and the two equations are subtracted, we get:

$$\frac{FzJ_1}{L_1} - \frac{FzJ_2}{L_2} = -\frac{RT}{FzX_1}\cdot\frac{dX_1}{dx} + \frac{RT}{FzX_2}\cdot\frac{dX_2}{dx} \tag{18}$$

and after rearrangement, taking again the condition of electroneutrality into account:

$$\frac{FzJ_1X_1}{L_1}\cdot X_2 - \frac{FzJ_2X_2}{L_2}\cdot X_1 = -\frac{RT}{Fz}\cdot\frac{dX_1}{dx} \tag{19}$$

If the functions X_1/L_1 and X_2/L_2 are calculated as functions of X_1 and stored on function generators, it is seen from equations (17) and (19) that the problem can be programmed relatively easily on an analogue computer and solved numerically.[1] Typical current voltage characteristics calculated in this manner for a glass membrane are shown in Fig. 3 and compared with experimental observations.

2. *General Case of a Constant Permeability Ratio*

Some useful expressions may also be obtained by combining the flux ratio relationship (10) with equation (19). Rearranging equation (19), we first introduce the permeability ratio from equation (8):

$$\frac{F^2z^2J_1}{L_1}\cdot X_1\left(X_2 - \frac{J_2}{J_1}\cdot\frac{P_1}{P_2}\cdot X_1\right) = -RT\frac{dX_1}{dx}. \tag{20}$$

