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Theory for Carrier-Mediated Zero-Current Conductance of Bilayers Extended to Allow for Nonequilibrium of Interfacial Reactions, Spatially Dependent Mobilities and Barrier Shape

S. Ciani, R. Laprade, G. Eisenman, and G. Szabo

Dept. of Physiology, UCLA, Center for the Health Sciences,
Los Angeles, California and
C.N.R. Laboratory of Cybernetics and Biophysics, Camogli, Italy

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Summary. A generalized form of the electrodiffusion equation, allowing for any shape of symmetrical energy barrier and any spatial dependence of the diffusion coefficient, is used to deduce theoretically the carrier-mediated conductance for thin (e.g., bilayer) membranes in the limit of low applied current. Both the Nernst-Planck and the Eyring single-barrier treatments are special cases of this more general approach, which allows for the effect of non-uniform properties of the lipid and non-uniform profiles of the forces acting within the membrane interior. Two independent mechanisms for ions to cross the membrane-solution interfaces are considered; namely, (1) the reaction at the interface between ions from solution and carriers from the membrane, and (2) the partition across the interfaces of complexes already formed in the solution. The rates of these reactions are taken into account using the rate equations of chemical kinetics; and the Poisson-Boltzmann equation is integrated in the aqueous solutions to evaluate the effect of charged polar head groups of the lipid. The analysis leads to an expression for the conductance, which, in the approximation of constant field, is an explicit function of such experimentally variable parameters as the concentrations and types of permeant ions and carriers in the aqueous phases, the total ionic strength and the nature of the polar head groups of the lipid. The functional relationship observable in an unknown membrane can, in principle, enable one to deduce such information as the mechanism of ion permeation across the interfaces, the magnitude of the surface charge, and the degree of ion-carrier complexation in the aqueous solutions.

This paper presents a theoretical analysis of the zero-current conductance of a thin membrane in the presence of neutral carriers of ions. For this restricted, but important, membrane property the analysis presented here is more general and comprehensive than others heretofore (Ciani, Eisenman & Szabo, 1969; Markin, Kristalik, Liberman & Topaly, 1969;

Markin, Pastushenko, Kristalik, Liberman & Topaly, 1969; Läuger & Stark, 1970), since it not only takes into account the kinetics of the interfacial processes, as well as the presence of fixed charges and dipoles at the membrane surfaces, but also starts from a form of the electrodiffusion equation which allows for any shape of symmetrical energy barrier[1] and any spatial dependence of the diffusion coefficient. Both the Nernst-Planck (Planck, 1890) and the Eyring (Parlin & Eyring, 1954) treatments are special cases of this more general approach, which allows for the effect of non-uniform properties of the lipid and non-uniform profiles of the forces acting within the membrane interior. Two independent mechanisms for ions to cross the membrane-solution interfaces are considered; namely, (1) the reaction at the interface between ions from solution and carriers from the membrane, and (2) the partition across the interfaces of complexes already formed in the solution. For a clearer physical understanding of the theoretically deduced behavior, the effects of the rate-processes on the driving forces are discussed explicitly. Subsequent papers will deal with the zero-current potential in the presence of ionic gradients and nonzero current properties (Ciani, Eisenman, Laprade & Szabo, 1973; Ciani, Laprade, Szabo & Eisenman, *unpublished*) and compare the theoretical expectations with experiments on a variety of carrier molecules and lipids (Ciani *et al.*, 1973; Laprade, Ciani, Eisenman & Szabo, *unpublished*; Laprade, Ciani, Szabo & Eisenman, 1972; Laprade, Eisenman, Szabo & Ciani, *unpublished*).

Despite the generality of the starting assumptions and the indeterminacy as to the profiles of the energy barrier and the transport coefficients, the ohmic conductance for small applied voltages is deduced here as an explicit function of such experimental variables as the aqueous concentration and type of ions, the concentration and type of carrier, and the density and type of polar head groups. A certain number of parameters, most of which consist of combinations of fundamental equilibrium and rate constants, are deduced as being measurable, at least in principle, from the behavior of the conductance observed in appropriately designed experiments. The values of such parameters may indicate whether diffusion in the membrane or across the interfaces is predominantly rate-determining, which type of mechanism governs the transport of ions across the interfaces, as well as provide such useful information about the system as whether the polar head groups of the lipid bear a net charge or produce a dipolar poten-

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[1] The shape of the energy barrier for any diffusible particle is defined as the profile of its free-energy inside the membrane in the absence of any applied field; and can be formally described by the spatial dependence of the "standard chemical potential."

tial, and how much ion-carrier complexation occurs in the aqueous phase.

At the outset, it will be useful to recapitulate briefly the history of the previous theoretical approaches to the problem of carrier-mediated permeation. The relation between the results of the present paper and the important results in the existing literature will be made clear throughout the text. The Nernst-Planck equation was used to deduce the effects of neutral carriers on the zero-current membrane potential and conductance of lipid bilayer membranes under the assumption that the membrane interior could be regarded as a uniform liquidlike phase (Eisenman, Ciani & Szabo, 1968; Ciani *et al.*, 1969). This treatment was restricted to zero-current properties and by the assumption that the interfacial processes were at equilibrium. Both of these restrictions were removed by Markin, Kristalik *et al.* (1969) and Markin, Pastushenko *et al.* (1969) who not only deduced the voltage-current relationship using the Nernst-Planck formalism, but also introduced kinetic considerations at the interfaces. Läuger and Stark (1970) also dealt with the kinetics of the interfacial processes, but used a single-barrier Eyring model to describe the migration of the complexes and carriers across the membrane, and Stark, Ketterer, Benz and Läuger (1971) have recently extended this model to include time-dependent relaxation phenomena.

All of these treatments make implicit simplifications about the forces to which the diffusible particles are subjected inside the membrane. Thus, use of the Nernst-Planck equation implies that the applied electric field is the only force acting on the single charged particle; so that, in the absence of transmembrane voltage and in the approximation of constant field, the shape of the energy barrier is implicitly assumed to be uniform inside the membrane (i.e., representable by a rectangular barrier). On the other hand, the single-barrier Eyring model corresponds to an alternative extreme in which the barrier presents a sharp peak in the middle of the membrane. The theory of the present paper not only encompasses both of these extremes, but also allows for the possibility of variable mobilities and any symmetrical barrier shape, thereby encompassing those deduced theoretically from "image charge" effects (Neumcke & Läuger, 1969), as well as inferred experimentally from voltage-current data (Hall, Mead & Szabo, 1973). Because the derivation is lengthy, we suggest that the reader interested primarily in the results proceed to Eq. (64), which gives the dependence of conductance on concentrations and rate constants. His attention is also directed to the important recent work of LeBlanc (1971), Neumcke (1971), and Haydon and Hladky (1972), which deal with aqueous polarization phenomena not considered here.

Description of the Model

(a) The Aqueous Phases

The chemical composition of the aqueous phases will be described in terms of concentrations (moles/volume) of the ions and of the neutral ion-binding species, s. Considering, for simplicity, that only one of the ionic species present is capable of forming charged complexes with s, we shall denote it by i and shall use the symbol z to indicate its valence.

The chemical reaction between i and s leading to formation of complexes in the aqueous phases will be described formally by

$$i+s \underset{K_{is}^B}{\overset{K_{is}^F}{\rightleftarrows}} is \tag{1}$$

where K_{is}^F and K_{is}^B, the rate constants of association and dissociation of the complex, are related to the equilibrium constant[2] K_{is} and to the activities at equilibrium, $a_i(\mathrm{eq})$, $a_s(\mathrm{eq})$, $a_{is}(\mathrm{eq})$, by

$$\frac{K_{is}^F}{K_{is}^B}=K_{is}=\frac{a_{is}(\mathrm{eq})}{a_i(\mathrm{eq})\,a_s(\mathrm{eq})}. \tag{1a}$$

It is worth calling the attention of the reader on the fact that throughout this paper the symbols i and is will be used to denote ionic species which bear the same charge z and which will be referred to as the "permeant ion" and the "charged complex," respectively; s is neutral and will be denominated simply as the "carrier."

Two main assumptions will be made: (a) All the solutes, either neutral or ionic, behave ideally. This means that the chemical potential of the neutral carrier s, μ_s and the electrochemical potential of any present ion r, $\bar{\mu}_r$ can be written in the simple forms

$$\mu_s=\mu_s^0(T,P)+RT\ln c_s \tag{2}$$

$$\bar{\mu}_r=\mu_r^0(T,P)+RT\ln c_r+z_r F\psi \tag{3}$$

where $\mu_s^0(T,P)$ and $\mu_r^0(T,P)$ are the so-called standard chemical potentials, c_s and c_r are concentrations and $z_r F\psi$ denotes the electrostatic energy per mole of the r^{th} ionic component. The subscript r is used to indicate any of the present ions, regardless of their sign, including the species i and is.

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[2] Taking into consideration the experimental finding (Diebler, Eigen, Ilgenfritz, Maas & Winkler, 1969) that the rates of association and dissociation between the alkali cations and the macrotetralide actin antibiotics in methanol are very high, we generalize the validity of this result also for the aqueous phases, and shall consequently assume throughout that the homogeneous reaction (1) is always at equilibrium. This implies that the sole parameter characterizing reaction (1) is the equilibrium constant K_{is}, whereas the rate constants K_{is}^F and K_{is}^B will never be introduced explicitly.

As a consequence of assumption (a), the equilibrium of the aqueous association reaction can be rewritten in terms of concentrations

$$K_{is}=\frac{c_{is}}{c_i c_s}. \tag{4}$$

Noting that the total concentration of the carrier c_s^T is

$$c_s^T=c_s+c_{is} \tag{5}$$

and solving Eqs. (4) and (5) for c_{is} and c_s, we find

$$c_{is}=\frac{K_{is}c_i c_s^T}{1+K_{is}c_i}; \quad c_s=\frac{c_s^T}{1+K_{is}c_i}. \tag{6}$$

We shall also assume that: (b) A displacement from equilibrium of the whole membrane-solution system, such as when an electric current is driven through the membrane by an applied voltage, does not perturb significantly the thermodynamic equilibrium existing within *each* of the two solutions. This implies that the chemical and electrochemical potentials μ_s and $\bar{\mu}_r$ remain constant in the aqueous phases, despite the occurrence of irreversible processes within the membrane as well as at the membrane-solution boundaries. Denoting by c_s', c_r', ψ' and c_s'', c_r'', ψ'' the concentrations and the electric potential in the bulk aqueous phases, the constancy of μ_s and $\bar{\mu}_r$ can be expressed, by use of Eqs. (2) and (3), in the form

$$c_s=\begin{cases} c_s'; & -\infty<x\leqq 0 \\ c_s''; & d\leqq x<+\infty \end{cases} \tag{7}$$

and

$$c_r\exp[z_rF\psi/RT]=\begin{cases} c_r'\exp[z_rF\psi'/RT]; & -\infty<x\leqq 0 \\ c_r''\exp[z_rF\psi''/RT]; & d\leqq x<+\infty \end{cases}. \tag{8}$$

Eq. (7) implies that, when $c_s'=c_s''$, the aqueous concentrations of the carrier in the immediate proximities of the interfaces $c_s(0)$ and $c_s(d)$ are also equal, even though transport phenomena are occurring across the membrane.[3]

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[3] Assumption (b) implies that we are neglecting any effect of "diffusion polarization," namely the effect of diffusion of the carriers and complexes across the aqueous unstirred layers. This assumption sets an obvious limitation to the generality of the treatment. Nevertheless, it can be shown that for the experimentally important case, in which the permeation of the ion across the interfaces is due solely to the "heterogeneous complex forming reaction" defined in Eq. (17), the consequence of including "diffusion polarization" into the model amounts to no more than merely replacing in the definition of $\Delta_{is}^*(0)$ or N_i [*see* Eqs. (60) and (68)] the constant term $k_s^B+2A_s^*$ with another constant $[k_s^B+2A_s^*(1+k_s^F\delta/D_s)]/(1+k_s^F\delta/D_s)$; k_s^F, k_s^B and A_s^* are defined in Eqs. (16) and (35), while δ_s and D_s are the thickness of the unstirred layer and the diffusion coefficient of the neutral carrier in water, respectively. Note that, since both of those terms are constant, diffusion polarization will not affect the functional dependence of our results on concentrations; moreover, when the rate constant k_s^B is lower than A_s^*, $k_s^B<A_s^*$, they both reduce to the same quantity $2A_s^*$; which means that in this approximation no complications are expected from diffusion across the unstirred layers.

