MEMBRANE POTENTIALS AT ZERO CURRENT

THE SIGNIFICANCE OF A CONSTANT IONIC PERMEABILITY RATIO

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ABSTRACT The possibility has been examined that the Goldman-Hodgkin-Katz equation for V_0, the total membrane potential at zero current, can be derived with constant permeability ratios from a thermodynamic treatment. The flux equations have been integrated under zero current conditions subject only to the restriction that the total membrane potential should be independent of internal concentration profiles, which is the requirement for the premeability ratios to be phenomenological constants, independent of solution conditions. No assumptions have been made concerning the electric potential profile. It was found that a constant permeability ratio can only be characteristic of systems satisfying certain relationships between ionic conductances and chemical potentials. From these relationships it was possible to define the permeability ratio in terms of the thermodynamic properties of the membrane quite generally and to identify the permeability ratio as the product of mobility ratio and ratio of partition coefficients. Moreover, the ionic conductance ratio at any point in the membrane has been shown to be expressable explicitly in terms of the permeability ratio and the activities of an external solution which would be in equilibrium with the point under consideration. Lastly, a number of conclusions have been reached regarding the range of applicability of the Goldman-Hodgkin-Katz equation with constant permeability ratios.

INTRODUCTION

The total electric potential difference, V_0, observed under zero current flow between two aqueous solutions separated by a membrane has been one of the most widely characterized of electrochemical and bioelectric phenomena. For biological membranes this potential difference is usually described in terms of the Goldman-Hodgkin-Katz equation (Goldman, 1943; Hodgkin and Katz, 1949):

$$V_0 = \frac{RT}{F} \ln \frac{a_{\text{K}}' + \frac{P_{\text{Na}}}{P_{\text{K}}} a_{\text{Na}}' + \frac{P_{\text{Cl}}}{P_{\text{K}}} a_{\text{Cl}}''}{a_{\text{K}}'' + \frac{P_{\text{Na}}}{P_{\text{K}}} a_{\text{Na}}'' + \frac{P_{\text{Cl}}}{P_{\text{K}}} a_{\text{Cl}}'}, \tag{1}$$

where a_i are the activities of the indicated ions in the solutions (') and ('') on the two sides of the membrane and the permeability ratios $P_{\text{Na}}/P_{\text{K}}$ and $P_{\text{Cl}}/P_{\text{K}}$ represent a set of coefficients defined from measurements under various external solution

conditions. The permeability ratio is said to be constant when these coefficients are independent of external solution conditions.

For certain ion exchange membranes permeable solely to species of one sign (e.g. cations) V_0 is described by a generalized Nernst equation in which the permeability ratios are independent of external solution conditions (Nicolsky, 1937; Eisenman, 1965):

$$V_0 = \frac{RT}{F} \ln \frac{\sum_i a_i' \dfrac{P_i}{P_j}}{\sum_i a_i'' \dfrac{P_i}{P_j}}. \tag{2}$$

However, in the usual biological situation in which permeable anions as well as cations are present it is generally observed that equation (1) is only approximate in that the permeability ratios are not constant (Baker, Hodgkin, and Meves, 1964).

Depending on the transport mechanism, or the assumptions made in the derivations, 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 ion exchange equilibrium constant (Helfferich, 1962; Karreman and Eisenman, 1962). Derivations of the above equations have been made from various theoretical considerations. Hodgkin and Katz (1949), following Goldman (1943), derived equation (1) for the steady state with constant permeability ratios assuming a constant electric field to exist within the membrane. An equation for V_0 valid also in the nonsteady state was derived by Henderson (1907) assuming a mixture boundary. In the case of equal total concentrations and a common anion, the Henderson equation becomes identical in form to equation (1). When the total concentrations on either side of the membrane are equal, equation (1) is also derived from Teorell's fixed charge theory for a membrane with a uniform site distribution (Teorell, 1953). For this case, Finkelstein and Mauro (1963) have shown that the electric field is constant regardless of the fixed site density. However, when the fixed site density is high enough to exclude coions, the field is constant regardless of the external solution concentrations (Teorell, 1951).

On the other hand, equation (2) has been derived by Conti and Eisenman (1965) without assuming a constant field for ion exchange membranes, permeable solely to ions of one sign and having nonuniformly distributed fixed sites of constant chemical properties. In this case they deduced that not only are the permeability ratios constant in the steady state but also time-independent. Equation (2) has also been shown to describe the potential of a completely dissociated liquid ion exchange membrane, although only in the steady state (Conti and Eisenman, 1966).

Because of the wide variety of systems to which equations (1) and (2) appear to apply and because equations of a similar form can be deduced from such diverse initial assumptions, it seemed reasonable to ask whether such equations might not represent a general phenomenological property of a wide class of membranes, independent of the details of their ionic permeation mechanism. If so, it should be possible to derive an expression for V_0 very generally with the permeability ratios treated as phenomenological coefficients without specifying the permeation mechanisms. Such derivations have, indeed, been accomplished for the steady-state case by Patlak (1960) and by Kimizuka (1964). Their derivations of equation (1), however, did not define the conditions under which the permeability ratio would be independent of concentration profiles and the electric field within the membrane, which is the requirement for the existence of constant permeability ratio and which is desirable for an equation for V_0 to be most useful.

We will therefore start our treatment by requiring that the membrane potential be independent of internal profiles[1] and derive the mathematical conditions necessary for this requirement to be satisfied. Using these conditions, an expression for V_0 is deduced from a general thermodynamic treatment; although it should be noted that the derivation is not completely phenomenological since the mathematical conditions imply that certain relationships must exist between ionic conductances and chemical potentials within the membrane. We have evaluated the physical situations under which these mathematical conditions might be satisfied and discuss the significance and implications of an observed constant permeability ratio. From this evaluation *we conclude that the existence of an expression for V_0 with constant permeability ratios cannot be characteristic of all membranes but is restricted to certain physical situations, most notably those in which a membrane is permeable only to species of one sign.*

DESCRIPTION OF THE SYSTEM

The system to be treated consists of a membrane interposed between two external homogeneous solution phases and in which a coordinate system is arbitrarily fixed at some well defined point within the membrane, for example, at one of the membrane-solution interfaces. Note that we do not assume the pressure, volume, or temperature of the system to be constant; nor is electroneutrality assumed either.

The membrane may be composed of any number of quasi-homogeneous phases in each of which the membrane properties are continuous functions of the space coordinates. Thus any diffusion layers next to the membrane are considered to be part of it. We shall limit ourselves to the case in which no external forces such as magnetic, centrifugal, or gravitational act on the system, whose behavior is then

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[1] Although Planck's derivation does not require an explicit calculation of the profiles, it is restricted to a particular physical situation (namely a homogeneous junction between solutions of equal total concentrations having a common anion).

completely determined by the pressure, temperature, and concentrations of all the components. The potential difference between the external solutions is called the membrane potential and is said to be profile-independent when defined uniquely by external solution conditions.

THE FLUX EQUATIONS

We shall proceed from the macroscopic linear laws of nonequilibrium thermodynamics (see, for example, de Groot and Mazur, 1962):

$$J_i = \sum_{k=0}^{N} L_{ik} X_k , \qquad (3)$$

where J_i are the fluxes, X_k the thermodynamic forces, and L_{ik} the phenomenological coefficients, which are, in general, functions of the thermodynamic properties of the system. The nondiagonal elements of the matrix L_{ik} are called coupling coefficients, since they describe the coupling between different kinds of thermodynamic processes (e.g. heat flow and diffusion), and are interrelated by the Onsager reciprocal relationships.

$$L_{ik} = L_{ki} \qquad (4)$$

Equations (3) describe quite generally the flow of matter and heat through a system in which any number of chemical reactions may take place and are valid for ordinary transport phenomena such as heat conduction and electric conduction even under rather extreme experimental conditions (de Groot and Mazur, 1962, p. 31). These equations may be used to relate the fluxes and forces both within the membrane and at the phase boundaries, depending on how the forces X_k are defined. Within the membrane the forces become gradients of the thermodynamic potentials, written explicitly as:

$$J_i = \sum_{k=0}^{n} L_{ik} \frac{\mathrm{grad}\ \bar{\mu}_k}{T} + l_{iq} \frac{\mathrm{grad}\ T}{T} , \qquad (5)$$

where $\bar{\mu}_k$ is the electrochemical potential, T is the absolute temperature, and l_{iq} is the thermal diffusion coefficient. $\bar{\mu}_k$ is defined as the sum of electrical and chemical potentials:

$$\bar{\mu}_k = \mu_k + z_k F \psi, \qquad (6)$$

where μ_k is the chemical potential of component k, z_k the corresponding valence, ψ the electrical potential, and F the Faraday constant.