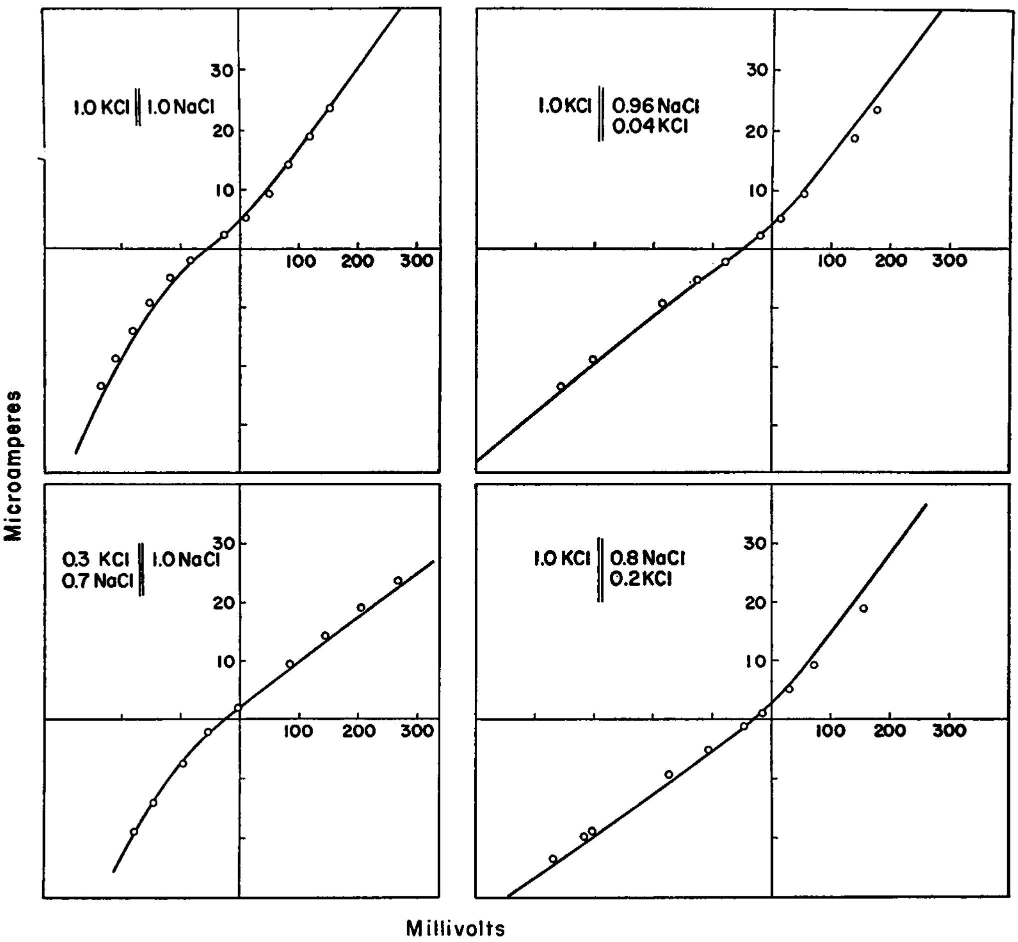

FIGURE 3 Computed and observed steady-state current-voltage relationships of a thin (ca. 0.1 μ) hydrated NAS 27-4 glass membrane when interposed between solutions having the indicated compositions. The continuous curves are computed from the data of Fig. 2 and equations (17) and (19); while the open circles are the experimentally measured values. Positive current flows from left to right of the indicated systems. The data are to be published.[1]

When the variables in equation (20) are separated and integrated, the following expression is obtained:

$$J_1 \cdot S = -\frac{RT}{F^2 z^2} \int_{X_1'}^{X_1''} \frac{L_1(X_1) \cdot dX_1}{X_1\left(X_2 - \frac{J_2}{J_1} \cdot \frac{P_1}{P_2} X_1\right)}, \qquad (21)$$

where L_1 is given as a function of X_1.

We can now use the condition of electroneutrality (4) and the flux ratio equation (10) to eliminate the flux ratio and X_2 from the numerator in equation (21):

$$X_2 - \frac{J_2}{J_1} \cdot \frac{P_1}{P_2} X_1 = X_2 - \frac{X_2'' e^{FzV/RT} - X_2'}{X_1'' e^{FzV/RT} - X_1'} \cdot X_1$$

$$= 1 - \frac{e^{FzV/RT} - 1}{X_1'' e^{FzV/RT} - X_1'} \cdot X_1 . \quad (22)$$

If $L_1(X_1)$ is known, it is now possible by numerical methods to evaluate the integral in equation (21) in terms of the total membrane potential V.

The fluxes can also be expressed in terms of the electric current:

$$Fz(J_1 + J_2) = I, \quad (23)$$

where I is the electric current.

Combining equations (21) and (22) gives:

$$I = -\frac{RT}{S \cdot Fz}\left(1 + \frac{J_2}{J_1}\right) \int_{X_1'}^{X_1''} \frac{L_1(X_1)\, dX_1}{X_1\left(1 - \dfrac{e^{FzV/RT} - 1}{X_1'' e^{FzV/RT} - X_1'} \cdot X_1\right)}, \quad (24)$$

and after inserting equation (10) in equation (24), we get a relationship between the total electric current, the external boundary conditions, and the total membrane potential:

$$I = -\frac{RT}{Fz \cdot S}\left[X_1'' e^{FzV/RT} - X_1' + \frac{P_2}{P_1}(X_2'' e^{FzV/RT} - X_2')\right]$$

$$\cdot \int_{X_1'}^{X_1''} \frac{P_1(X_1)\, dX_1}{[X_1'' e^{FzV/RT} - X_1' - (e^{FzV/RT} - 1)X_1]}, \quad (25)$$

where we have defined the permeability P_1 as:

$$P_1(X_1) = \frac{L_1(X_1)}{X_1} \text{ ohm}^{-1} \text{ cm}. \quad (26)$$

We have previously only defined a permeability ratio assumed to be constant, thereby leaving the individual permeabilities undefined with respect to a function or a constant, which is now introduced in equation (26).

Equations (25) and (26) also illustrate the fundamental character of the ionic permeabilities and conductances. From the extended definition of an ionic permeability coefficient (equation 26), a precise physical meaning can be given to both the permeabilities and ionic conductances in terms of coefficients appearing in the flux equations for the corresponding solvent membrane. Combining equations (6) and (26) gives:

$$I_i = -P_i \frac{RT}{Fz} \cdot \frac{\partial X_i}{dx} - L_i \frac{\partial \phi}{\partial x}, \tag{27}$$

where I_i is the ionic current, and where P_i is here defined to include the dependence on x.