Eq. (8) shows that the constancy of $\bar{\mu}_r$ leads to a relationship between the two variables $c_r(x)$ and $\psi(x)$, the separate knowledge of which requires an additional equation. This is obtained combining Eq. (8) with the Poisson equation

$$\frac{d^2\psi}{dx^2} = -\frac{4\pi\rho}{\varepsilon}. \tag{9}$$

Following a well-known procedure (Verwey & Overbeek, 1948), and restricting considerations to the case of symmetrical electrolytes in which all the ions present, whether permeant or impermeant, bear the same valence $|z|$, we find in solution (′)

$$\frac{d^2\phi'}{dx^2} = \frac{\sinh\phi'}{L'^2}; \qquad -\infty < x \leqq 0 \tag{10}$$

where

$$\phi'(x) = \frac{|z|F}{RT}[\psi(x) - \psi']; \qquad -\infty < x \leqq 0 \tag{11}$$

$$L'^2 = \frac{RT\varepsilon}{8\pi F^2 \Gamma'} \tag{12}$$

and Γ', the ionic strength of solution (′), is defined as

$$\Gamma' = \frac{z^2}{2}\sum_r c'_r. \tag{13}$$

$\sum$ stands for the sum over the concentrations of all the ionic species (negative and positive) present in the solution. A set of equations entirely analogous to Eqs. (10) through (13) can be written for solution (″) when $\phi''(x)$ is defined as $\phi''(x) = \frac{|z|F}{RT}[\psi(x) - \psi'']$.

(b) The Membrane-Solution Interfaces

The membrane-solution interfaces are represented schematically as ideal surfaces of separation between two dielectric media, having dielectric constants ε and ε^*, respectively; *(the asterisk (*) will be used consistently to denote any quantity referring to the membrane phase).*

To simplify the treatment, we shall make the three following additional assumptions: (c) The dielectric constant changes abruptly at the interfaces. (d) A net fixed charge, resulting from the dissociation of the ionizable polar head groups of the phospholipids, is distributed with uniform surface density σ at the surfaces. (e) A sheet of dipoles is also distributed uniformly

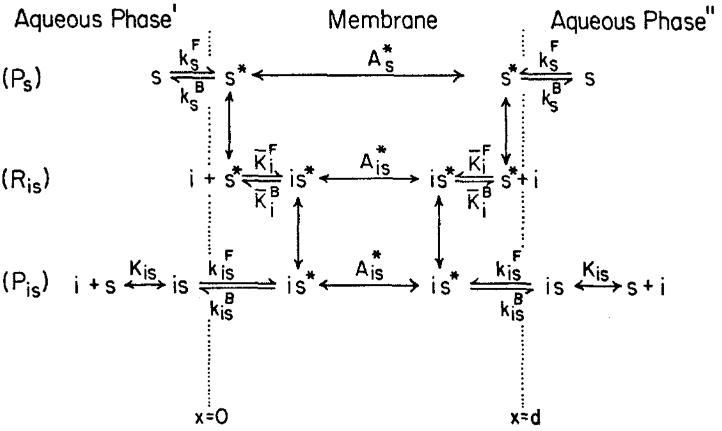

Fig. 1. Schematic diagram of the processes by which neutral carriers mediate permeation of ions through the membrane. The membrane is indicated to be interposed between two aqueous phases (′) and (″) and extends from $x=0$ to $x=d$. (P_s) refers to the partition process of the neutral carrier [Eq. (16)]. (R_{is}) refers to the interfacial reaction process [Eq. (17)], which involves the reaction at the interface between an ion in the aqueous phase and a carrier molecule already present in the membrane. (P_{is}) refers to the partition process of the complex [Eq. (18)], which involves the transfer of an already formed complex from the aqueous phase into the membrane

on both surfaces, determining a discontinuous change of the electric potential across the interfaces.

As a consequence of assumption (d) and of Gauss' theorem of electrostatics, applied to an ideal phase separation boundary between two dielectric media, the discontinuous change of the electric field across the interfaces is described by

$$\varepsilon\left[\frac{d\psi}{dx}\right]_0-\varepsilon^*\left[\frac{d\psi^*}{dx}\right]_0=4\pi\sigma;\qquad \varepsilon^*\left[\frac{d\psi^*}{dx}\right]_d-\varepsilon\left[\frac{d\psi}{dx}\right]_d=4\pi\sigma. \tag{14}$$

From assumption (e) we deduce

$$\psi(0)-\psi^*(0)=\delta;\qquad \psi(d)-\psi^*(d)=\delta \tag{15}$$

where δ takes into account the possible existence of a discontinuous change of the electric potential and is assumed to be a constant, independent of the applied electric field as well as of the composition of the solutions.

The three following processes indicated schematically in Fig. 1 are assumed to occur at both interfaces, which are considered identical.[4] (1.) The "carrier partitioning process" diagrammed in the upper line of Fig. 1

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[4] This follows from our assumption of a symmetric energy profile inside the membrane, as we will see in Section *C*.

[called (P_s)], by which a neutral carrier molecule s is transferred from the aqueous into the membrane phase

$$S \underset{k_s^B}{\overset{k_s^F}{\rightleftarrows}} S^* \tag{16}$$

where

$$\frac{c_s^*(0)_{\text{eq}}}{c_s(0)_{\text{eq}}} = \frac{c_s^*(d)_{\text{eq}}}{c_s(d)_{\text{eq}}} = \frac{k_s^F}{k_s^B} = k_s \quad \text{(partition coefficient of the carrier).} \tag{16a}$$

k_s^F and k_s^B denote the rate constants of the forward and backward transfer of s across the interfaces. Forward is meant to indicate from the aqueous into the membrane, and backward from the membrane into the aqueous phase. k_s is the partition coefficient of the s species, and is related to the equilibrium concentrations according to Eq. (16a).

(2.) The "interfacial reaction process" [called (R_{is})], diagrammed in the second line of Fig. 1, by which a complex is assumed to be formed in the membrane according to

$$i + s^* \underset{\bar{K}_i^B}{\overset{\bar{K}_i^F}{\rightleftarrows}} is^* \tag{17}$$

where

$$\frac{c_{is}^*(0)_{\text{eq}}}{c_i(0)_{\text{eq}}\, c_s^*(0)_{\text{eq}}} = \frac{c_{is}^*(d)_{\text{eq}}}{c_i(d)_{\text{eq}}\, c_s^*(d)_{\text{eq}}} = \frac{\bar{K}_i^F}{\bar{K}_i^B} = \bar{K}_i \tag{17a}$$

(equilibrium constant of the interfacial complex-forming reaction).

(3.) The "complex partitioning process" [called (P_{is})], by which the charged complex is transferred across the interface

$$is \underset{k_{is}^B}{\overset{k_{is}^F}{\rightleftarrows}} is^* \tag{18}$$

where

$$\frac{c_{is}^*(0)_{\text{eq}}}{c_{is}(0)_{\text{eq}}} = \frac{c_{is}^*(d)_{\text{eq}}}{c_{is}(d)_{\text{eq}}} = \frac{k_{is}^F}{k_{is}^B} = k_{is} \quad \text{(partition coefficient of the complex).} \tag{18a}$$

This is diagrammed in the lowest part of Fig. 1 and, aside from the fact that it refers to a charged species, is entirely analogous to the carrier partition process (P_s) described in Eqs. (16) and (16a). The two processes, described in Eqs. (17) and (18), represent two independent mechanisms for ion permeation, one of which may prevail over the other in the particular system studied, but which can generally operate in parallel and contribute additively to the total current across the interfaces.

It should be emphasized that the partition coefficients k_s, k_{is} and the equilibrium constant $\bar{K}_i$, as defined in Eqs. (16a)–(18a), refer to concentrations immediately adjacent to the interfaces, and in no way do they imply that the concentrations of the carrier and of the complex are homogeneous across the membrane interior.

To facilitate the task of following the details of the calculations presented in the mathematical derivation of the results, it will be useful to note that the equilibrium constant $\bar{K}_i$ of the heterogeneous reaction (17a) is related to the equilibrium constant of the homogeneous complexation reaction in the aqueous phase K_{is} and to the partition coefficients k_s and k_{is} by[5]

$$\bar{K}_i = K_{is} \frac{k_{is}}{k_s}. \tag{19}$$

Expressing $\bar{K}_i$ and k_{is} in terms of rate constants, as in Eqs. (17) and (18), relation (19) can be written in the form

$$\frac{\bar{K}_i^F}{\bar{K}_i^B} = \frac{K_{is}}{k_s} \frac{k_{is}^F}{k_{is}^B}. \tag{20}$$

From Eq. (20) we deduce the two following relationships, which will be recurrently used later

$$\frac{\bar{K}_i^F}{\bar{K}_i^B} k_s = \frac{k_{is}^F}{k_{is}^B} K_{is} = \frac{\bar{K}_i^F k_s + k_{is}^F K_{is}}{k_{is}^B + \bar{K}_i^B}. \tag{21}$$

Assuming that the three interfacial processes (P_s), (R_{is}) and (P_{is}), [Eqs. (16)–(18)] can be described by the equations of the chemical kinetics, the total rightward flux of the ionic species i across the left interface $(x=0)$, due to mechanisms (P_{is}) and (R_{is}), will be

$$J_i^{\mathrm{Tot}}(0) = k_{is}^F c_{is}(0) + \bar{K}_i^F c_i(0)\, c_s^*(0) - k_{is}^B c_{is}^*(0) - \bar{K}_i^B c_{is}^*(0). \tag{22}$$

Recalling that the complexation reaction in the aqueous phase is assumed to be at equilibrium up to the interfaces, so that $c_{is}(0) = K_{is} c_s c_i(0)$, Eq. (22) can be conveniently rewritten in the form

$$J_i^{\mathrm{Tot}}(0) = k_{is}^F K_{is} c_s' c_i(0) + \bar{K}_i^F c_i(0)\, c_s^*(0) - (k_{is}^B + \bar{K}_i^B)\, c_{is}^*(0). \tag{23}$$

At the other interface $(x=d)$, the total rightward flux will be

$$J_i^{\mathrm{Tot}}(d) = (k_{is}^B + \bar{K}_i^B)\, c_{is}^*(d) - k_{is}^F K_{is} c_s'' c_i(d) - \bar{K}_i^F c_i(d)\, c_s^*(d). \tag{24}$$

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[5] Eq. (19) follows from the consideration that reaction (17) can be formally decomposed into a sum of reactions (1), (16) and (18), namely $[i + s^* \xrightleftharpoons{\bar{K}_i} is^*] = [i + s \xrightleftharpoons{K_{is}} is] + [is \xrightleftharpoons{k_{is}} is^*] - [s \xrightleftharpoons{k_s} s^*]$, recalling that the equilibrium constant of a chemical reaction, which can be obtained as the algebraic sum of other reactions, is given by the product of the equilibrium constants of the reactions that are added, divided by those of the reactions that are subtracted.