For each phase boundary (including unstirred layers), which may be regarded as a surface of discontinuity separating two adjacent phases, the forces X_k simply be-

come the differences in thermodynamic potentials between the phases. We assume, however, as is usually done, that the thermodynamic potentials are continuous functions throughout the system, implying thereby that the difference of electro-chemical potential and the difference of temperature across each discontinuity are zero,[2] a situation usually referred to as "local thermodynamic equilibrium."

The fluxes J_i appearing in equations (3) are referred to the center of gravity of the system and hence we may write

$$J_i = C_i(v_i - v) = J'_i - C_i v, \tag{7}$$

v_i and v being the velocities of the ith component and the center of gravity, respectively; C_i being the concentration; and J'_i being the flux relative to the fixed coordinate system.

Since we are considering the system only after mechanical equilibrium has been

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[2] This is a mathematical statement, however, which involves two physical assumptions about the system. First, the frictional coefficients of each boundary must be zero, for otherwise there would have to be a definite difference in potential across each boundary in order to overcome the frictional forces. Second, and for the same reason, we must assume the inertial forces to be zero at the boundaries. They are not zero, however, until the whole system has come to mechanical equilibrium; i.e., when the internal frictional forces are counterbalanced by all other forces in the system. Establishment of mechanical equilibrium usually occurs very quickly in comparison with thermodynamic processes and is therefore (except for extremely thin systems) reached virtually at the beginning of a process, long before the stationary state is reached; and, indeed, the time constant for establishing mechanical equilibrium may be taken as the time constant for establishing boundary conditions. In fact, by writing the force balance equation for a single ion, it can be shown that the time constant τ_m for establishing mechanical equilibrium is given by:

$$\tau_m = \frac{M}{N} u = M \frac{D}{RT},$$

where u is the mobility of the ion, M the molecular weight, N Avogardo's number, D is the self-diffusion coefficient, and R is the universal gas constant. To assess when the time constant of mechanical equilibrium is short compared with the time constant of diffusion we will consider an example relevant to the thinnest system in which we are interested at present, the cell membrane. The time constant of diffusion is given by (K. S. Cole, 1965):

$$\tau_D = \frac{d^2}{\pi^2 D};$$

and we can estimate the ratio between τ_m and τ_D :

$$\frac{\tau_m}{\tau_D} = \frac{\pi^2 M}{RT} \left(\frac{D}{d}\right)^2 = \frac{M}{RT} \left(\frac{d}{\pi \tau_D}\right)^2$$

Note that τ_m/τ_D is proportional to the square of the ratio of the diffusion coefficient to membrane thickness. Using Cole's estimate of the time constant $\tau_D = 3 \times 10^{-8}$ sec for diffusion of potassium across the squid axon membrane and taking M as 39 g per mole and d as 100 A, this ratio is found to be 2×10^{-17} even for this very thin membrane.

established, the fluxes and forces appearing in equations (3) are no longer independent, and consequently one of the components may be eliminated from the flux equations. In particular, we shall choose to eliminate the "solvent" (i.e. the matter *in which* the ions are dispersed) whether this is a liquid or a solid. The method of elimination is described in textbooks on irreversible thermodynamics (see de Groot and Mazur, 1962, pp. 345–347), and we shall here only quote the result:

$$J_i' = \sum_{k=1}^{n} L_{ik} \frac{\text{grad } \bar{\mu}_k}{T} + l_{iq} \frac{\text{grad } T}{T} + C_i \, v_0 \tag{8}$$

where v_0 is the velocity of the solvent and where L_{ik} are now related to the inverse frictional coefficients with respect to the solvent but are still subject to the Onsager relationships of equation (4). The fluxes J_i' are now referred to the fixed coordinate system as opposed to the fluxes J_i appearing in equation (3) which refer to the center of gravity of the system.

At first we shall consider a single phase membrane, separating two homogeneous (e.g. aqueous) phases, and later extend the theory to a multiphase membrane. In order to solve for the electric potential, we use the definition of electric current:

$$I = \sum_i J_i' z_i F. \tag{9}$$

After multiplying the flux equations (8) by Fz_i and adding them we get, using equation (9):

$$I = \sum_i \frac{L_i}{Fz_i} \text{grad } \mu_i + \sum_i L_i \text{grad } \psi + l_q \text{grad } T + Fv_0 \sum_i z_i \, C_i, \tag{10}$$

where L_i, the partial specific conductance, is defined (Fitts, 1962), as

$$L_i = \frac{F^2 z_i}{T} \sum_k z_k L_{ik} \tag{11}$$

and l_q as:

$$l_q = \frac{F}{T} \sum_i z_i l_{iq}. \tag{12}$$

In equation (10), we have taken the Onsager reciprocal relationships (4) into account (see Fitts, 1962, pp. 74–76). Note also that the summations include all components in the system except the solvent.

Since z_i is equal to zero for a neutral component, it is seen from equations (10) and (11) that the coefficient in front of gradient of μ_i corresponding to a neutral component contains only the coupling coefficients with charged components and does not depend on the neutral component's self-diffusion coefficient. (Although

z_i for a neutral component is zero, it is seen from equation (11) that L_i/z_i is a finite quantity.) Thus, a neutral component only contributes to the current by virtue of its interaction with charged components. The same conclusion applies to the gradient of temperature which may produce an electric current only by virtue of the coupling between temperature and diffusion of charged components.

THE ELECTRIC POTENTIAL

Our task is to integrate equation (10) under zero current conditions; i.e., when there is no transport of charge at any point in the system. This means that for non-steady-state systems the time constant of diffusion must be large in comparison with the time constant for the Planck charging process (Cole, 1965). Furthermore we can only consider situations in which the last term (the charge transport created by a moving medium) in equation (10) vanishes.

For all solid membranes this term vanishes since v_0 is zero. For liquid (i.e. solvent) membranes the last term vanishes only when the condition of electroneutrality applies:

$$\sum_{i=1}^{n} z_i C_i = 0. \tag{13}$$

The membrane could also consist of a porous solid medium containing a liquid (Teorell, 1953), in which case we treat the fixed medium as the eliminated solvent and regard the solvent in the pores as one of the n components.