Apart from proportionality constants, the ionic permeability coefficients and ionic conductances are seen to be defined as the coefficients of the concentration gradient and the potential gradient respectively in the corresponding solvent membrane.

If in equation (25) we substitute for $P_1(X_1)$, the expression R_{symm} obtained from equations (15) and (26), we get:

$$I = -\frac{RT}{zF}\left[X_1''e^{FzV/RT} - X_1' + \frac{P_2}{P_1}(X_2''\,e^{FzV/RT} - X_2')\right]$$
$$\cdot \int_{X_1'}^{X_1''} \frac{dX_1}{R_{\text{symm}}\left[X_1 + \frac{P_2}{P_1}X_2\right]\left[X_1''e^{FzV/RT} - X_1'(e^{FzV/RT} - 1)X_1\right]}, \tag{28}$$

and we see that S cancels out and does not appear in the equation. Consequently, it is not necessary to determine the function $f(x)$ or the membrane thickness d in order to evaluate the I-V curves. The integral in equation (28) may be evaluated numerically for each value of the potential V by plotting the integrand as a function of X_1 (R_{symm} is obtained from the empirical curve in Fig. 2) and measuring the area under the curve from X_1' to X_1''.

The current-voltage relationship is uniquely defined by the external solution conditions, R_{symm} and V_0, and we have shown that this result follows without making any assumptions about mobilities and equilibrium thermodynamic properties other than that they are functions of local mole fractions. Although the I-V curves may have a very complex appearance (see Fig. 3), the R_{symm} curve (Fig. 2) and V_0 contain enough information to characterize completely the steady-state properties of the system.

3. *Special Case of a Constant Mobility Ratio*

For comparison, we shall evaluate the integral in equation (25) for one particular case, namely when the mobility ratio is constant. In this case, $P_1(X_1)$ can be expressed in terms of X_1 in the following way; using the definition of L_1 and equation (8):

$$\frac{L_1}{F^2z^2} = u_1 X_1^m \bar{c} = u_1 \bar{c}(1 - X_2^m) = u_1 \bar{c} - r\frac{L_2}{F^2z^2}$$

$$\frac{L_1}{F^2z^2}\left(1 + r\,\frac{L_2}{L_1}\right) = u_1\,\bar{c}$$

$$\frac{L_1}{F^2z^2X_1}\left[X_1\left(1 - r\,\frac{P_2}{P_1}\right) + r\,\frac{P_2}{P_1}\right] = u_1\,\bar{c} = f(x) \tag{29}$$

where r is the mobility ratio and $\bar{c}$ the fixed charge concentration. The superscript m refers to the membrane phase. Inserting equation (29) into equation (25) and integrating, gives

$$I = -\frac{zF\cdot RT}{S}\,\frac{\left[X_1''e^{FzV/RT} - X_1' + \frac{P_2}{P_1}\left(X_2''e^{FzV/RT} - X_2'\right)\right]}{\left(X_1'' + r\,\frac{P_2}{P_1}X_2''\right)e^{FzV/RT} - \left(X_1' + r\,\frac{P_2}{P_1}X_2'\right)}$$
$$\cdot\left[\frac{FzV}{RT} + \ln\frac{X_1' + r\,\frac{P_2}{P_1}X_2'}{X_1'' + r\,\frac{P_2}{P_1}X_2''}\right], \tag{30}$$

which is the same result as that obtained by Conti and Eisenman (1965) using the classical method of solving for the internal profiles, and which may be considered to represent a special case of the present treatment.