Adding the contributions of the processes (P_s) and (P_{is}), the total flux of the carrier s across the two interfaces will be

$$J_s^{\mathrm{Tot}}(0)=k_s^F c_s'+k_{is}^F K_{is} c_s' c_i(0)-k_s^B c_s^*(0)-k_{is}^B c_{is}^*(0) \tag{25}$$

and

$$J_s^{\mathrm{Tot}}(d)=k_s^B c_s^*(d)+k_{is}^B c_{is}^*(d)-k_s^F c_s''-k_{is}^F K_{is} c_s'' c_i(d). \tag{26}$$

The use of Eqs. (22)–(26) is required for the derivation of the conductance when the rate constants of the interfacial processes are comparable with the rate of migration across the membrane.

(c) The Membrane Phase

The membrane will be represented as a homogeneous phase, whose properties are considered continuous functions of the distances from the interfaces, symmetrical with respect to a central plane at $x=d/2$. Consistently with this point of view, the transport of the permeant species will be described in terms of flux equations, which are more general than any used heretofore, in that they allow for any symmetrical shape of the free-energy barrier as well as for any dependence of the diffusion coefficients on the distances from the interfaces.

Recalling that the neutral carrier s and the charged complex is are the only diffusible species present in the membrane, the respective fluxes J_s^* and J_{is}^* will be given by

$$-J_s^*=\frac{D_s^*(x)}{RT}\,c_s^*(x)\,\frac{d\mu_s^*}{dx} \tag{27}$$

and

$$-J_{is}^*=\frac{D_{is}^*(x)}{RT}\,c_{is}^*(x)\,\frac{d\bar{\mu}_{is}^*}{dx}. \tag{28}$$

$D_s^*(x)$, $D_{is}^*(x)$ are the x-dependent coefficients, generally considered constant in previous treatments, and $c_s^*(x)$, $c_{is}^*(x)$ are the concentrations of the neutral carriers and of the charged complexes inside the membrane; in the general case of a non-uniform profile of the free-energy barrier, the chemical potential of the carrier $\mu_s^*(x)$ and the electrochemical potential of the complex $\bar{\mu}_{is}^*(x)$ will be written in the form

$$\mu_s^*(x)=\mu_s^{0*}(x)+RT\ln c_s^*(x) \tag{29}$$

and

$$\bar{\mu}_{is}^*(x)=\mu_{is}^{0*}(x)+RT[\ln c_{is}^*(x)+\phi^*(x)] \tag{30}$$

where $\phi^*(x)$ is the normalized electric potential

$$\phi^*(x)=\frac{zF}{RT}\,\psi^*(x) \tag{31}$$

and the lack of uniformity of the energy profile is taken into account by the dependence on distance of the standard chemical potentials $\mu_s^{0*}(x)$ and $\mu_{is}^{0*}(x)$.[6]

Eq. (28) reduces to the classic Nernst-Planck equation when both D_{is}^* and μ_{is}^{*0} are assumed constant. Less apparent but not difficult to show[7] is that the Eyring single-barrier model corresponds to the case in which the profile of the energy has a sharp maximum in the middle of the membrane.

Defining

$$w_s^*(x)=\mu_s^{0*}(x)/RT; \qquad w_{is}^*(x)=\mu_{is}^{0*}(x)/RT+\phi^*(x) \tag{32}$$

and observing that, because of the symmetry of the energy barrier, the standard chemical potentials must have equal values at the two interfaces, integration of Eqs. (27) and (28) gives at steady state

$$J_s^*=A_s^*[c_s^*(0)-c_s^*(d)] \tag{33}$$

and

$$J_{is}^*=B_{is}^*[c_{is}^*(0)\,e^{\phi^*(0)}-c_{is}^*(d)\,e^{\phi^*(d)}] \tag{34}$$

where

$$\frac{1}{A_s^*}=e^{-w_s^*(0)}\int_0^d \frac{e^{w_s^*}}{D_s^*}\,dx \tag{35}$$

and

$$\frac{1}{B_{is}^*}=e^{-\mu_{is}^{0*}(0)/RT}\int_0^d \frac{e^{w_{is}^*}}{D_{is}^*}\,dx. \tag{36}$$

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[6] Taking into account image charge effects, Neumcke and Läuger (1969) have derived the shape of the energy barrier and have analyzed its consequences on the expectations of the current-voltage relationships. Hall *et al.* (1973) have deduced the profile of the barrier for the nonactin-K$^+$ complex in phosphatidyl ethanolamine membranes, by fitting experimental voltage-current data with Eq. (34), and solving the integral Eq. (36).

[7] Recalling the definition of $w_{is}^*(x)$ given in Eq. (32), and assuming that $\mu_{is}^{0*}(x)$ attains high values only in a thin region of thickness δ, centered around the middle of the membrane, the integral

$$\int_0^d \frac{e^{w_{is}^*}}{D_{is}^*}\,dx \text{ can be approximated by } \delta\,\frac{e^{w_{is}^*\left(\frac{d}{2}\right)}}{D_{is}^*\left(\frac{d}{2}\right)}. \tag{a}$$

Taking into account approximation (a) and recalling Eq. (32), Eqs. (34) and (36) give

$$J_{is}^*=\frac{D_{is}^*\left(\frac{d}{2}\right)}{\delta}\left\{\exp\left[\left(\mu_{is}^{0*}(0)-\mu_{is}^{0*}\left(\frac{d}{2}\right)\right)\Big/RT\right]\right\} \tag{b}$$
$$\cdot\,\{c_{is}^*(0)\,e^{\phi^*(0)-\phi^*\left(\frac{d}{2}\right)}-c_{is}^*(d)\,e^{\phi^*(d)-\phi^*\left(\frac{d}{2}\right)}.$$

Eq. (b) is formally identical to the flux equation in the Eyring single-barrier model [e.g., *see* Läuger and Stark, 1970; Eqs. (6)–(9)].

From the definitions of $w_s^*(x)$, $w_{is}^*(x)$, A_s^* and B_{is}^*, given in Eqs. (32), (35) and (36), it should be clear that A_s^* is a constant, dependent only on the type of carrier and the structural properties of the membrane, whereas B_{is}^* depends also on the profile of the electric potential and is therefore in general also a function of the concentration of ions. A paper by Ciani, Laprade, Szabo, and Eisenman is now in preparation in which there will be a rigorous evaluation of B_{is}^* at equilibrium and in the particular case where the profiles of the energy barrier and the diffusion coefficient are uniform. The results presented in this paper will be restricted to the case of constant field, where B_{is}^*, at equilibrium, can be approximated by the expressions (62) and (65).

Derivation of the Expression for the Conductance in the Limit of Zero Current

We consider a membrane interposed between solutions of identical composition ($c_i' = c_i'' = c_i$; $c_s' = c_s'' = c_s$) and restrict the analysis to the case in which only one ionic species, that we denote by i, is capable of forming membrane-soluble charged complexes with the neutral carrier s. Since the complex is is the only charged species present in the membrane, the current density I is related to its flux by

$$I = zFJ_{is}^* \tag{37}$$

and the zero-current conductance for unit surface of the membrane will be

$$G_0(i) = \lim_{V\to 0} \frac{I}{V} = zF \lim_{V\to 0} \frac{J_{is}^*}{V}. \tag{38}$$

V is the potential difference between the two aqueous solutions ($V = \psi' - \psi''$), which is not necessarily identical to the potential difference between the interfaces of the membrane. It is actually worth emphasizing that the following derivation is formulated so as to allow for the possibility that a portion of the applied potential is distributed in the diffuse double layers, thereby perturbing the ion concentrations from their initial values, according to the condition for equilibrium given by Eq. (8).

We can start from the observation that, when a current density I is passing through the membrane, the boundary concentrations $c_i(0)$, $c_s^*(0)$, $c_{is}^*(0)$ and $c_i(d)$, $c_s^*(d)$, $c_{is}^*(d)$[8] will be displaced from their equilibrium values

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[8] In agreement with assumption (b) and Eq. (7) we neglect the perturbation of the aqueous concentrations of the neutral carriers $c_s(0)$ and $c_s(d)$ from their equilibrium value c_s.

by amounts that can be considered functions of the current density and that we shall define in the following way

$$c_i(0)=c_i(0)_{\mathrm{eq}}+\delta_i^0[I]\,I, \tag{39}$$

$$c_s^*(0)=k_s c_s+\delta_s^{0*}[I]\,I, \tag{40}$$

$$c_{is}^*(0)=\bar{K}_i k_s c_s c_i(0)_{\mathrm{eq}}+\delta_{is}^{0*}[I]\,I. \tag{41}$$

$\bar{K}_i$ and k_s have been defined in Eqs. (16a) and (17a), and $\delta_i^0(I)$, $\delta_s^{0*}[I]$ and $\delta_{is}^{0*}[I]$ are functions of the current density I, which, for $I=0$, reduce to the derivatives of $c_i(0)$, $c_s^*(0)$ and $c_{is}^*(0)$ with respect to I, calculated at $I=0$ (e.g., $\delta_i^0[0]=[\partial c_i(0)/\partial I]_{I=0}$); the superscript ^{0} indicates that these quantities refer to the left interface ($x=0$). A set of equations analogous to Eqs. (39)–(41) can be written for the right interface, introducing the functions $\delta_i^d[I]$, $\delta_s^{d*}[I]$ and $\delta_{is}^{d*}[I]$. Observing, however, that the system, when at equilibrium, is symmetrical with respect to the middle of the membrane, the values of $c_i(d)$, $c_s^*(d)$ and $c_{is}^*(d)$, corresponding to a given current density I, must be identical to the values that $c_i(0)$, $c_s^*(0)$ and $c_{is}^*(0)$ would have if we simply inverted the sign of the current, namely if we passed across the membrane the current density $-I$. Therefore, when the current $+I$ flows across the membrane, we can write for the right interface ($x=d$)

$$c_i(d)=c_i(0)_{\mathrm{eq}}+\delta_i^0[-I]\cdot(-I), \tag{42}$$

$$c_s^*(d)=k_s c_s+\delta_s^{0*}[-I]\cdot(-I), \tag{43}$$

$$c_{is}^*(d)=\bar{K}_i k_s c_s c_i(0)_{\mathrm{eq}}+\delta_{is}^{0*}[-I]\cdot(-I). \tag{44}$$