From this point on, our treatment applies strictly to liquid membrane systems only under the condition of electroneutrality (i.e. when the number of sites per unit volume is large compared to the space charge density). It applies to solid and porous membrane systems without this restraint. If we integrate equation (10) between the two internal membrane boundaries $x = 0$ and $x = d$ under conditions (13) for liquid systems and without this restraint for solid systems, we obtain the internal potential (commonly called the diffusion potential to distinguish it from the internal potential under nonzero current conditions):

$$\psi_d - \psi_0 = -\int_0^d \frac{\sum_i \frac{L_i}{z_i} d\mu_i}{\sum_i L_i} - \int_0^d \frac{l_q}{\sum_i L_i} dT. \tag{14}$$

It should be apparent from equation (14) that in order for the potential to be independent of the concentration profiles the expressions within the integral signs must be total differentials. Only then is the potential a function only of the end points of the integral and thereby defined uniquely by external solution. It is proven in Appendix I that a necessary and sufficient condition for the expressions within the

integral signs to be total differentials is that equations (15) be satisfied:

$$L_i = G(x, t)\beta_i \exp\left(\frac{\mu_i}{z_i \alpha}\right), \qquad z_i \neq 0; \quad (15\ a)$$

$$\frac{L_i}{z_i}\, d\mu_i = 0, \qquad z_i = 0; \quad (15\ b)$$

$$l_q\, dT = 0. \qquad (15\ c)$$

This proof is valid for a general thermodynamic system in which any set of $n + 1$ variables chosen arbitrarily from $\mu_1, \cdots \mu_n$; T; $L_1/z_1, \cdots L_n/z_n$; l_q is necessary and sufficient to specify the system (i.e. they constitute a set of true independent variables). In equation (15 a) α is a constant which is the same for all components, independent of x and of the time variable t, $G(x, t)$ can be any function of the parameters in the system and therefore depends on x and t but is the same for all components, and β_i are integration constants which are independent of x and t but different for different components.[3]

The Physical Meaning of Equations (15). Before using equations (15) to integrate equation (14), we shall examine their physical meaning. Let us compare equations (15 a) with the usual expression for the chemical potential:

$$\mu_i = RT \ln C_i + RT \ln f_i + \mu_i^0(P, T), \qquad (16)$$

where f_i is the activity coefficient and μ_i^0 the standard chemical potential, which is a function only of temperature and pressure. Eliminating μ_i between equations (15 a) and (16) gives the expression

$$G(x, t) \cdot \beta_i = L_i \exp\left(\frac{\mu_i^0}{z_i \alpha}\right) \cdot f_i^{-RT/z_i\alpha} \cdot C_i^{-RT/z_i\alpha}. \qquad (17)$$

Taking the ratio of equation (17) for the i species relative to j, we obtain:

$$\frac{\beta_i}{\beta_j} = \frac{\lambda_i}{\lambda_j} \cdot \frac{\exp\dfrac{-\mu_i^0}{z_i \alpha}}{\exp\dfrac{-\mu_j^0}{z_j \alpha}} \cdot \frac{f_i^{-RT/z_i\alpha}}{f_j^{-RT/z_j\alpha}} \cdot \frac{C_i^{1-RT/z_i\alpha}}{C_j^{1-RT/z_j\alpha}}, \qquad (18)$$

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[3] Equations (15 a) and (15 b) can mathematically be comprised in the following equation

$$\left(z_i \frac{d \ln \dfrac{L_i}{z_i}}{d\mu_i}\right)_G = \frac{1}{\alpha} \qquad (15\ ab)$$

where the differentiation is carried out at constant G.

where λ_i is the partial equivalent conductance of the ith component obtained by dividing L_i by C_i.

Condition (15 *a*) is therefore equivalent to requiring that the thermodynamic variables appearing in the right-hand side of equation (18) combine to yield a constant, which will be shown in equation (22) to be proportional to the permeability ratio. It should be noted that all the particular conditions assumed in the various derivations for equation (2) described in the Introduction are included as special cases of this condition.

Equations (15 *b*) and (15 *c*) simply require that there be no contribution to the electric current by the neutral species or the temperature. The meaning of equation (15 *b*), pertaining to neutral components, is then either that μ_i must be constant or that the coefficient of $d\mu_i$ must be zero. The latter condition is reasonable if the neutral components are nonpolar solutes (e.g. sugars, dissolved gases, or associated ion pairs) in fairly low concentrations; for then the frictional forces or coupling coefficients between charged and neutral components can be neglected, in which case the coefficient of $d\mu_i$ becomes zero. The neutral components may, however, be solvent molecules [as in diffusion in a porous plug (Teorell, 1959)] in which case the frictional forces can no longer be neglected. Instead μ_i must then be constant throughout the system to satisfy equations (15 *b*). As an example, consider water as the solvent and write:

$$d\mu_w = v_w(dP - d\pi),$$

where v_w is the partial molar volume of water and π the osmotic pressure in the system. μ_w is seen from this expression to be constant only when the pressure P counterbalances the osmotic pressure, namely when the system is in osmotic equilibrium. Of course, equations (15 *b*) always apply if the membrane is completely impermeable to the solvents.

Equation (15 *c*), pertaining to the temperature, leads to conclusions similar to those for neutral components. Either l_q or dT must be zero, implying either that thermal diffusion effects are negligible or that the temperature is uniform throughout the system.

Integration of Equation (14) and Expression for V_0. Inserting equations (15 *a*) into equation (14) and integrating from 0 to d gives

$$\psi_d - \psi_0 = -\frac{\alpha}{F} \ln \frac{\sum\limits_i \beta_i \exp\left(\dfrac{\mu_i(d)}{z_i \alpha}\right)}{\sum\limits_i \beta_i \exp\left(\dfrac{\mu_i(0)}{z_i \alpha}\right)}, \tag{19}$$

where $\mu_i(0)$ and $\mu_i(d)$ are the chemical potentials of species i just within the membrane at the two membrane-solution interfaces at 0 and d respectively. Equation

(19) can also be rearranged using the definition (6) of $\bar{\mu}_i$:

$$\sum_i \beta_i \exp\left(\frac{\bar{\mu}_i(d)}{z_i\,\alpha}\right) = \sum_i \beta_i \exp\left(\frac{\bar{\mu}_i(0)}{z_i\,\alpha}\right).$$ (20)

Since, by the condition of continuity of the electrochemical potential, the electrochemical potentials $\bar{\mu}_i$ are the same in the solution phases as within the membrane, we can solve explicitly directly for the total membrane potentials, V_0, from equation (20) to obtain:

$$V_0 = -\frac{\alpha}{F}\ln\frac{\sum_i \beta_i \exp\dfrac{\mu_i''}{z_i\,\alpha}}{\sum_i \beta_i \exp\dfrac{\mu_i'}{z_i\,\alpha}}$$

$$= -\frac{\alpha}{F}\ln\frac{\sum_i \beta_i\, a_i''^{(RT/z_i\alpha)} \exp\dfrac{\mu^{0''}_i}{z_i\,\alpha}}{\sum_i \beta_i\, a_i'^{(RT/z_i\alpha)} \exp\dfrac{\mu^{0'}_i}{z_i\,\alpha}}.$$

Dividing through by one of the exponential terms we get the desired expression for V_0 :

$$V_0 = -\frac{\alpha}{F}\ln\frac{\sum_i a_i''^{(RT/z_i\alpha)}\cdot\dfrac{\beta_i \exp\left(\dfrac{\mu_i^0(\text{sol})}{z_i\,\alpha}\right)}{\beta_j \exp\left(\dfrac{\mu_j^0(\text{sol})}{z_j\,\alpha}\right)}}{\sum_i a_i'^{(RT/z_i\alpha)}\cdot\dfrac{\beta_i \exp\left(\dfrac{\mu_i^0(\text{sol})}{z_i\,\alpha}\right)}{\beta_j \exp\left(\dfrac{\mu_j^0(\text{sol})}{z_j\,\alpha}\right)}},$$ (21)

provided that the solvent is the same on the two sides of the membrane, in which case $\mu^{0''}_i = \mu^{0'}_i = \mu_i^0\,(\text{sol})$.

Equation (21) can be seen, by comparison with equations (2) and (18), to be identical in form to equation (2) with the permeability ratio a constant defined in terms of the thermodynamic properties of the membrane by:

$$\frac{P_i}{P_j} = \frac{\beta_i}{\beta_j}\cdot\frac{\exp\left(\dfrac{\mu_i^0(\text{sol})}{z_i\,\alpha}\right)}{\exp\left(\dfrac{\mu_j^0(\text{sol})}{z_j\,\alpha}\right)} = \frac{\exp\left(\dfrac{\mu_i^0(\text{sol})-\mu_i^0}{z_i\,\alpha}\right)}{\exp\left(\dfrac{\mu_j^0(\text{sol})-\mu_j^0}{z_j\,\alpha}\right)}\cdot\frac{\lambda_i}{\lambda_j}\cdot\frac{f_i^{-RT/z_i\alpha}}{f_j^{-RT/z_j\alpha}}\cdot\frac{C_i^{1-RT/z_i\alpha}}{C_j^{1-RT/z_j\alpha}},$$ (22)

where μ^0 (sol) and μ^0 refer to the solution and membrane phases, respectively.