Asymptotic Behavior

Equation (25) can also be used to evaluate the asymptotes of the I-V curve. Examining this expression as zV tends to minus infinity, we see that the integrand has a singular point at $X_1 = X_1'$, and similarly, when zV tends to plus infinity, the integrand has a singular point at $X_1 = X_1''$. If for large *negative* zV, we therefore expand $P_1(X_1)$ in Taylor series around X_1' and integrate, we get for the asymptote:

$$I = -\frac{1}{S}\left(P_1(X_1')X_1' + P_2(X_2')X_2'\right)\left[V + \frac{RT}{Fz}\sum_{n=1}^{\infty}\frac{P_1^n(X_1')}{P_1(X_1')}\,\frac{(X_1'' - X_1')^n}{n\cdot n!}\right], \tag{31 a}$$

where $P^n(X_1')$ is the nth derivative of P_1 with respect to X_1 at the point $X_1 = X_1'$. The same procedure applies for large *positive* zV when the I-V curve has the following asymptote:

$$I = -\frac{1}{S}(P_1(X_1'')X_1'' + P_2(X_2'')X_2''\left[V + \frac{RT}{Fz}\sum_{n=1}^{\infty}\frac{P_1^n(X_1'')}{P_1(X_1'')}\,\frac{(X_1' - X_1'')^n}{n\cdot n!}\right]. \tag{31 b}$$

It is seen by comparing these expressions with equation (15) for the symmetrical resistance and definition (26) of the permeabilities, that the slopes of the asymptotes are identical with the symmetrical resistances for the corresponding solution conditions. It is therefore not necessary to measure the quantity R_{symm} directly in symmetrical solution conditions, which can be experimentally difficult (e.g. in living cell membranes). Instead, the limiting slopes for various exterior solution conditions could be used to calculate $R_{\text{symm}}(X_1)$ from equation (31 *b*).

Another interesting relationship is obtained by dividing the two limiting conductances from equations (31):

$$\frac{G(zV \to -\infty)}{G(zV \to +\infty)} = \frac{P_1(X_1')X_1' + P_2(X_2')X_2'}{P_1(X_1'')X_1'' + P_2(X_2'')X_2''} = \frac{P_1(X_1')}{P_1(X_1'')}$$

$$\cdot \frac{X_1' + \dfrac{P_2}{P_1}X_2'}{X_1'' + \dfrac{P_2}{P_1}X_2''} = \frac{P_1(X_1')}{P_1(X_1'')}e^{FzV_0/RT} \tag{32}$$

If the permeabilities are constant-independent of X_1, the ratio of the limiting conductances is seen from equation (32) to become equal to the exponent of V_0. This was also found by Teorell (1951, 1953) to be the case for a fixed site membrane with equal total concentrations on the two sides of the membrane. However, in those dense membranes mentioned in the introduction where the mobilities are not constant, this simple relationship is no longer valid and must be substituted for by the more general relationship expressed in equation (32).

It is also seen from equations (25), that whereas the slopes of the asymptotes depend only on the end points (X_1' and X_1'') of the permeabilities, the intercepts of the asymptotes with the V-axis depend not only on the end points, but also on the shape of the permeability curves (L_1/X_1 and L_2/X_2 plotted as functions of X_1) in the vicinity of these points as expressed by the derivatives.

It is also apparent that the intercepts of the asymptotes provide an alternate way of expressing the permeabilities in terms of external solution conditions. Let us consider namely the intercepts of the asymptotes for small differences in external solution conditions, in which case the intercept potential can be written according to equation (31):

$$\Delta V_\infty = \frac{RT}{Fz}\frac{\partial \ln P_1(X_1)}{\partial X_1}(X_1'' - X_1'), \tag{33}$$

where higher orders of $X_1'' - X_1'$ have been neglected. In the limit where X_1'' approaches X_1', equation (33) can be written:

$$\frac{\partial V_\infty}{\partial X_1} = \frac{RT}{Fz} \cdot \frac{\partial \ln P_1(X_1)}{\partial X_1},$$

and when this equation is integrated, we get:

$$V_\infty = \frac{RT}{Fz} \ln \frac{P_1(X_1)}{P_1(1)}, \tag{34}$$

where $P_1(1)$ is the conductance of the membranes in pure solutions of species 1, according to equation (26). Note that the potential V_∞ does not correspond to an actual measured potential but rather to an integrated form of the intercept potential ΔV_∞ measured at small $X_1'' - X_1'$.