Solving Eqs. (39) and (42) for $c_i(0)_{\mathrm{eq}}$ and substituting in Eqs. (41) and (44), we get

$$c_{is}^*(0)=\bar{K}_i k_s c_s c_i(0)+\Delta_{is}^{0*}[I]\cdot I, \tag{45}$$

$$c_{is}^*(d)=\bar{K}_i k_s c_s c_i(d)+\Delta_{is}^{0*}[-I]\cdot(-I) \tag{46}$$

where

$$\Delta_{is}^{0*}[\pm I]=\delta_{is}^{0*}[\pm I]-\bar{K}_i k_s c_s \delta_i^0[\pm I]. \tag{47}$$

From Eqs. (45) and (46) it is seen that the terms Δ_{is}^{0*} can be regarded as a measure of the effect a current I has on displacing the interfacial complex concentrations $c_{is}^*(0)$ and $c_{is}^*(d)$ from their respective values $\bar{K}_i k_s c_s c_i(0)$ and $\bar{K}_i k_s c_s c_i(d)$, expected if the interfacial reactions were at equilibrium.

Substituting Eqs. (45) and (46) in Eq. (34) we find

$$I=\frac{zF\,\bar{K}_i k_s c_s B_{is}^*[c_i(0)\,e^{\phi^*(0)}-c_i(d)\,e^{\phi^*(d)}]}{1-zFB_{is}^*[\Delta_{is}^{0*}[I]\,e^{\phi^*(0)}+\Delta_{is}^{0*}[-I]\,e^{\phi^*(d)}]}. \tag{48}$$

On the other hand, by use of Eqs. (8) and (15), and setting $\psi''=0$, the bracketed quantity of the numerator becomes

$$c_i(0)\,e^{\phi^*(0)}-c_i(d)\,e^{\phi^*(d)}=c_i\,e^{-\frac{zF}{RT}\delta}\left[e^{\frac{zF}{RT}\psi'}-1\right] \tag{49}$$

or, recalling that $\psi'=V$ and defining

$$\omega=e^{-\frac{zF}{RT}\delta}, \tag{50}$$

$$c_i(0)\,e^{\phi^*(0)}-c_i(d)\,e^{\phi^*(d)}=\omega\,c_i\left[e^{\frac{zF}{RT}V}-1\right]. \tag{51}$$

Substituting Eq. (51) in Eq. (48) and dividing both sides by $\frac{RT}{zF}\left[e^{\frac{zFV}{RT}}-1\right]$ we find

$$\frac{zF}{RT}\,\frac{I}{e^{\frac{zF}{RT}V}-1}=\frac{z^2F^2}{RT}\,\frac{\omega\,B_{is}^*\,\overline{K}_i\,k_s\,c_s\,c_i}{1-zF\,B_{is}^*\left[\Delta_{is}^{0\,*}[I]\,e^{\phi^*(0)}+\Delta_{is}^{0\,*}[-I]\,e^{\phi^*(d)}\right]}. \tag{52}$$

Carrying out now the limit of both sides of Eq. (52) for $V\to 0$ (and therefore $I\to 0$), taking into account the fact that

$$\lim_{V\to 0} B_{is}^*=B_{is}^*(\mathrm{eq})=e^{\mu_{is}^{0\,*}(0)/RT}\left[\int_0^d \frac{e^{\mu_{is}^{0\,*}(x)/RT+\phi^*(x)_{\mathrm{eq}}}}{D_{is}^*(x)}\,dx\right]^{-1} \tag{53}$$

where $\phi^*(x)_{\mathrm{eq}}$ is the profile of the electric potential at equilibrium ($I=0$ and $V=0$), and that

$$\lim_{V\to 0}\Delta_{is}^{0\,*}[I]\,e^{\phi^*(0)}=\lim_{V\to 0}\Delta_{is}^{0\,*}[-I]\,e^{\phi^*(d)}=\Delta_{is}^{0\,*}[0]\,e^{\phi^*(0)_{\mathrm{eq}}} \tag{54}$$

we find

$$\begin{aligned}\frac{zF}{RT}\lim_{V\to 0}\frac{I}{e^{zFV/RT}-1}&=\lim_{V\to 0}\frac{I}{V}\\ &=G_0(i)=\frac{z^2F^2}{RT}\,\frac{\omega\,B_{is}^*(\mathrm{eq})\,\overline{K}_i\,k_s\,c_s\,c_i}{1-2zF\,B_{is}^*(\mathrm{eq})\,\Delta_{is}^{0\,*}[0]\,e^{\phi^*(0)_{\mathrm{eq}}}}.\end{aligned} \tag{55}$$

Eq. (55) not only is the most general expression for the membrane conductance in the limit of zero current which can be deduced under the assumptions of the present treatment, but, as long as $\Delta_{is}^{0\,*}$ is defined as generally as in Eq. (47), its validity could be extended also to a more comprehensive model which would take into account the effects of diffusion polarization in the aqueous unstirred layers. In fact, $\Delta_{is}^{0\,*}[0]$, which appears in the denominator of Eq. (55), expresses the effects on the conductance of deviations from equilibrium at the interfaces, regardless of their particular physical origin, and, in particular, regardless of whether they are caused by rate limitations at the interfaces, by diffusion polarization, or by the presence of both such phenomena. On the other hand, $B_{is}^*(\mathrm{eq})$, defined in Eq. (53), allows for any profile of the electric potential, for any symmetrical shape of the energy barrier, as well as for a variable diffusion coefficient of the permeant complex; therefore it takes into account implicitly the possible contribution of "image charge" effects (Neumcke & Läuger, 1969) to the forces acting on the diffusible particles, and the possible variations of fluidity (McConnell, 1970) with position.

To obtain a more useful and relatively simple expression for the conductance in terms of experimentally accessible variables, we shall, however, restrict the generality of Eq. (55) to those cases in which the perturbation from equilibrium at the boundaries is due to the finite rates of the interfacial processes rather than to diffusion polarization, and, in addition, the space charge inside the membrane is sufficiently low that the constant field approximation can be used. The first of these two restrictions simplifies greatly the evaluation of $\Delta_{is}^{0\,*}[0]$; the second will enable us to find the explicit dependence of $B_{is}^{*}(\mathrm{eq})$ on parameters such as the fixed-charge density and the ionic strength.

Determination of $\Delta_{is}^{0\,*}[0]$

Recalling that at steady state the interfacial and transmembrane flows of current must be identical, we have at the left interface

$$\frac{zF\,J_i^{\mathrm{Tot}}(0)}{I}=1. \tag{56}$$

Using the right-hand side of Eq. (23) for the explicit expression of $J_i^{\mathrm{Tot}}(0)$, substituting $c_i(0)$, $c_s^*(0)$ and $c_{is}^*(0)$ with the right-hand sides of Eqs. (39), (40) and (45), and finally taking the limit of Eq. (56) for $I\to 0$, we obtain, with the help of Eqs. (19), (20) and (21),

$$\overrightarrow{K}_i^F\, c_i(0)_{\mathrm{eq}}\, \delta_s^{0\,*}[0]-(k_{is}^B+\overrightarrow{K}_i^B)\,\Delta_{is}^{0\,*}[0]=1/zF. \tag{57}$$

Moreover, the steady-state requirement that the total flux of the carrier species across the left interface be equal to its total transmembrane flux gives

$$\frac{J_s^{\mathrm{Tot}}(0)}{J_s^*+J_{is}^*}=\frac{J_s^{\mathrm{Tot}}(0)}{J_s^*+I/zF}=1. \tag{58}$$

With the help of Eqs. (25), (33), (35), (40) and (47), and following the same sequence of steps as for the derivation of Eq. (57), we get

$$(k_s^B+2A_s^*)\,\delta_s^{0\,*}[0]+k_{is}^B\,\Delta_{is}^{0\,*}[0]=-1/zF. \tag{59}$$

Solving Eqs. (57) and (59) for $\Delta_{is}^{0*}[0]$ we obtain finally[9]

$$\Delta_{is}^{0*}[0] = -\frac{1}{zF} \frac{1}{k_{is}^{B} + \dfrac{\overline{K}_i^{B}(k_s^{B} + 2A_s^{*})}{\overline{K}_i^{F} c_i(0)_{\text{eq}} + (k_s^{B} + 2A_s^{*})}}. \tag{60}$$

We have already seen in Eqs. (45) and (46) that the quantity Δ_{is}^{0*} could be regarded as a measure of the displacement of the boundary concentrations of complexes from their equilibrium value, caused by the passage of a unit current across the membrane. Now, Eq. (60) indicates the extent of this displacement in terms of the rate constants which are responsible for it. A clearer physical explanation for the presence of the term $\Delta_{is}^{0*}[0]$ in the expression of the conductance is given in Appendix B, and we will now proceed to derive the conductance expression for the particular case in which the constant field approximation can be made.

Explicit Expression for the Conductance in the Constant Field Approximation

Substitution in Eq. (55) of the explicit expression for $\Delta_{is}^{0*}[0]$ given by Eq. (60) does not lead to an explicit dependence of the conductance on the aqueous parameters because $e^{\phi^*(0)}$eq and $B_{is}^{*}(\text{eq})$ are, in general, unknown functions of the aqueous compositions. However, assuming that the space charge inside the membrane is so low that the profile of the electric potential can be approximated by a constant (*see* Appendix A), we shall have

$$\phi^*(x)_{\text{eq}} = \text{const} = \phi^*(0)_{\text{eq}}. \tag{61}$$

Eq. (53) can then be written as

$$B_{is}^{*}(\text{eq}) = A_{is}^{*} e^{-\phi^*(0)_{\text{eq}}} = A_{is}^{*} \eta/\omega \tag{62}$$

where

$$A_{is}^{*} = e^{\mu_{is}^{0*}(0)/RT} \left[\int_0^d \frac{e^{\mu_{is}^{0*}(x)/RT}}{D_{is}^{*}(x)} \, dx \right]^{-1} \tag{63}$$

and η from Eqs. (62), (50), (15) and (31) is recognized as being equal to $\exp\left[-\frac{zF}{RT}\psi(0)\right]$. Since $\mu_{is}^{0*}(x)$ and $D_{is}^{*}(x)$ are assumed to be independent of concentration, A_{is}^{*}, defined in Eq. (63), will be so too. Substituting Eqs.