Equation (21), for the membrane potential at zero current, and equation (22), expressing the thermodynamic conditions for the existence of a constant permeability ratio, are the principal results of this paper. Some general features of these expressions will be examined below and their applicability to experimental systems will be considered in the Discussion. It can be seen immediately that the permeability ratio is a function of the standard chemical potentials of the ions in the membrane (μ_i^0) and the solutions $(\mu_i^0 \text{ (sol)})$, of the partial equivalent conductances in the membrane (λ_i), of the ionic activity coefficients in the membrane (f_i), and of the ionic concentrations in the membrane (c_i). It should also be apparent that the existence of a constant permeability ratio does not require the individual constancy of any of these terms but only the constancy of their products as defined in equation (22).

The proportionality factor relating P_i/P_j to β_i/β_j is seen to be:

$$\exp\left[\frac{\mu_i^0(\text{sol})}{z_i\,\alpha} - \frac{\mu_j^0(\text{sol})}{z_j\,\alpha}\right].$$

Since an undetermined factor $G(x, t)$ appears in equations (15 a), this means that equations (2) and (21) can only define a constant *ratio* between permeabilities whereas the individual permeabilities remain as undefined variables.

Equation (21) is somewhat more general than equation (2) in that neither z nor RT/α need be unity; and the problem is now solved in that a general form of equation (2) with constant permeability ratios has been derived by requiring only a *profile independence* of the membrane potential.

We have shown how this requirement is only fulfilled for systems in which equations (15) are satisfied, and *it is for this reason that a purely phenomenological derivation of equation (2) cannot be obtained when specifying that the permeability ratios are independent of solution conditions.*

Equation (21) for the total membrane potential is also identical in form to equation (19) for the internal potential but with the chemical potentials of equation (19) replaced by the standard chemical potentials and ionic activities of the external solution phases. Furthermore, equations (19), (20), and (21) can be extended to include any number of phases within the membrane using the conditions of the continuity of electrochemical potentials, provided that one can assume that the parameters α and β_k have the same value for all membrane phases. Since no assumption has been made about stationary states except the existence of mechanical and charge equilibrium, equations (19), (20), and (21) are time-independent as long as equations (15) are valid.

THE RELATIONSHIP BETWEEN IONIC CONDUCTANCE RATIO AND PERMEABILITY RATIO

Before discussing the above results it should be noted that P_i/P_j can be related to the ratio of partial specific conductances L_i/L_j. By dividing equations (15 a) we

get, recalling equation (22):

$$\frac{P_i}{P_j} = \frac{\beta_i}{\beta_j} \cdot \frac{\exp\left(\dfrac{\mu_i^0(\text{sol})}{z_i\,\alpha}\right)}{\exp\left(\dfrac{\mu_j^0(\text{sol})}{z_j\,\alpha}\right)} = \frac{L_i}{L_j} \cdot \frac{\exp\left(\dfrac{\mu_i^0(\text{sol}) - \mu_i}{z_i\,\alpha}\right)}{\exp\left(\dfrac{\mu_j^0(\text{sol}) - \mu_j}{z_j\,\alpha}\right)} ; \tag{23}$$

and, due to the continuity of electrochemical potentials and the fact that the electric potential difference across the interface is the same for species i and j, the ratio of exponential terms appearing in equation (23) will be the same, whether the chemical potentials refer to just inside or just external to the membrane. We can therefore write equation (23) at each interface as:

$$\frac{P_i}{P_j} = \frac{L_i(0)}{L_j(0)} \cdot \frac{a_j'^{(RT/z_j\alpha)}}{a_i'^{(RT/z_i\alpha)}} = \frac{L_i(d)}{L_j(d)} \cdot \frac{a_j''^{(RT/z_j\alpha)}}{a_i''^{(RT/z_i\alpha)}} . \tag{24 a}$$

One may also, however, visualize any point in the membrane as being in equilibrium with a particular set of external solution conditions. The conductance ratio at any point in the membrane can then be expressed explicitly in terms of the permeability ratio and the activities in the external solution which would be in equilibrium with the point under consideration as:

$$\frac{P_i}{P_j} = \frac{L_i(x, t)}{L_j(x, t)} \cdot \frac{a_j(x, t)^{RT/z_j\alpha}}{a_i(x, t)^{RT/z_i\alpha}} , \tag{24 b}$$

where the activities refer to external solutions. This procedure has proved useful in evaluating the current-voltage characteristics of membranes whose thermodynamic properties, while unknown, are functions only of the local mole fractions (Sandblom, 1967).

For univalent ions and $z\alpha = RT$, equation (24 b) reduces to:

$$\frac{P_i}{P_j} = \frac{\lambda_i}{\lambda_j} \cdot \frac{c_i}{a_i} \cdot \frac{a_j}{c_j} , \tag{24 c}$$

using the definition of partial equivalent conductance λ given above (see equation 18). If we define the *partition coefficient* of an ion as the ratio between its concentration in the membrane and its corresponding activity in the external solution, equation (24 c), and therefore equations (15), imply that the permeability ratio can be regarded, quite generally, as the product of the equivalent conductance ratio and the ratio of partition coefficients. This also follows from the definition of the individual permeabilities given by Hodgkin and Katz (1949).

DISCUSSION

Properties of Certain Physical Systems

Although we have concluded that equation (21) follows from a general thermo-dynamic treatment, the restrictions imposed by equations (15), which are necessary conditions for the existence of a constant permebaility ratio, will limit the number of systems to which equation (21) applies, as will be discussed below.

The Effect of Coions. Equations (15) which have been derived without any assumptions regarding the valences can, however, be satisfied only in membranes permeable to species of one sign (i.e. membranes which are impermeable to coions). This follows from the thermodynamic requirement that $(\partial L_i/\partial \mu_i)_{P,T,\mu_{j\neq i}} > 0$ which is only fulfilled if all $z_i\alpha > 0$ in equations (15 a).

There is one important exception to the above conclusion, however, namely when the total ionic concentrations on both sides of the membrane are equal. For permeant monovalent species, V_0 is then described by the following equation in the steady state (see Appendix II):

$$V_0 = \frac{RT}{F} \ln \frac{\sum_i a_i^{+\prime} \dfrac{P_i^+}{P_j^+} + \sum_k a_k^{-\prime\prime} \cdot \dfrac{P_k^-}{P_j^+}}{\sum_i a_i^{+\prime\prime} \dfrac{P_i^+}{P_j^+} + \sum_k a_k^{-\prime} \cdot \dfrac{P_k^-}{P_j^+}}, \qquad (25)$$

where the plus and minus superscripts refer to cations and anions respectively. Equation (1) is seen to be a special case of equation (25) for a single anion and two cations.

The derivation of equation (25) has been carried out in Appendix II for univalent ions assuming that $RT\,\alpha = z_i$. This means that α is positive for cations and negative for anions; and, since the potential is then no longer profile-independent, equation (25) is valid only in the steady state. The validity is further restricted to membranes in which the cross-coefficients can be neglected (i.e. $L_{ik} = 0$, $i \neq k$). In deriving equation (25) we have defined a constant ionic permeability ratio from equation (24), recalling that α is positive for cations and negative for anions. This definition implies, as we have seen, that the permeability ratio is equal to the product of the mobility ratio and the ratio of partition coefficients. If the system satisfies the boundary conditions of Hodgkin and Katz, the ratio of partition coefficients is a constant. Similarly, if ideal Donnan conditions prevail at the membrane-solution interfaces, it can be shown that the ratio of partition coefficients is a constant when the total ionic strength is constant (see Appendix II).