Individual Ionic Currents

The individual ionic currents are obtained directly by combining equations (23) and (28):

$$I_1 = -\frac{RT}{Fz}(X_1'' e^{FzV/RT} - X_1') \int_{X_1'}^{X_1''} \frac{dX_1}{\cdot R_{\text{symm}} \left[X_1 + \frac{P_2}{P_1} X_2\right] \left[X_1'' e^{FzV/RT} - X_1' - (e^{FzV/RT} - 1)X_1\right]} \tag{35 a}$$

$$I_2 = -\frac{RT}{Fz}\frac{P_2}{P_1}(X_2'' e^{FzV/RT} - X_2') \int_{X_1'}^{X_1''} \frac{dX_1}{\cdot R_{\text{symm}} \left[X_1 + \frac{P_2}{P_1} X_2\right] \left[X_1'' e^{FzV/RT} - X_1' - (e^{FzV/RT} - 1)X_1\right]} \tag{35 b}$$

These equations show also that the individual ionic currents are related to and completely determined by the permeability ratio and R_{symm}. Since we have shown that R_{symm} and V_0 can be obtained from a set of I-V curves when the solution composition is varied only on one side of the membrane, it is possible to use these to calculate both the ionic conductances and steady-state ionic currents as functions of membrane potential and external solution conditions without actually knowing the mechanism of ion transport. This should be particularly useful in biological membranes where the structure is unknown and where the ionic currents and conductances have been used to describe the dynamic behavior of the membranes (Hodgkin and Huxley, 1952).

General Cases

We shall also briefly discuss the case in which the valences of the two permeant species can have any arbitrary value and the permeability ratio need not be constant. The method is not altered and still consists of finding the ionic conductances as functions of mole fractions in external solutions for the purpose of solving equations (6). Equation (15) for the symmetrical resistance is still valid provided that L_1 and L_2 have the same dependence on distance. This assumption is valid when the membrane has a uniform fixed-charge distribution, and could be valid also with nonuniform fixed-site distribution when the ions have the same valence in which case the ion-site interactions are independent of site spacing. When the valences are different, this is no longer true and the assumption is then probably only valid when the membrane is uniform.

The additional relationship necessary to solve for the individual conductances in the case of constant permeability ratio was seen to be derived in a simple way from V_0 measurements. In the case of variable permeability ratio, Conti and Ciani[8] have shown that it is possible to express the ionic conductance ratio in terms of external solution conditions by a method, in which small changes in V_0 are measured following a small step change in solution conditions. This treatment is not based on any assumptions on transport mechanisms other than those made in the present treatment, and makes no assumption about the valences or the permeability ratio. *For a fixed-site membrane containing two permeable ions we can therefore generalize the statement that R_{symm} and V_0 are two independent sets oj measurements from which the ionic conductances can be expressed in terms of external solution compositions. This is then sufficient to solve equations (6) to obtain the fluxes and currents in terms of external solution conditions and total membrane potential.*

DISCUSSION

Assumptions

The most important assumption made in this treatment is that the local thermodynamic properties depend only on the local mole fractions, the validity of which can be tested experimentally. There are several conceivable cases, however, in which this assumption will not be true. One type of system already mentioned for which the theory does not hold is a membrane containing mobile sites. In this case the local thermodynamic properties will then not only depend on local mole fractions, but also on the field since the sites will rearrange under the influence of the electric field. The same argument also applies if the condition of electroneutrality is not valid, the ionic conductances depending then not only on local mole fractions but also on the electric field. The electroneutrality condition is therefore implicitly contained in the local mole fraction-dependence of thermodynamic properties. In these cases, the conductance appearing in equations (6) does not correspond to the conductance measured in symmetrical solutions and calculated from equation (15).