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[9] The validity of relationships (42)–(44) relieves us of the necessity of considering explicitly the equations of continuity for the current and the total flux of carriers at the other interface ($x = d$). It can be shown that, if we write Eqs. (56) and (58) for the right interface and carry out the limit for $I \to 0$, we merely reobtain Eqs. (57) and (59).

(62) and (60) in Eq. (55) we find

$$G_0(i)=\frac{z^2F^2}{RT}A_{is}^*\overline{K}_i k_s\frac{c_s^T c_i\eta}{1+K_{is}c_i}\,\frac{1}{1+2\dfrac{A_{is}^*}{k_{is}^B+\dfrac{\overline{K}_i^B(k_s^B+2A_s^*)}{\overline{K}_i^F c_i\eta+(k_s^B+2A_s^*)}}} \tag{64}$$

where η, in the case of electrolytes all having the same valence, is shown in Appendix A to be given by

$$\eta=\left\{\sqrt{1+\frac{\pi z^2\sigma^2}{2RT\varepsilon\Gamma}}+\sqrt{\frac{\pi z^2\sigma^2}{2RT\varepsilon\Gamma}}\right\}^{-2\frac{\sigma}{z}\left|\frac{z}{\sigma}\right|}. \tag{65}$$

Eqs. (64) and (65) represent our principle result in the constant field approximation, which gives the explicit dependence of the conductance on parameters such as the concentration of the permeant ion c_i, the total concentration of the carrier c_s^T, the ionic strength Γ, and the fixed-charge density σ. The denominator of the last fraction in Eq. (64) expresses the effect of rate limitation at surfaces; η accounts for the presence of the fixed charges, while the effects of non-uniformity of the energy barrier and the diffusion coefficient are contained in the parameters A_{is}^* and A_s^*. It should be noted that the contribution of dipole effects does not appear explicitly, since it is already included in the definition of the equilibrium and rate constants of the interfacial processes.

To analyze the dependence of $G_0(i)$ on the aqueous composition, Eq. (64) can be rewritten more conveniently in the form

$$G_0(i)=\frac{z^2F^2}{RT}k_s L_i c_s^T c_i\eta\frac{1+M_ic_i\eta}{(1+K_{is}c_i)(1+N_ic_i\eta)} \tag{64'}$$

where the parameters L_i, M_i and N_i are defined in the following way

$$L_i=\frac{\overline{K}_iA_{is}^*}{1+2A_{is}^*/(k_{is}^B+\overline{K}_i^B)}, \tag{66}$$

$$M_i=\frac{k_{is}^B}{k_{is}^B+\overline{K}_i^B}\,\frac{\overline{K}_i^F}{k_s^B+2A_s^*}, \tag{67}$$

$$N_i=M_i+\frac{2L_i}{\left(1+\dfrac{k_{is}^B}{\overline{K}_i^B}\right)^2(k_s^B+2A_s^*)}. \tag{68}$$

The form of the conductance given by Eq. (64′) is particularly apt for identifying the parameters deducible from conductance measurements. For example, when η is maintained constant by holding the ionic strength to a fixed level, it is simple to realize that four parameters at most, namely $k_s L_i \eta$, $M_i \eta$, $N_i \eta$ and K_{is}, can be derived by varying the concentration of the permeant ion c_i.

The two equivalent expressions for the conductance, Eqs. (64) and (64′), even though less general than Eq. (55), since restricted to the limit of constant field, are still considerably more general than these deduced previously from the classic Nernst-Planck (Markin, Pastushenko *et al.*, 1969) or the Eyring models (Läuger & Stark, 1970). In fact, both such models are special cases of Eq. (28) used in the present paper, and correspond to the particular circumstances in which the diffusion coefficients are constant and the energy barrier is either a step function with discontinuities at the interfaces (Nernst-Planck), or a symmetrical function with a sharp peak in the center of the membrane (Eyring). Moreover, as compared to the results reported in previous treatments, Eqs. (64) and (64′) take into account explicitly the contribution to the conductance of the interfacial partitioning of the charged complexes between the aqueous solutions and the membrane [mechanism (P_{is})]. The consequences of this mechanism were not analyzed in detail in previous treatments (Läuger & Stark, 1970; Stark & Benz, 1971; Stark *et al.*, 1971), mainly because no effects of complexation in the aqueous phases could be detected in systems where valinomycin or monactin were used as carriers. Nevertheless, the recent finding that trinactin does form complexes in an aqueous phase with monovalent cations (Feinstein & Felsenfeld, 1971; Ciani *et al.*, *unpublished*; Laprade *et al.*, 1972; Laprade, Ciani *et al.* and Laprade, Eisenman *et al.*, *unpublished*) implies that, at least for certain types of carriers, the contribution of the partition mechanism (P_{is}) should not be neglected *a priori*, and therefore justifies the extension of the theory to include its expected effects.

Finally, it is worth mentioning that Eqs. (64) and (64′) generalize the expressions of the carrier-mediated conductance, found in the previous literature, to allow for the redistribution of concentrations and potential in the aqueous diffuse double layers, expected when a potential difference is applied between the two solutions (i.e., the "ion injection effect" of Walz, Bamberg and Läuger, 1969). The peculiar circumstance that the contribution of such effects does not appear tangibly in the results, so that Eqs. (64) and (64′) are actually identical to the equations that one would obtain by simply ignoring them, does by no means imply that they are necessarily negligible, but is rather a consequence of the exponential dependence of the aqueous ion concentrations on the potential, expressed in Eq. (8) and used in Eq. (49) to replace the internal potential and concentrations with the total applied potential and the concentrations in the bulk solutions.

The Main Features of the Conductance Equation

(a) Definition of the "Equilibrium" and "Kinetic Domains"

For the discussion of the general result in the constant field approximation, as given by Eqs. (64) or (64′), it will now be useful to consider separately the two alternative cases in which

$$-2zF\Delta_{is}^{0*}[0]=\frac{2}{k_{is}^{B}+\dfrac{\overline{K}_i^B(k_s^B+2A_s^*)}{\overline{K}_i^F c_i\eta+(k_s^B+2A_s^*)}}\ll\frac{1}{A_{is}^*} \tag{69}$$

and

$$-2zF\Delta_{is}^{0*}[0]=\frac{2}{k_{is}^{B}+\dfrac{\overline{K}_i^B(k_s^B+2A_s^*)}{\overline{K}_i^F c_i\eta+(k_s^B+2A_s^*)}}\geqq\frac{1}{A_{is}^*}. \tag{70}$$

All the cases in which condition (69) is satisfied will be referred to as defining an "*equilibrium domain*," whereas the opposite condition (70) will be taken as the definition of a "*kinetic domain*." From Eqs. (45), (46) and (55) it should be clear that the "equilibrium domain" encompasses all the cases in which the displacement of the interfacial concentrations of complexes, $c_{is}^*(0)$ and $c_{is}^*(d)$, from the values that they would have if local equilibrium existed at the membrane-solution boundaries can be considered negligible; while the "kinetic domain" refers to the cases in which this approximation is not valid.

(b) Dependence of the Zero-Current Conductances $G_0(i)$ on the Concentration of the Permeant Ion at Constant Ionic Strength and Constant Fixed-Charge Density

In this section we analyze the dependence of Eq. (64) [or (64′)] on the concentration of the permeant ion c_i assuming that any variation of c_i is accompanied by an equal variation in the opposite direction of the concentration of an impermeant ion, to maintain the ionic strength constant.

Equilibrium Domain

When condition (69), defining the equilibrium domain, is satisfied, Eq. (64) reduces to

$$G_0(i)=\frac{z^2F^2}{RT}A_{is}^*\overline{K}_i k_s\frac{c_s^T c_i\eta}{1+K_{is}c_i}. \tag{71}$$

As illustrated in the dashed curve of Fig. 2, Eq. (71) predicts a linear dependence of $G_0(i)$ on c_i when complexation in the aqueous phases is negligible

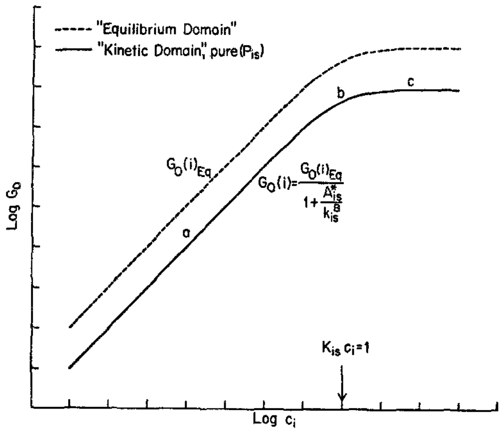

Fig. 2. Theoretically expected dependence on the permeant ion concentration of the membrane conductance in the "kinetic domain" when only a pure partition mechanism (P_{is}) operates at the interfaces, as well as in the "equilibrium domain." η is assumed constant. The dashed curve corresponds to the "equilibrium domain" and the solid curve to the "kinetic domain." For this figure, as well as for Figs. 3 through 6, the logarithm of the conductance is plotted as a function of the logarithm of the permeant ion concentration. Each division on the ordinate and the abscissa corresponds to a 10-fold change in conductance and a 10-fold change in the permeant ion concentration, respectively. Absolute values of conductance and concentration are arbitrary. The regions denoted by lower case letters are discussed in the text

($K_{is}c_i=c_{is}/c_s \ll 1$); at higher ionic concentrations for which formation of the complexes in the aqueous solutions becomes appreciable ($K_{is}c_i=c_{is}/c_s > 1$), $G_0(i)$ approaches to a constant finite value, which, from Eqs. (71) and (19) is seen to be equal to

$$G_0(i)_L=\frac{z^2F^2}{RT}A^*_{is}k_{is}c^T_s. \tag{72}$$

The saturation of the conductance, expected from Eq. (71) and shown in Fig. 2, is due to the fact that at equilibrium the concentration of charged complexes at any point x inside the membrane is given by

$$c^*_{is}(x)=k_{is}c_{is}(0)\,e^{-[w^*(x)-w^*(0)]}=k_{is}\frac{K_{is}c^T_s\,c_i\eta}{1+K_{is}c_i}\,e^{-[w^*(x)-w^*(0)]} \tag{73}$$

and thus can never exceed the maximum value $k_{is}c^T_s\eta e^{-[w^*(x)-w^*(0)]}$, regardless of how high the concentration c_i in the aqueous phase might be.

Kinetic Domain

When relationship (70) is satisfied, the second term in the denominator of Eq. (64) cannot be neglected. Before considering the behavior of the general expression (64) [or (64')], which allows for both mechanisms (P_{is})

and (R_{is}) to function in parallel at the interfaces, we shall analyze separately the two limiting cases in which only one or the other of these is operative in a given membrane.