Equation (25), valid for equal total concentrations ($a'' = a'$), has also been derived under more restricted conditions by Planck (1890) for a homogeneous uncharged membrane and by Teorell (1951) for a membrane with a uniform fixed site distribution and ideal behavior. For these cases, Finkelstein and Mauro (1963)

have deduced that the electric field is constant in the membrane. It is shown, however, in Appendix II that equation (25) is valid more generally; and since the individual mobilities need not be constant, the electric field need not be constant. Therefore equation (25) does not imply a constant electric field.

In the case of a single common anion and the same total ionic strength throughout the system, equation (25) also follows from the Henderson derivation for mixture boundaries and is also valid in the nonsteady state. It is evident, however, that for this particular case the anion satisfies equation (15 b) and that therefore this case also follows from the present treatment.

Despite the above exceptions, the necessity of fulfilling conditions (15) of the present treatment indicates that in general a membrane can only have a constant permeability ratio in the absence of coion permeation, which is why we have defined the permeability ratio from equations (2) or (21) rather than from equations (1) or (25).

Zero Current Conditions and Mosaic Membranes. Another important assumption of the present treatment is that the electric current is zero *everywhere* in the membrane. In a mosaic membrane, for example, we may still have local circulating currents (e.g. resting currents, Teorell, 1959; Eisenman and Conti, 1965) although the total membrane current is zero. The membrane potential is then no longer profile-independent since the profiles will influence the potential by the IR drop as seen from equation (14), and equation (25) is no longer valid.

Fixed Sites vs. Mobile Sites and Time-Dependent Permeability Ratios. In applying the present results to ion exchange membranes it is useful to distinguish between those having sites which are restricted to the membrane phase but free to move within it (which we shall call "mobile site" membranes) as opposed to those in which the ion exchange sites are fixed spatially at particular locations within the membranes (i.e. "fixed site" membranes). The former are essentially liquid ion exchangers; the latter correspond to the more conventional solid ion exchangers. A constant permeability ratio can be characteristic of these membranes in three situations pertaining to time:[4] (a) at steady state, (b) instantaneously, and (c) transiently. The permeability ratio may be constant in all three cases in some systems, or only in certain of these cases, as will be discussed below.

(a) *Fixed site membranes.* The particular physical situations for the applicability of equation (21) are all consequences of satisfying equations (15), and in certain fixed site membranes these equations are satisfied at all times. For example, equations (15 a) can easily be shown to be satisfied for the usually assumed ideal membrane behavior, in which mobilities and standard chemical potentials are constant,

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[4] In the following treatment we will have occasion to refer to a "time-independent" behavior of the equations. By this we will mean that the observed potential becomes independent of time, attaining steady-state values even while ionic concentration profiles and electric potential profiles within the membrane are changing with time.

activity coefficients are unity, and the cross-terms vanish. In this case, equations (22) for univalent ions are satisfied with $\alpha = RT$. Consequently, the permeability ratios are constant, equation (2) describes the potentials, and equation (22) reduces to:[5]

$$\frac{P_i}{P_j} = \frac{\exp \dfrac{\mu_i^0(\text{sol}) - \mu_i^0}{RT}}{\exp \dfrac{\mu_j^0(\text{sol}) - \mu_j^0}{RT}} \cdot \frac{u_i}{u_j} = K_{ji} \frac{u_i}{u_j}, \tag{26}$$

where K_{ji} is the ion exchange equilibrium constant defined by $K_{ji} = 1/K_{ij} = (a_j/a_i)(c_i'/c_j')$ in which the primed quantities refer to the concentration of the ions in the membrane while the unprimed quantities refer to the activities in the solution phases.

More generally, a constant permeability ratio can also be characteristic of certain kinds of nonideal behavior, for example when $\alpha = nRT$. One such type of non-ideal behavior is that considered by Conti and Eisenman (1965) in which $d \ln a/d \ln c$ was assumed to equal n, and for which the equilibrium is described by $K_{ji} = (a_j/a_i)[(c_i'/c_j')]^n$. Another type of nonideal behavior not considered by these authors, for which $\alpha = nRT$, is that in which $d \ln L/d \ln c = \dfrac{1}{n}$ (see equation 15 ab).

In either of these cases the electric potential is described by equation (21) at all times (i.e. in the steady state, instantaneously, and transiently); and equation (22) simplifies to:

$$\frac{P_i}{P_j} = \frac{\exp \dfrac{\mu_i^0(\text{sol}) - \mu_i^0}{nRT}}{\exp \dfrac{\mu_j^0(\text{sol}) - \mu_j^0}{nRT}} \cdot \frac{u_i}{u_j} = K_{ji}^{1/n} \cdot \frac{u_i}{u_j}. \tag{27}$$

We therefore conclude that V_0 of a variety of fixed site membranes is described by equations (21) or (2) with constant permeability ratios which are independent of time. It can also be seen why the existence of a constant permeability ratio does not require the individual constancy of the equilibrium constant or of the mobility ratio but merely that their products be constant.

(b) *Mobile site membranes.* On the other hand, equation (21) generally does not apply to mobile site membranes in a time-independent form. This is because in a membrane containing mobile sites their movement contributes to the electric current and the sites must therefore be treated as additional components in the system

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[5] Note that in such an ideal membrane the absence of cross-terms implies that the equivalent conductance ratio λ_j/λ_i equals the mobility ratio u_j/u_i.

(Conti and Eisenman, 1966; Sandblom, Eisenman, and Walker[6]). In consequence, we find that a mobile site membrane does not obey equation (21) except at steady state, provided that all charged species are completely dissociated, in which case the sites behave effectively as fixed, a conclusion already reached by Conti and Eisenman (1966). Even at steady state, equation (21) does not apply precisely to mobile site membranes if dissociation is incomplete since in this case the sites will "circulate" in the membrane moving as free species in one direction and combined with counterions in the other direction (Sandblom, Eisenman, and Walker[6]). Indeed, the treatment of Sandblom, Eisenman, and Walker indicates that, in the limiting case of strong association, V_0 is given by a linear combination of two logarithmic terms:

$$V_0 = \frac{RT}{z_i F}\left(1 - \frac{\bar{t}_s}{|z_s|}\right) \ln \frac{\sum_i a_i' \frac{P_i^1}{P_j^1}}{\sum_i a_i'' \frac{P_i^1}{P_j^1}} + \frac{RT}{z_i F}\cdot\frac{\bar{t}_s}{|z_s|}\ln \frac{\sum_i a_i'^{|z_s|}\cdot\frac{P_i^2}{P_j^2}}{\sum_i a_i''^{|z_s|}\cdot\frac{P_i^2}{P_j^2}}, \qquad (28)$$

where z_i and z_s are the valences of counterions and sites respectively, and t_s is an average value of the transference number of the sites in the interval 0 to d. The first set of permeability ratios, indicated by the superscripts 1, is determined by the properties of the membrane solvent alone; while the second set, indicated by the superscripts 2, is determined by the site properties.

Temporal Behavior of the Membrane Potential. One of the most important characteristics of the membrane potential is its time course subsequent to a step change in solution conditions. From the preceding sections we can conclude that in general the permeability ratio can be constant and V_0 can be time-independent during the transition from one steady state to another only if there is no coion movement in the membrane and if, moreover, the sites are fixed. Under these circumstances equations (21) and (2) are valid at all times subsequent to establishment of the boundary conditions. An exception to this restriction is that the same behavior is expected for coion-permeable membranes in systems having a single common anion, or cation, and the same total concentrations everywhere. In this case the Henderson (1907) derivation applies and equation (2) is valid at all times.