One might also conceive of stress gradients produced in the membrane as a result of profile rearrangement, and these stress gradients will not only produce local

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[8] Conti, F., and S. Ciani. Data to be published.

effects but also distant effects as well, and thereby change the properties in other parts of the membrane.

Effect of Cross-Coefficients

It was also assumed from the beginning that the frictional drags between ions could be neglected. If this is not so, the flux equations (2) must be rewritten in the following way:

$$J_1 = -L_{11}\frac{\partial\bar{\mu}_1}{\partial x} - L_{12}\frac{\partial\bar{\mu}_2}{\partial x}, \tag{36 a}$$

$$J_2 = -L_{21}\frac{\partial\bar{\mu}_1}{\partial x} - L_{22}\frac{\partial\bar{\mu}_2}{\partial x}, \tag{36 b}$$

and due to the Onsager relationships the cross-coefficients are equal, or $L_{12} = L_{21}$. Since we are now dealing with three independent conductances, we need a third independent measurement to determine how L_{12} varies with external solution conditions. From V_0 and R_{symm} it is only possible to calculate the total ionic conductances

$$\frac{L_1}{F^2 z_1} = z_1 L_{11} + z_2 L_{12} \quad \text{and} \quad \frac{L_2}{F^2 z_2} = z_1 L_{21} + z_2 L_{22}$$

(Sandblom and Eisenman, 1967)

and in order to solve equations (29) a third relationship is needed involving the three phenomenological coefficients L_{11}, L_{22}, and L_{12}.

Experimental Verification

The theory has been tested on thin, completely hydrated glass membranes whose thermodynamic properties are very complex functions of local mole fractions, and where the classical theory has proven to be inadequate.[1] The present method, however, applies surprisingly accurately to these membranes (see Fig. 3) which demonstrates the usefulness of the assumptions and the procedures. A further advantage is that the thermodynamic properties of the membrane (mobilities, ion exchange isotherms) can be calculated from the ionic conductances provided that some function, relating the chemical potentials or other suitable thermodynamic properties of the membrane to the mole fractions, is known or may be assumed.[1]

Information Contained in the Current-Voltage Relationships

We have shown that the current-voltage relationship can be calculated from the ionic conductances (given by R_{symm} and V_0) for any set of solution conditions. We have also shown how conversely the I-V curves can be used to calculate the ionic conductances as functions of external solution conditions and how the conductance functions can be used to predict all the steady-state properties of the system. The agreement between theory and experiment will then test the validity of the assumption that the thermodynamic properties depend only on local mole fractions, and no information is obtained from which it is possible to calculate the equilibrium properties of the system. Measurements of ionic fluxes as well as electrical measurements are dynamic measurements (*extra* membrane quantities) and serve to predict the dynamic behavior of the system, but cannot give information about the equilibrium properties. In order to determine these in an unknown membrane, another class of measurements is needed *equivalent* to measuring the actual concentrations in the membranes (i.e. *intra*membrane quantities).

Attempts have been made to determine the equilibrium properties from I-V relationships (Conti and Eisenman, 1965), but the method used involves an initial knowledge about the system and therefore permits all the properties to be determined, as pointed out in the preceding section.

Biological Membranes

Some implications relevant to biological membranes have already been pointed out. There is, however, a special case of equations (25) and (35) which is of particular interest in connection with the ionic theory of Hodgkin and Katz (1949). If the permeability $P_1(X_1)$ appearing in equation (25) is a constant, equations (25) and (28) can be integrated directly and combined with equations (10) and (23) to give:

$$I = -V \cdot \frac{P_1}{S} \frac{\left(X_1'' + \frac{P_2}{P_1} X_2''\right) e^{FzV/RT} - \left(X_1' + \frac{P_2}{P_1} X_2'\right)}{e^{FzV/RT} - 1}, \qquad (37\,a)$$

$$I_1 = -V \cdot \frac{P_1}{S} \frac{X_1'' \, e^{FzV/RT} - X_1'}{e^{FzV/RT} - 1}, \qquad (37\,b)$$

$$I_2 = -V \cdot \frac{P_2}{S} \frac{X_2'' \, e^{FzV/RT} - X_2'}{e^{FzV/RT} - 1}. \qquad (37\,c)$$