(1) *Mechanism* (P_{is}). When only mechanism (P_{is}) operates at the interfaces, $(\bar{K}_i^B=0)$, so that $M_i=N_i$, Eq. (64′) becomes, with the help of Eq. (66)

$$G_0(i)=\frac{z^2F^2}{RT}\,\frac{A_{is}^*\bar{K}_i k_s}{1+2A_{is}^*/k_{is}^B}\,\frac{c_s^T c_i \eta}{1+K_{is}c_i}. \tag{74}$$

As illustrated by the solid line of Fig. 2, Eq. (74) predicts the same functional dependence on c_i as Eq. (71), and the physical interpretation for the saturation, when $K_{is}c_i>1$, is also entirely analogous. Nevertheless, for comparable values of $A_{is}^*\bar{K}_i k_s$ both the proportionality coefficient of the concentration dependent term and the value of the limiting conductance are reduced by the factor $(1+2A_{is}^*/k_{is}^B)$. The physical justification for this result will be clarified in Appendix B. Note that, because of the identical functional dependence of Eqs. (71) and (74) on the concentration c_i, any experimentally determined behavior of the conductance which is found to be consistent with the expectations of either of these equations cannot provide information as to whether local equilibrium does or does not exist at the interfaces.

(2) *Mechanism* (R_{is}). When only mechanism (R_{is}) is operative at the interfaces, $k_{is}^B=0$, so that

$$M_i=0 \tag{75}$$

we will have

$$L_i=\frac{\bar{K}_i A_{is}^*}{1+2A_{is}^*/\bar{K}_i^B};\qquad N_i=\frac{2L_i}{k_s^B+2A_s^*} \tag{76}$$

and Eq. (64′) reduces to

$$G_0(i)=\frac{z^2F^2}{RT}\,k_s L_i c_s^T\,\frac{c_i\eta}{(1+K_{is}c_i)(1+N_i c_i \eta)}. \tag{77}$$

Eq. (77), which is a particular case of Eq. (64′), extends the validity of formally similar expressions obtained with the use of a Nernst-Planck (Markin, Pastushenko *et al.*, 1969) and the Eyring single-barrier model (Läuger & Stark, 1970), to the more general case in which both the diffusion coefficient and the shape of the energy barrier are non-uniform functions of distance. It turns out that allowing for such effects requires the introduction of parameters of less intuitive physical significance, such as A_s^* and A_{is}^*, but does not affect the functional dependence of the conductance on concentrations. The behavior of Eq. (77), illustrated in Fig. 3, is more complex than that of either Eq. (71) or (74). It predicts a linear dependence

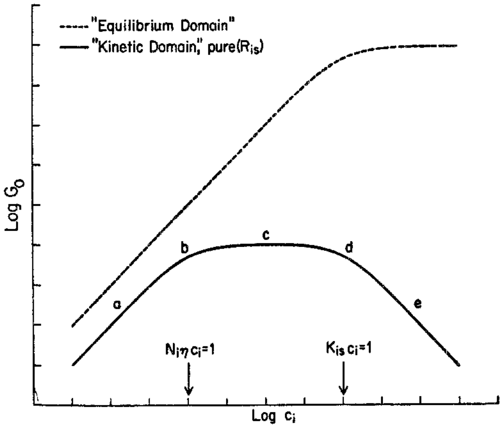

Fig. 3. Theoretically expected dependence on permeant ion concentration of the membrane conductance in the "kinetic domain" with a pure (R_{is}) mechanism (solid curve). Ordinate and abscissa as in Fig. 2. η is assumed constant. For comparison, the behavior in the "equilibrium domain" is shown by the dashed curve. The regions denoted by lower case letters are discussed in the text

of $G_0(i)$ on c_i for $K_{is}c_i \ll 1$ and $N_i\eta c_i \ll 1$, the attainment of a maximum at

$$c_i = (K_{is} N_i \eta)^{-\frac{1}{2}} \tag{78}$$

and the tendency for it to vanish at higher concentrations. Such behavior is exemplified in experimental data obtained for glyceryl-dioleate membranes by using trinactin as the carrier and K^+ or NH_4^+ as the permeant ions (Laprade *et al.*, 1972; Ciani *et al.*, *unpublished*).

In the absence of aqueous complexation ($K_{is}c_i \ll 1$), Eq. (77) becomes

$$G_0(i) = \frac{z^2 F^2}{RT} k_s L_i \frac{c_s^T c_i \eta}{1 + N_i c_i \eta}. \tag{79}$$

Eq. (79) is only formally similar to either Eq. (71) or (74); in fact, if $N_i\eta \gg K_{is}$, the saturation of $G_0(i)$, predicted by Eq. (79), will occur much before the ion-carrier complexation in the aqueous solutions sets a limit to the concentration of complexes inside the membrane. As is shown in Appendix B, this property can be accounted for in terms of the effects of rate limitation at the surfaces on the driving force within the membrane interior. At concentrations sufficiently high that $N_i\eta c_i > 1$, Eq. (79) shows that the conductance approaches to a constant finite level given by

$$G_0(i)_L = \frac{z^2 F^2}{RT} \frac{k_s c_s^T}{2} (k_s^B + 2A_s^*). \tag{80}$$

Note that the expression on the right-hand side depends on the properties of the neutral carrier and is independent of the particular cation bound. This means that for a given carrier, whatever the ion bound, the conductance will never exceed the value given by Eq. (80).

(3) *Mechanism* (P_{is}) *and* (R_{is}) *Acting in Parallel.* In (1) and (2) we have considered the extreme cases in which ion permeation across the interfaces was due to only one of the two mechanisms (P_{is}) and (R_{is}). We shall consider here the more complex case in which both mechanisms operate simultaneously, but will analyze separately the two alternative situations in which either one or the other is predominant.

If we consider first the situation ($k^B_{is} \gg \bar{K}^B_i$) where, at low concentration of the permeant ion, (P_{is}) is more effective than (R_{is}), Eq. (64) as well as a comparison between Figs. 2 and 3 indicate that it will remain so in the whole concentration range. This can be intuitively understood if one considers that the contribution of (R_{is}) to the conductance in the range of low concentrations can increase at most at the same rate as that of (P_{is}), but will eventually reach a "plateau," or even decrease at higher concentrations; at this stage, noting that the two mechanisms operate in parallel, the only effective contribution will be that of (P_{is}), and the behavior of the conductance will consequently be the same as that of Fig. 2.

In the alternative case in which $\bar{K}^B_i \gg k^B_{is} (N_i \gg M_i)$, where at low concentrations (R_{is}) is predominant, the conductance will be described either by curve *1* or by curve *2* of Fig. 4, depending on whether $M_i \eta < K_{is}$ or $M_i \eta > K_{is}$, respectively. For curve 1, up to point e_1, the situation is identical to that of Fig. 3 (up to point *e*). However, further increase of the concentration of the permeant ion will lead to a region $f_1 g_1$ where the only effective mechanism is (P_{is}). Since in this concentration range the aqueous ion-carrier complexation is already substantial, we finally reach a "plateau."

For curve *2* of Fig. 4, which refers to the situation in which $M_i \eta > K_{is}$, the behavior up to point *c* is identical to that of curve *1*. However, further increase of the concentration c_i will lead to a region in which $M_i \eta c_i > 1$, while $K_{is} c_i$ is still less than 1, so that the conductance will increase further[10] (region "cd_2") and even become strictly proportional to c_i (region $d_2 e_2$). This simply means that at such concentrations, before significant ion-carrier association takes place, the contribution of mechanism (P_{is}) has already become more important than that of (R_{is}). Finally, when association in the aqueous phase becomes substantial ($K_{is} c_i > 1$), the conductance will reach a "plateau." In both curves *1* and *2*, at concentrations sufficiently high that

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[10] The inflection shown in curve *2* of Fig. 4 is a consequence of using log-log plots; it would not be seen in a linear plot.

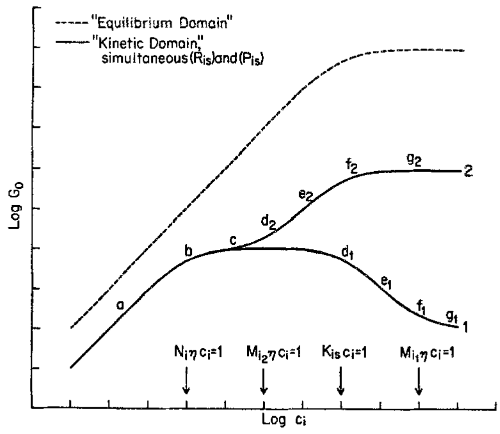

Fig. 4. Theoretically expected dependence on permeant ion concentration of the membrane conductance in the "kinetic domain" when both mechanism of permeation (P_{i_s}) and (R_{i_s}) operate simultaneously. For comparison, the "equilibrium domain" behavior is shown by the dashed curve. Solid curves 1 and 2 both refer to situations in which, at low permeant ion concentration, mechanism (P_{i_s}) is less important than mechanism (R_{i_s}). Curve 1 describes the case where substantial aqueous ion-carrier association occurs before the contribution of mechanism (P_{i_s}) becomes more significant than that of mechanism (R_{i_s}) $(M_i\eta \ll K_{i_s})$, whereas curve 2 describes the converse situation $(M_i\eta \gg K_{i_s})$. The regions denoted by lower case letters are discussed in the text. η is assumed constant

$K_{i_s}c_i > 1$ and $M_i\eta c_i > 1$, $G_0(i)$ will approach to a constant finite value given by

$$G_0(i)_L = \frac{z^2F^2}{RT} k_s c_s^T \frac{L_i M_i}{K_{i_s} N_i} = \frac{z^2F^2}{RT} \frac{A_{is}^* k_{is} c_s^T \eta}{1+2A_{is}^*/k_{is}^B}. \tag{81}$$

Note that the limiting value given by Eq. (81) is identical to that expected from Eq. (74), which refers to the case in which only mechanism (P_{i_s}) operates at the interfaces.

This follows from the fact that, even when mechanism (R_{i_s}) predominates at low concentrations, ultimately, at higher concentrations (which might not be experimentally accessible) (P_{i_s}) will always be the only effective mechanism, since the contribution of (R_{i_s}) to the conductance is in any case limited by the value given in Eq. (80). From curve *1* of Fig. 4 we can see that the conditions $N_i \gg M_i$ and $K_{i_s} > M_i\eta$ are sufficient for the conductance to have a maximum. More generally, it can be shown by derivation of Eq. (64′) with respect to c_i that the conductance will have a maximum only if

$$K_{i_s}(M_i - N_i) + M_i N_i \eta < 0. \tag{82}$$

It should be clear that relation (82) reduces, as expected, to $K_{i_s} > M_i$, when $N_i \gg M_i$.

(c) Dependence of the Conductance on the Surface Fixed-Charge Density and the Ionic Strength

Eqs. (64) and (65) show that in the constant field approximation the dependence of the conductance on the fixed-charge density σ and the ionic strength Γ is lumped into the single parameter σ^2/Γ. Substituting Eq. (65) into Eq. (64), it is found by straightforward derivation that

$$\left[\frac{\partial G_0(i)}{\partial(\sigma^2/\Gamma)}\right]_{c_i}>0 \quad \text{if} \quad \frac{\sigma}{z}<0; \qquad \left[\frac{\partial G_0(i)}{\partial(\sigma^2/\Gamma)}\right]_{c_i}<0 \quad \text{if} \quad \frac{\sigma}{z}>0 \tag{83}$$

where z is the valency of the permeant ion i.