Thermally Insulated Systems. It has been shown above that the permeability ratio must be constant and the same everywhere in the membrane in order for equations (2) or (21) to apply with a permeability ratio independent of solution conditions. In reaching this conclusion, it was explicitly assumed that all portions of the membrane are in thermodynamic contact which, however, need not be the case experimentally. The situation may well be that two or more regions of the membrane

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[6] Sandblom et al. Electrical phenomena associated with the transport of ions and ion pairs in liquid ion exchange membranes. I. Steady state theory. To be submitted to *J. Phys. Chem.*

(e.g., the left-hand side and the right-hand side) are isolated thermodynamically from each other and yet are maintained in contact electrically (each membrane region could be separated from the other by an electrically conducting reservoir of constant composition). In such a situation, in which there is no transfer of matter or heat between the two regions, V_0 is still defined; and equation (21) is still valid, but with permeability ratios which can be *different* for the two sides of the system. This conclusion also applies to systems in which thermodynamic isolation exists transiently (e.g., because of their thickness).

Some Comments on Biological Resting Potentials

Despite the fact that the Goldman-Hodgkin-Katz equation (1) has been used extensively in describing the resting potential of biological membranes, it has been noted that the permeability ratios are not constant under conditions in which ionic strength is unequal on the two sides of the membrane (Baker, Hodgkin, and Meves, 1964; Chandler, Hodgkin, and Meves, 1965). This behavior is not surprising in the light of the present demonstration (see equation 25 and Appendix II) that equation (1) is expected to apply with constant permeability ratios only when a membrane separates solutions of the same ionic strength if permeable species of more than one sign are present. On the other hand, our results indicate that V_0 is expected generally to be describable using constant permeability ratios only when the membrane is permeable to species of one sign (in which case equations (2) or (21) apply). Therefore, it would seem worthwhile to test the adequacy of equations (2) or (21) under conditions of varying ionic strength, after replacing the relatively permeable Cl^- by an anion to which the membrane is less permeable. Recent experiments on the crayfish axon, which has an appreciable chloride permeability, indicate that the permeability ratios vary considerably with external solution conditions even when these are kept at constant ionic strength.[7]

In describing bioelectric resting potentials such as those of the squid giant axon, the Goldman-Hodgkin-Katz equation has generally been used with the explicit assumption that the ratios of the permeabilities are the same for both surfaces of the membrane (*see* equation (3) of Baker, Hodgkin, and Meves, 1964). When one is dealing with a (thin) membrane which is not capable of providing thermal insulation between its two surfaces, the present analysis indicates that the permeability ratios *must* be the same on both sides of the membrane if equation (2) is found to describe the data satisfactorily for variations of solution conditions on one side. It would, therefore, be of interest to assess the extent to which the permeability ratio (e.g. of Na to K) is observed to be the same for the perfused axon when measured by variations of internal as opposed to external solution concentrations, particularly when precautions have been taken to use anions to which the membrane is relatively impermeable.

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[7] Strickholm, A., and G. Wallin. Submitted for publication.

In contrast to this situation is the one observed for the isolated frog skin in which it has been found that equation (2) is obeyed reasonably well for variations of either the external or internal solution conditions separately, but in which the permeability ratios defined by equation (2) are found to be very different for the outer vs. inner surfaces (Lindley and Hoshiko, 1964). These findings can only be reconciled with the conclusions of the present treatment if the inner and outer surfaces of the frog skin are effectively separated from each other by a reservoir (presumably the cell interior) which serves to isolate them thermodynamically from each other while providing electrical contact between them.

CONCLUSIONS

1. A purely phenomenological derivation of an equation for V_0, the membrane potential for zero current, cannot be obtained when one requires that the permeability ratios be constant.

2. Equations (15) define the mathematical conditions for a thermodynamic derivation of an equation for V_0 with constant permeability ratio.

3. Equation (21) gives an explicit integral expression for V_0 whose only parameters are a set of constant permeability ratios defined in terms of the requirements given by equation (22) for the membrane's thermodynamic properties. Equation (21) is a generalized Nernst equation.

4. An examination of the physical situations in which equations (15) are satisfied indicates that, in general, an expression for V_0 with constant permeability ratios cannot be characteristic of all membranes but is restricted to certain physical situations, most notably those in which a membrane is permeable solely to ionic species of one sign. In certain fixed site membranes equation (21) is not restricted to the steady state, but may also be valid transiently. In mobile site membranes, equation (21) only applies to the steady state in the limit of complete dissociation; while for incomplete dissociation an equation having an additional logarithmic term (equation (28)) is needed even in the steady state.

5. However, when the total ionic concentrations on both sides of the membrane are equal, V_0 is describable by equation (25), an equation of the Goldman-Hodgkin-Katz type, for a variety of permeation mechanisms even when the membrane is permeable to species of both signs, provided there are no local resting currents.

6. It has been shown that a constancy of the permeability ratios does not require an individual constancy of the mobility ratios and partition coefficient ratios but merely that their product be constant. This result also indicates that the Goldman-Hodgkin-Katz equation does not imply a constant electric field.

7. It has been demonstrated that permeability ratios can be independent of solution conditions for certain kinds of nonideal behavior, one of which is exemplified when $d \ln a/d \ln c$ is constant.

8. A number of conclusions were reached relevant to biological resting potentials. First, the Goldman-Hodgkin-Katz equation is not expected to apply when the ionic strength is not the same on both sides of a membrane and there are present ionic species of more than one sign to which the membrane is permeable. Second, a membrane which obeys the Goldman-Hodgkin-Katz equation for variation of solution conditions on one side must have the same set of permeability ratios for both faces; and vice versa, unless the faces are thermodynamically isolated from each other. Conversely, if thermodynamic isolation is maintained between the two faces (e.g. by a suitable reservoir of constant ionic composition), each face can obey the Goldman-Hodgkin-Katz equation with a different set of permeability ratios.

APPENDIX I

THE CONDITIONS UNDER WHICH $d\psi$ IS A TOTAL DIFFERENTIAL

JOHN P. SANDBLOM

The problem is to find the conditions necessary for the following expression to be a total differential:

$$d\psi = \frac{\sum \dfrac{L_i}{z_i} d\mu_i}{\sum L_i} + \frac{l_q}{\sum L_i} dT. \tag{29}$$

When these conditions are fulfilled, the integral will be a function only of the end points and independent of the integration path.

In order to simplify the equations, we shall denote the μ_i by x_i, T by x_{n+1}, (L_i/z_i) by y_i and l_q by y_{n+1}, in which case equation (29) reduces to:

$$dh = \frac{\sum\limits_{i=1}^{n+1} y_i\, dx_i}{\sum\limits_{i=1}^{n+1} z_i\, y_i}. \tag{30}$$

where $\psi = h$ and $z_{n+1} = 0$.

The problem is solved if we can find the conditions on y_i and x_i which will make dh a total differential. In general, this problem has infinitely many solutions but we shall limit ourselves to the case in which the system is uniquely determined by $n + 1$ variables chosen arbitrarily from the set y_i, x_i. To each point in the $n + 1$ dimensional space therefore a unique set of values $y_1, \cdots, y_{n+1}, x_1, \cdots, x_{n+1}$ corresponds. Mathematically this means that we can write $n + 1$ functional relationships between these variables or in particular

$$x_i = x_i(y_1, \cdots, y_{n+1}) \tag{31}$$

where $y_1, \cdots, y_{n+1}$ are chosen as the independent variables. The functions x_i are assumed to be well behaved.