Similar equations in which activities appear instead of equivalent fractions have already been derived and used by Hodgkin and Katz (1949) under the following basic assumptions: (*a*) that ions in the membrane move under the influence of diffusion and the electric field in a manner which is essentially similar to that in free solutions; (*b*) that the electric field may be regarded as constant throughout the membrane; (*c*) that the concentrations of ions at the edges of the membrane are directly proportional to those in the aqueous solutions surrounding the membrane; and (*d*) that the membrane is homogeneous.

In our treatment we have shown that the same equations can be derived by replacing the first three highly idealized assumptions of Hodgkin and Katz by the more general thermodynamic assumptions (*a*) thermodynamic properties are functions of local mole fractions only, (*b*) complete coion exclusion, and (*c*) constant permeabilities.

In its general form the present theory gives no information about the concentration profiles and the potential profile, by the nature of the method employed. *Equations (37) do therefore not necessarily imply a constant field.*

However, in the special case of constant mobilities and a uniform fixed-site distribution, the potential profile in the membrane is proportional to the potential profile in the corresponding solvent membrane. This profile is linear when the permeabilities are constant (see equation 17) and the present treatment reduces to previous treatments of fixed-site membranes (Teorell, 1953; Conti and Eisenman, 1965).

It is interesting to note that the original constant field theory (Goldman, 1943; Hodgkin and Katz, 1949) does not explicitly take into account the presence of fixed sites, although some effects of a possible fixed-site distribution have been considered in a recent publication by Hodgkin and Chandler (1965).

In deriving equations (37) it has been assumed that the permeabilities are constant. This assumption can be tested by calculating the ratios of outside to inside permeabilities from the limiting slope conductances (equation 32).

If we use the data given by Hodgkin et al. (1952) for the limiting conductances of the squid axon in sea water, we get from equation (32):

$$\frac{P_{\mathrm{K}}^{o}}{P_{\mathrm{K}}^{i}} = \frac{P_{\mathrm{Na}}^{o}}{P_{\mathrm{Na}}^{i}} \simeq 8,$$

a result which is also obtained from the constant field theory without assuming coion exclusion. Although the permeability ratio is constant, the permeabilities are evidently not constant and the calculations of the ionic currents at steady state and at the peak of the action potential made by Hodgkin and Katz (1949) using equations (37), are therefore uncertain. For this reason it would be of interest to test the validity of more general treatments (e.g. the present treatment and the treatment of Conti and Eisenman, 1965) on biological membranes. However, the measurements must be carried out in the presence of impermeable coions. Another disadvantage is that measurements of the resistance in identical solutions must be carried out over a wide range of solution compositions in which the cell membrane is not likely to remain intact. Recent perfusion studies on squid axons, however, (Tasaki, 1965; Tasaki et al., 1965), seem to indicate that with the proper choice of internal and external anions the membrane can withstand changes in the ionic environment sufficiently well that it might be possible to measure the symmetric resistance for various compositions of cations at least over a certain range.

Another alternative has been suggested in the text, namely to use the limiting conductances for various solution compositions to calculate the ionic conductances as functions of solution compositions without having to measure R_{symm} directly.

This method is not without difficulties since the cell membrane breaks down at high fields (Rudolph and Stämpfli, 1958). For certain ionic compositions, however, the I-V curves seem to be sufficiently well characterized to calculate the limiting conductances (Hodgkin et al., 1952) with them.

It is therefore suggested that a proper choice of perfusion composition combined with measurements of limiting conductances might permit the complete characterization of the steady-state fluxes of the cell membrane using the present method. The criterion is that measurements of V_0 and R_{symm} should permit an adequate prediction of the I-V curves.

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