The following property can therefore be stated as applying to the conductance both in the equilibrium and in the kinetic domain: when the concentration c_i of the permeant ion is held constant, an increase of σ^2/Γ (obtained by either increasing σ or lowering Γ) will cause an increase or a decrease of the conductance, according as to whether the sign of σ is opposite or equal to that of z, respectively. Such property becomes apparent when the system is studied in the region of concentrations sufficiently low that $K_{is}c_i \ll 1$, $M_i\eta c_i \ll 1$ and $N_i\eta c_i \ll 1$. When these conditions are satisfied, Eq. (64′) can be approximated by

$$G_0(i)=\frac{z^2F^2}{RT}\,k_s L_i c_s^T c_i \eta \tag{84}$$

or, taking the logarithm of both sides and recalling the explicit expression of η, Eq. (65),

$$\begin{aligned}\log G_0(i)=&\log\left[\frac{z^2F^2}{RT}\,k_s L_i c_s^T c_i\right]\\ &-2\frac{\sigma}{z}\left|\frac{z}{\sigma}\right|\log\left\{\sqrt{1+\frac{\pi z^2\sigma^2}{2RT\varepsilon\Gamma}}+\sqrt{\frac{\pi z^2\sigma^2}{2RT\varepsilon\Gamma}}\right\}.\end{aligned} \tag{85}$$

It should be apparent from Eq. (85) that the second term on the right-hand side adds or subtracts from the first one, depending on whether $\frac{\sigma}{z}<0$ or $\frac{\sigma}{z}>0$, respectively. Since the parenthesized quantity in the right-hand side is an increasing function of σ^2/Γ, it should be clear from Eq. (85) that, for a given increase of σ^2/Γ, log $G_0(i)$ will increase when $-2\sigma/z$ is positive ($\sigma/z<0$) and will, conversely, decrease by the same amount when $-2\sigma/z$ is negative ($\sigma/z>0$).

The physical basis for this result can be understood if one considers that the conductance given by Eq. (84) is proportional to the interfacial concentration of the permeant ion, $c_i(0)=c_i\eta=c_i\exp\left[-\frac{zF}{RT}\,\psi(0)\right]$, rather than to its bulk concentration c_i. On the other hand, Eq. (65) shows that the exponential term, $\eta = \exp\left[-\frac{zF}{RT}\psi(0)\right]$, increases with σ^2/Γ when $\sigma/z<0$, and decreases when $\sigma/z>0$. By increasing σ^2/Γ we shall therefore either increase or decrease both the boundary concentration $c_i(0)$ and the conductance, according as to whether σ and z have opposite or equal signs, respectively.

Eq. (85) shows also that a proportionality between $G_0(i)$ and the concentration of the permeant ion c_i is expected only if the measurements are performed while maintaining the ionic strength constant with an impermeant electrolyte. This is due to the fact that only when the ionic strength is constant is the exponential term η constant and, therefore, the boundary concentration $c_i(0)$ proportional to the bulk concentration c_i. The expectations of Eq. (85) have been successfully verified by experimental results within the equilibrium domain (McLaughlin, Szabo, Eisenman & Ciani, 1970; McLaughlin, Szabo & Eisenman, 1971; Szabo, Eisenman, McLaughlin & Krasne, 1972) as well as in the kinetic domain (Laprade *et al.*, 1972; Laprade, Ciani *et al.* and Laprade, Eisenman *et al.*, *unpublished*). The charge density of the membrane was changed either by using different lipids or by varying the pH. The complexes between monovalent cations and the macrotetralide actin antibiotics were used as permeant cations, the iodide-iodine complexes as permeant anions.

It is interesting to observe that, as long as the approximate expression (85) is valid, the dependence of the conductance on the charge density σ and the ionic strength Γ is independent of whether the rate-limiting step for transport is determined by transmembrane diffusion or by the interfacial processes. The nature of the rate-limiting step will affect only the value and the physical meaning of the parameter L_i. Namely, if the rate-limiting step is diffusion through the membrane, so that $A^*_{is} < k^B_{is} + \bar{K}^B_i$, Eq. (66) shows that

$$L_i \simeq \bar{K}_i A^*_{is}. \tag{86}$$

In the other alternative, when $A^*_{is} \gg k^B_{is} + \bar{K}^B_i$,

$$L_i \simeq \frac{\bar{K}_i}{2}(k^B_{is} + \bar{K}^B_i) \quad \text{or, if} \quad k^B_{is} \ll \bar{K}^B_i, \quad L_i \simeq \tfrac{1}{2}\bar{K}_i \bar{K}^B_i = \tfrac{1}{2}\bar{K}^F_i. \tag{87}$$

Previously (McLaughlin *et al.*, 1970; McLaughlin *et al.*, 1971), experimental data on the dependence of the conductance on the surface charge density σ and the ionic strength Γ have been interpreted in terms of Eq. (85) with the additional assumption of local equilibrium at the interfaces, so that the explicit expression of L_i was that given by Eq. (86). It can now be seen that the assumption of local equilibrium at the interfaces was not essential, since the form of Eq. (85), valid at low concentrations, remains the same regardless of the nature of the rate-limiting process.

Discussion

(a) Information about the Permeation Mechanism Deducible from the Conductance Behavior

We have already seen that the relative values of N_i and M_i are indicative of the relative importance of mechanisms (R_{i_s}) and (P_{i_s}). From Eq. (64′) it is apparent that, if the conductance is strictly proportional to the concentration of the permeant ion in the whole range of the experimentally accessible values, no information about the permeation mechanism can be obtained; since nothing can be said about the relative values of N_i and M_i. On the other hand, if within the experimentally accessible range of concentrations the conductance curve presents only the portion *abc* of Figs. 2, 3 or 4, it will be impossible to distinguish from it alone between Eqs. (71), (74) and (79). However, if the membrane is charged, η can then be varied by altering the ionic strength.[11] The conductance curve can then be examined at different ionic strengths (corresponding to different values of η). If the plateau is due to simple aqueous association, varying η will merely displace the whole curve vertically without changing the range of linearity, as is shown in Fig. 5. If the plateau is due to the term $N_i \eta c_i$ of Eq. (79), varying η will alter the range of linearity of the conductance and not the plateau level, as can be seen in Fig. 6. In any case, if a decrease from linearity in the dependence of the conductance on the concentration c_i is detected for a particular test lipid, it is possible, in principle, by merely changing the lipid composition, to decide whether such effect is due to the term $K_{i_s} c_i$ or $N_i \eta c_i$. In fact, if due to $K_{i_s} c_i$, the same numerical value for K_{i_s} must be deduced from measurements on membranes of any lipid composition, since K_{i_s} is the complexation constant in the aqueous phase and is, by definition, independent of the membrane composition. On the other hand, if, by varying the type of lipid, we can find at least one in which no deviation from linearity can be detected, or the concentration at which the conductance starts to bend off is higher than that of the original test lipid, we can infer that the deviation from linearity in the test lipid was due to the term $N_i \eta c_i$, and, therefore, mechanism (R_{i_s}) was operating at the interfaces.

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[11] Alternatively, one can vary the charge of bilayers by varying the polar head groups of lipids having comparable lipid tails. This has been done by comparing negatively charged with neutral lipids (*cf.* Ciani *et al.*, 1973, Fig. 22).

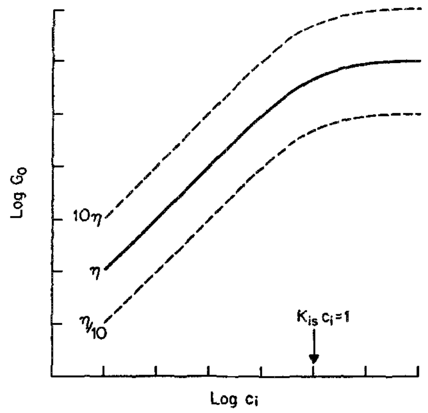

Fig. 5. Theoretically expected behavior [*cf.* Eqs. (71) and (74)] of the curve of log conductance *vs.* log permeant ion concentration for a 10-fold change in η, when the plateau is due to ion-carrier association. Each curve is drawn for a constant η

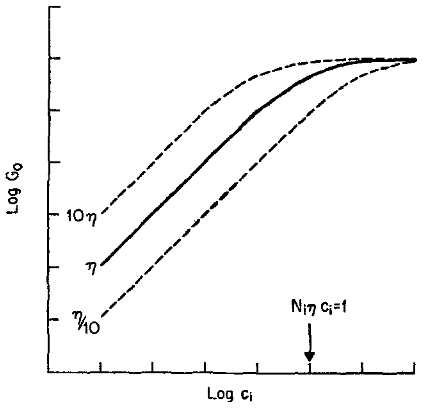

Fig. 6. Theoretically expected behavior [*cf.* Eq. (79)] of the conductance *vs.* log permeant ion concentration for a 10-fold change in η, when the plateau is due to the term $N_i\eta c_i$. Each curve is drawn for a constant η

Finally, if the conductance curve presents the behavior exemplified in Figs. 3 or 4, i.e. a "flattening" followed by either an increase or a decrease of conductance, the permeation mechanism can be determined unambiguously. Thus, if the "flattening" is followed by the tendency of the conductance to vanish, we can infer that only mechanism (R_{is}) operates at the interfaces, because such behavior is compatible only with Eq. (77). In the remaining two cases, exemplified by the two solid lines of Fig. 4, where the "flattening" in a log-log plot is followed by either an increase or a decrease of the conductance to constant finite levels, we can deduce that both mechanisms

(P_{is}) and (R_{is}) must be present, because either of these two behaviors can be accounted for only by Eq. (64′) with nonzero values for both the parameters M_i and N_i.

(b) Characterization of the System by Zero-Current Conductance Measurements

Eq. (64′) shows that, by varying the concentration of the permeant ion c_i, it is possible to evaluate at most four parameters, namely K_{is}, $k_s L_i \eta$, $M_i \eta$ and $N_i \eta$; k_s is the partition coefficient of the neutral carrier, K_{is} the association constant in the aqueous phase, and η, L_i, M_i and N_i are defined in Eqs. (65)–(68). As is shown clearly by Eq. (64′), the parameters K_{is}, M_i and N_i can be determined only if $K_{is} c_i$, $M_i \eta c_i$ and $N_i \eta c_i$ become of the order of unity in the range of experimentally accessible values of c_i.