The form of equation (30) suggests that equations (31) must have the following form:

$$x_i = x_i(y_i, g),$$

where g is a function of all y_i, in order to satisfy equation (31). To prove this we shall rearrange equations (31) in the following way:

$$x_i = \bar{x}_i(y_i, g_1, \cdots, g_{n+1}), \tag{32}$$

where $g_1, \cdots, g_{n+1}$ are functions of the independent variables y_i chosen so as not to alter the values of x_i. Taking the differentials of equations (32) we get:

$$dx_i = \frac{\partial \bar{x}_i}{\partial y_i} dy_i + \sum_j \frac{\partial \bar{x}_i}{\partial g_j} dg_j, \tag{33}$$

and after substituting equations (33) in equation (29) this yields:

$$dh = \frac{\sum_i y_i \frac{\partial \bar{x}_i}{\partial y_i} dy_i}{\sum_i z_i y_i} + \frac{\sum_{i,j} y_i \frac{\partial \bar{x}_i}{\partial g_j} dg_j}{\sum_i z_i y_i}. \tag{34}$$

Since dh is a total differential, it follows that h must be a function of all the variables appearing in equation (34), and we can therefore write this function as:

$$h = \bar{h}(y_1, \cdots, y_{n+1}, g_1, \cdots g_{n+1}), \tag{35}$$

The differential of this function then becomes:

$$dh = \sum_i \frac{\partial \bar{h}}{\partial y_i} dy_i + \sum_j \frac{\partial \bar{h}}{\partial g_j} dg_j. \tag{36}$$

If we now compare equations (34) and (36), we get:

$$\frac{\partial \bar{h}}{\partial y_i} = \frac{y_i \frac{\partial \bar{x}_i}{\partial y_i}}{\sum_i z_i y_i} \tag{37 a}$$

$$\frac{\partial \bar{h}}{\partial g_j} = \frac{\sum_i y_i \frac{\partial \bar{x}_i}{\partial g_j}}{\sum_i z_i y_i}. \tag{37 b}$$

Since h is a total differential, it follows from equation (36) that the following relationships must be fulfilled:

$$\frac{\partial^2 \bar{h}}{\partial y_j \, \partial y_i} = \frac{\partial^2 \bar{h}}{\partial y_i \, \partial y_j} \tag{38 a}$$

$$\frac{\partial^2 \bar{h}}{\partial g_j \, \partial y_i} = \frac{\partial^2 \bar{h}}{\partial g_j \, \partial y_i}, \tag{38 b}$$

where y_i and g_j may be treated as independent variables. Combining equations (37) and (38) gives:

$$\frac{\partial}{\partial y_j} \cdot \frac{y_i \frac{\partial \bar{x}_i}{\partial y_i}}{\sum_i z_i y_i} = \frac{\partial}{\partial y_i} \frac{y_j \frac{\partial \bar{x}_j}{\partial y_j}}{\sum_i z_i y_i} \tag{39 a}$$

$$\frac{\partial}{\partial g_j} \cdot \frac{y_i \frac{\partial \bar{x}_i}{\partial y_i}}{\sum_i z_i y_i} = \frac{\partial}{\partial y_i} \frac{\sum_i y_i \frac{\partial \bar{x}_i}{\partial g_j}}{\sum_i z_i y_i} \tag{39 b}$$

Although the functions g_i, are not independent variables, we have shown here that from the way in which they are constructed they must satisfy equations (39), when treated as independent variables.

After performing the differentiation of equations (39 b), we get, taking into account equations (32) and treating the functions g_i as independent variables:

$$\frac{y_i}{\sum_i z_i y_i} \cdot \frac{\partial^2 \bar{x}_i}{\partial g_j \partial y_i} = \frac{\frac{\partial \bar{x}_i}{\partial g_j}}{\sum_i z_i y_i} + \frac{y_i}{\sum_i z_i y_i} \cdot \frac{\partial^2 \bar{x}_i}{\partial y_i \partial g_j} - \frac{z_i \sum_i y_i \frac{\partial \bar{x}_i}{\partial g_j}}{(\sum_i z_i y_i)^2}, \tag{40}$$

which reduces to:

$$\frac{\partial \bar{x}_i}{\partial g_j} = \frac{z_i \sum_i y_i \frac{\partial \bar{x}_i}{\partial g_j}}{\sum_i z_i y_i}. \tag{41}$$

Equations (41) constitute $(n + 1)$ equations for each g_j and if we subtract any two of these equations we get if z_i, $z_k \neq 0$:

$$\frac{1}{z_i} \cdot \frac{\partial \bar{x}_i}{\partial g_j} - \frac{1}{z_k} \frac{\partial \bar{x}_k}{\partial g_j} = 0. \tag{42}$$

These equations can be integrated directly giving

$$\frac{\bar{x}_i}{z_i} - \frac{\bar{x}_k}{z_k} = r_{ik}(y_1, \cdots . y_{n+1}). \tag{43}$$

A comparison between equations (32) and (43) shows that the functions $\bar{x}_i$ must be of the following form:

$$\bar{x}_i = z_i f_i(y_i) + z_i U(g_1, \cdots, g_{n+1}), \tag{44}$$

where U is a function of the variables $g_1, \cdots, g_{n+1}$. These functions can now be introduced in equations (39 a); and after the differentiations are carried out, this yields

$$y_i \cdot \frac{\partial f_i(y_i)}{\partial y_i} = y_j \cdot \frac{\partial f_j(y_j)}{y_j}. \tag{45}$$

Since in equations (45) we have a complete separation of variables, each side of the equations must be equal to a constant α:

$$y_i \frac{\partial f_i(y_i)}{\partial y_i} = \alpha. \tag{46}$$

Integration of equations (46) finally gives:

$$f_i(y_i) = \alpha \ln y_i + \gamma_i, \tag{47}$$

where γ_i are integration constants. Eliminating $f_i(y_i)$ from equations (47), with the aid of equations (44), gives:

$$\frac{\bar{x}_i}{z_i} = \alpha \ln y_i + \gamma_i + U(g_i, \cdots, g_{n+1}). \tag{48}$$

This equation applies to all $\bar{x}_i$ whose corresponding valences z_i are different from zero.

From those $\bar{x}_i$ whose corresponding valences are zero we get from equations (39 a) and (41):

$$y_i \frac{\partial \bar{x}_i}{\partial y_i} = 0, \tag{49 a}$$

$$\frac{\partial \bar{x}_i}{\partial g_j} = 0. \tag{49 b}$$

Finally substituting the definitions of the variables y_i, g_i in equations (48) and (49), we get after rearrangement the conditions (15) which are the basis of our analysis:

$$L_i = \beta_i \, G(x, t) \, \exp \frac{\mu_i}{z_i \alpha} \qquad z_i \neq 0 \quad (15 \, a)$$

$$\frac{L_i}{z_i} \cdot d\mu_i = 0 \qquad z_i = 0 \quad (15 \, b)$$

$$l_q \, dT = 0 \qquad (15 \, c)$$

where:

$$G(x, t) = \frac{\exp \, U(g_1, \cdots, g_{n+1})}{\alpha}$$

and

$$\beta_i = z_i \exp \left(\frac{-\gamma_i}{\alpha} \right).$$

Note that the functions g_i are functions of the phenomenological coefficients and are therefore in general functions of time and distance in the membrane, expressed in $G(x, t)$.

APPENDIX II

A GENERAL DERIVATION OF THE GOLDMAN-HODGKIN-KATZ EQUATION WHEN TOTAL IONIC CONCENTRATIONS ARE THE SAME ON BOTH SIDES OF THE MEMBRANE

JOHN P. SANDBLOM

We shall solve the flux equations (8) in the steady state considering only isothermal systems and univalent ions. Assuming the cross-coefficients (L_{ik} $i \neq k$) to be zero and the electroneutrality condition (13) to be valid, the equations (8) reduce to:

$$J_i^+ = -L_i^+(x) \cdot \frac{d}{dx} (RT \ln a_i^+(x) + F\psi(x)), \qquad (50\ a)$$

$$J_k^- = -L_k^-(x) \cdot \frac{d}{dx} (RT \ln a_k^-(x) - F\psi(x)), \qquad (50\ b)$$

where the superscripts refer to positive and negative ions respectively.

Since the electrochemical potentials are continuous throughout the system, the electrochemical potential of an ion at a point x in the membrane is equal to the electrochemical potential of a solution which would be in equilibrium with the membrane phase at the point x. The activities $a_i(x)$ and the potential $\psi(x)$ in equations (50) therefore can equally well refer to the activities and the potential of such an hypothetical solution as to the more usually considered quantities in the membrane phase (Sandblom, 1967). Consequently, when equations (50) are expressed in terms of hypothetical solution values and integrated, they yield directly the fluxes in terms of the external solution conditions and the total membrane potential.

Dividing two of the equations (50 a) and rearranging, we get:

$$\frac{J_i^+}{J_j^+} \cdot \frac{L_j^+(x)}{L_i^+(x)} \cdot \frac{a_i^+(x)}{a_j^+(x)} = \frac{da_i^+(x) \cdot e^{F\psi(x)/RT}}{da_j^+(x) \cdot e^{F\psi(x)/RT}}. \qquad (51)$$

Recalling the relationship between ionic permeability ratios and ionic conductance ratios (equation 24) and assuming for simplicity that $RT/z_i\alpha = 1$, equation (51) can be integrated to yield:

$$\frac{J_i^+}{J_j^+} \cdot \frac{P_j^+}{P_i^+} = \frac{a_i^{+''} e^{FV/RT} - a_i^{+'}}{a_j^{+''} e^{FV/RT} - a_j^{+'}}. \qquad (52)$$

where the superscripts $'$ and $''$ refer to the two sides of the membrane and where V is the total membrane potential. Equation (52) is equivalent to the extended Behn formula, given by Teorell (1951), for which the above constitutes a more general derivation.

A corresponding equation may be derived for the anions:

$$\frac{J_k^-}{J_l^-} \cdot \frac{P_l^-}{P_k^-} = \frac{a_k'' - a_k^{-'} \cdot e^{FV/RT}}{a_l'' - a_l^{-'} \cdot e^{FV/RT}}. \qquad (53)$$

Next we multiply equations (50 a) by $a_i^+(x)/L_i^-(x)$ and equations (50 b) by $a_k^-(x)/L_k^-(x)$,

after which they are added separately to yield:

$$\sum_i J_i^+ \frac{a_i^+(x)}{L_i^+(x)} = -RT\frac{da}{dx} - Fa\frac{d\psi}{dx}, \tag{54 a}$$

$$\sum_i J_k^- \frac{a_k^-(x)}{L_k^-(x)} = -RT\frac{da}{dx} + Fa\frac{d\psi}{dx}, \tag{54 b}$$

where a is the total activity of cations and anions respectively which, taking into account the condition of electroneutrality, is:

$$a = \sum_i a_i^+ = \sum_k a_k^-. \tag{55}$$

Recall that since a_i^+ and a_k^- refer to an hypothetical solution in equilibrium with the membrane phase, equation (55) does not exclude the possible presence of fixed charges in the membrane.

Adding equations (54) and taking the relationships between ionic permeability ratios and ionic conductance ratios (equation 24) into account, we get:

$$\frac{a_j^+(x)}{L_j^+(x)}\left[\sum_i J_i^+ \frac{P_j^+}{P_i^+} + \frac{L_j^+(x)}{L_i^-(x)} \cdot \frac{a_i^-(x)}{a_j^+(x)} \cdot \sum_k J_k^- \frac{P_i^-}{P_k^-}\right] = -2RT\frac{da}{dx}. \tag{56}$$

If we now define a permeability ratio between cations and anions in analogy with equation (24) which was derived for each category separately, we get:

$$\frac{P_j^+}{P_i^-} = \frac{L_j^+(x)}{L_i^-(x)} \cdot \frac{a_i^-(x)}{a_j^+(x)}. \tag{57}$$

We emphasize that equation (57) is a *definition* and cannot be deduced in the same way as equation (24) was deduced from equations (15). For this reason equation (57) is not equivalent to equation (22) which is only valid for cations and anions separately if we define permeability ratios from equations (24) and (57). Further implications of definition (57) will be discussed at the end of this Appendix.

If we require the permeability ratio P_j^+/P_i^- to be constant, it is seen by integrating equation (56) that the term within the bracket must be zero when the total concentrations are equal on the two sides of the membrane (i.e. $a'' = a'$), or:

$$\sum_i J_i^+ \frac{P_j^+}{P_i^+} + \frac{P_j^+}{P_i^-} \cdot \sum_k J_k^- \cdot \frac{P_i^-}{P_k^-} = 0. \tag{58}$$

In order to use this result, we add the flux ratio equations (52) in the following two ways:

$$\frac{\sum_i J_i^+ \cdot \frac{P_j^+}{P_i^+}}{J_j^+} = \frac{a''e^{FV/RT} - a'}{a_j^{+''}e^{FV/RT} - a_j^{+'}}; \tag{59 a}$$

$$\frac{\sum_i J_i^+}{J_j^+} = \frac{\sum_i \frac{P_i^+}{P_j^+}[a_i^{+''}e^{FV/RT} - a_i^{+'}]}{a_j^{+''}e^{FV/RT} - a_j^{+'}}. \tag{59 b}$$

Equations (59 b) are then divided by equations (59 a) to give:

$$\frac{\sum_i J_i^+}{\sum_i J_i^+ \cdot \frac{P_j^+}{P_i^+}} = \frac{\sum_i \frac{P_i^+}{P_j^+}[a_i^{+\prime\prime}e^{FV/RT} - a_i^{+\prime}]}{a^{\prime\prime}e^{FV/RT} - a^{\prime}} \; ; \qquad (60 \, a)$$

and, similarly, for anions

$$\frac{\sum_k J_k^-}{\sum_i J_k^- \cdot \frac{P_l^-}{P_k^-}} = \frac{\sum_k \frac{P_k^-}{P_l^-}[a_l^{-\prime\prime} - a_l^{-\prime}e^{FV/RT}]}{a^{\prime\prime} - a^{\prime}e^{FV/RT}} \; . \qquad (60 \, b)$$

Finally, in order to obtain the membrane potential at zero current, V_0, equations (60) are divided and combined with equations (58) and (61), using the condition of zero current:

$$\sum_i J_i^+ = \sum_k J_k^-, \qquad (61)$$

to yield equation (25), which can therefore be valid with constant permeability ratios quite generally, subject only to the assumptions made above, the most important of which is that the total ionic concentrations are the same on both sides of the membrane.

Let us consider briefly certain physical conditions under which the permeability ratio as defined by equation (57) will be constant. From the relationship between ionic conductance and mobility ratio we get:

$$\frac{P_j^+}{P_l^-} = \frac{\lambda_j^+}{\lambda_l^-} \cdot \frac{c_j^+}{c_l^-} \cdot \frac{a_l^-}{a_j^+}. \qquad (62)$$

If the mobility ratio is constant it follows that the quantity

$$\frac{c_j^+}{c_l^-} \cdot \frac{a_l^-}{a_j^+} \qquad (63)$$

relating membrane and hypothetical solution concentrations (i.e. the ratio between distribution coefficients) must also be constant. This condition is fulfilled in the treatment of Hodgkin and Katz (1949) where membrane concentrations are assumed to be proportional to solution concentrations. It is also fulfilled in the treatment of Teorell (1951) where the membrane has a uniform fixed site distribution and obeys ideal Donnan boundary conditions. In this case the quantity in equation (63) reduces to r^2 where r is the Donnan ratio. However, since r is a function of a (the total activity in the external solutions), the term within the brackets in equation (56) is therefore also a function of a. Dividing equation (56) by this term, called $f(a)$, and integrating, we get:

$$\int_0^d \frac{a_j^+(x)}{L_j^+(x)} \, dx = -2RT \int_{a'}^{a''} \frac{da}{f(a)}. \qquad (64)$$

When $a' = a''$, the integral on the right side is zero if $f(a) \neq 0$. $f(a)$ must therefore be zero· and consequently both a and r are constant and equation (58) is satisfied.

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