On the other hand, η, which contains the square of the fixed charge density σ^2 as the only unknown, can be determined by measuring any of the three parameters, $k_s L_i \eta$, $M_i \eta$ and $N_i \eta$ at two different ionic strengths. For example, the ratio of the parameters $k_s L_i \eta_1$, and $k_s L_i \eta_2$ measured at two different ionic strengths, Γ_1 and Γ_2, will be

$$\frac{k_s L_i \eta_1}{k_s L_i \eta_2} = \frac{\eta_1}{\eta_2}. \tag{88}$$

Using the explicit expressions for η_1 and η_2 as given by Eq. (65), and solving for σ^2, we get

$$\sigma^2 = \frac{RT\varepsilon}{2\pi z^2} \left(\frac{\eta_1}{\eta_2}\right)^{\frac{1}{2}\frac{\sigma}{z}\left|\frac{z}{\sigma}\right|} \cdot \frac{\left[1 - \left(\frac{\eta_1}{\eta_2}\right)^{-\frac{\sigma}{z}\left|\frac{z}{\sigma}\right|}\right]^2}{\left[\frac{1}{\sqrt{\Gamma_1}}\left(\frac{\eta_1}{\eta_2}\right)^{-\frac{1}{2}\frac{\sigma}{z}\left|\frac{z}{\sigma}\right|} - \frac{1}{\sqrt{\Gamma_2}}\right]\left[\frac{1}{\sqrt{\Gamma_1}} - \frac{1}{\sqrt{\Gamma_2}}\left(\frac{\eta_1}{\eta_2}\right)^{-\frac{1}{2}\frac{\sigma}{z}\left|\frac{z}{\sigma}\right|}\right]}. \tag{89}$$

Moreover, if $k_s L_i \eta_1$ and $k_s L_i \eta_2$ are determined by measuring the conductance in the region of linear dependence on c_i, where the approximate expression (84) is valid, we shall have

$$\frac{\eta_1}{\eta_2} = \frac{G_{01}(i)}{G_{02}(i)} \tag{90}$$

where $G_{01}(i)$ and $G_{02}(i)$ denote the conductances measured at the two different ionic strengths Γ_1 and Γ_2, but for the same values of c_i and c_s. Substituting η_1/η_2 with $G_{01}(i)/G_{02}(i)$ in the right-hand side of Eq. (89)

would give an expression relating directly σ^2 to the ionic strengths Γ_1 and Γ_2 and to the ratio of the conductances. Once the surface charge density σ is determined, so is also the value of η for any ionic strength; and therefore the list of parameters deducible from conductance measurements becomes K_{is}, $k_s L_i$, M_i, N_i, σ (or η). It should be noted that among these parameters only K_{is} and σ represent quantities of immediate physical meaning, whereas $k_s L_i$, M_i and N_i are combinations of equilibrium and kinetic constants.

(c) The Driving Force in the Equilibrium and Kinetic Domains

When a small voltage V is applied between the two solutions, the effect or rate limitations at the membrane-solution boundaries is to create a gradient of concentration of the diffusible species inside the membrane, such as to decrease the value that the driving force would have if the system were in the equilibrium domain. Since the contribution of this gradient to the driving force is directly proportional to the applied potential, its effect will be present regardless of how small this potential is, and will be either negligible or substantial, depending on whether the proportionality coefficient is smaller than unity (equilibrium domain) or comparable to unity (kinetic domain). The effect on the driving force for the different permeation mechanisms (P_{is}, R_{is}) is examined in detail in Appendix B; but the physical consequences of the gradient may be visualized as slowing down the rate of migration of charged complexes across the membrane, as compared to their rate if the interfaces offered no resistance, while also displacing the interfacial concentrations from equilibrium so as to equalize the rate of crossing the interfaces and the rate of migration across the membrane.

Appendix A

Constant Field Approximation

The purpose of the Appendix is to discuss more rigorously the constant field approximation and to express the conditions for its validity in terms of physically meaningful parameters.

Let us first recall that the constant field approximation[12] is used in two different places: (*a*) to replace Eq. (53) with Eq. (62), and (*b*) to derive Eq. (65).

(*a*) *Discussion of the Validity of Eq.* (62). We start by showing that Eq. (62) will be certainly an adequate approximation of Eq. (53) whenever

$$|\phi^*(x)_{\mathrm{eq}} - \phi^*(0)_{\mathrm{eq}}| \ll 1 \qquad 0 \leqq x \leqq d. \tag{A.1}$$

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[12] For the present symmetrical solution conditions this corresponds to zero field at equilibrium.

In fact, Eq. (53) can be rewritten in the form

$$B_{is}^*(\mathrm{eq})=e^{\frac{\mu_{is}^{0*}(0)}{RT}-\phi^*(0)_{\mathrm{eq}}}\cdot\left[\int\frac{e^{\frac{\mu_{is}^{0*}(x)}{RT}+\phi^*(x)_{\mathrm{eq}}-\phi^*(0)_{\mathrm{eq}}}}{D_{is}^*(x)}\,dx\right]^{-1}. \tag{A.2}$$

As a consequence of relationship (A.1) we shall have

$$e^{\phi^*(x)_{\mathrm{eq}}-\phi^*(0)_{\mathrm{eq}}}\simeq 1 \tag{A.3}$$

so that, inserting Eq. (A.3) into (A.2), we obtain directly Eq. (62).

To discuss the physical requirement for the validity of relationship (A.1), consider the Poisson equation within the membrane. Since the complex *is* is the only charged species present in the membrane, the Poisson equation can be written

$$\frac{d^2\phi^*}{dx^2}=-\frac{4\pi z^2F^2}{RT\varepsilon^*}\,c_{is}^*(x) \tag{A.4}$$

where

$$\phi^*(x)=\frac{zF}{RT}\,\psi^*(x). \tag{A.5}$$

From Eq. (A.4) we realize that $d^2\phi^*/dx^2$ is negative, regardless of the sign of z as well as of the particular profile of the barrier shape. This implies that the profile of $\phi^*(x)$ inside the membrane has the convexity directed downward. Moreover, given the symmetry of the barrier shape, it also means that, at equilibrium ($I=0$) and for identical compositions of the two aqueous solutions, $\phi^*(x)$ is symmetrical with respect to the middle of the membrane ($x=d/2$) and has a maximum at that point. Therefore, the inequality (A.1) will be satisfied *a fortiori* if

$$\phi^*\left(\frac{d}{2}\right)_{\mathrm{eq}}-\phi^*(0)_{\mathrm{eq}}\ll 1. \tag{A.6}$$

Let us denote by $c_{is}^*(x_M)$ the maximum value of the concentration $c_{is}^*(x)$ inside the membrane. Since by definition $c_{is}^*\leqq c_{is}^*(x_M)$, we deduce from Eq. (A.4)

$$-\frac{d^2\phi^*}{dx^2}\leqq\frac{4\pi z^2F^2c_{is}^*(x_M)}{RT\varepsilon^*}. \tag{A.7}$$

Integrating relationship (A.7) between $x\left(0\leqq x\leqq\frac{d}{2}\right)$ and $\frac{d}{2}$ and recalling that at equilibrium $d\psi^*/dx$ vanishes at $x=d/2$, we find

$$\left(\frac{d\phi^*}{dx}\right)_{\mathrm{eq}}\leqq\frac{4\pi z^2F^2c_{is}^*(x_M)}{RT\varepsilon^*}\left(\frac{d}{2}-x\right). \tag{A.8}$$

Integrating once more Eq. (A.8) from 0 and $d/2$ we get

$$\phi^*\left(\frac{d}{2}\right)_{\mathrm{eq}}-\phi^*(0)_{\mathrm{eq}}\leqq\frac{\pi z^2F^2d^2c_{is}^*(x_M)}{2RT\varepsilon^*}. \tag{A.9}$$

From the relationships (A.6) and (A.9) it should be clear that condition (A.6) is certainly satisfied if

$$c_{is}^*(x_M)\ll\frac{2RT\varepsilon^*}{\pi z^2F^2d^2}. \tag{A.10}$$

The requirement expressed by the inequality (A.10) is therefore a sufficient condition for the validity of relationship (A.1) and consequently of Eq. (62).

Using the following set of numbers ($\varepsilon^* = 3 \times 1.1 \times 10^{-10}$ coulomb^2/Joule meter, $T = 300$ °K, $d = 70$ Å), relationship (A.10) gives

$$c_{is}^*(x_M) \ll 10^{-3} \text{ moles/liter.} \tag{A.11}$$

If we assume now that c_{is}^* attains its maximum at the interfaces, we have

$$c_{is}^*(x_M) = c_{is}^*(0) = \overline{K}_i k_s c_s c_i e^{-\frac{zF}{RT}\psi(0)}. \tag{A.12}$$

Considering the numerical values reported by Stark *et al.* (1971) for the K$^+$-valinomycin complex in a phosphatidyl-inositol membrane ($\overline{K}_i e^{-\frac{zF}{RT}\psi(0)} = k_R/k_D = 1$ liter/mole, $k_s = \gamma_s = 6 \times 10^4$), the condition (A.11) becomes, with the help of Eq. (A.12),

$$c_i c_s \ll 1.7 \times 10^{-8} \text{ moles}^2/\text{liter}^2. \tag{A.13}$$

The last inequality is seen not to hold for experimentally common situations where $c_s \geqq 10^{-7}$ mole/liter and $c_i \geqq 10^{-1}$ mole/liter. It should be emphasized, however, that relationship (A.10) is only a sufficient and not a necessary condition; this implies that the approximation of constant field might be valid also in some situations where the condition (A.10) is not satisfied. For instance, if the complexes are located predominantly on the membrane surfaces and the internal region of the membrane is practically devoid of charges, it follows from the Poisson equation that the field must be constant inside the membrane, despite the fact that the average volume concentration, $c_{is}^* \simeq N_{is}^* 2/d$, might be too high to satisfy condition (A.10).

(*b*) *Derivation and Discussion of Eq.* (65). To derive Eq. (65) we must integrate the Poisson-Boltzmann equation in the aqueous phases, Eq. (10). Carrying out a first integration and imposing the condition that the electric potential and the electric field vanish at $x \to -\infty$, we find

$$\frac{d\phi}{dx} = \frac{2}{L} \sinh \frac{\phi}{2}. \tag{A.14}$$

In particular, for $x = 0$, and recalling that $\phi = \frac{|z|F}{RT}\psi$, Eq. (A.14) gives

$$\left[\frac{d\psi}{dx}\right]_{x=0} = \frac{2RT}{|z|FL} \sinh \frac{\phi(0)}{2}. \tag{A.15}$$

Defining for brevity

$$\beta = \frac{|z|FL}{2RT\varepsilon} \left\{ 4\pi\sigma + \varepsilon^* \left[\frac{d\psi^*}{dx}\right]_{x=0} \right\} \tag{A.16}$$

the first of the two boundary conditions (A.14) becomes with the help of Eq. (A.15)

$$\sinh \frac{\phi(0)}{2} = \beta \tag{A.17}$$

or, solving for $e^{\phi(0)}$

$$e^{\phi(0)} = \left\{ \sqrt{1+\beta^2} + |\beta| \right\}^{2\frac{\beta}{|\beta|}}. \tag{A.18}$$

If we assume now that the electric field inside the membrane is sufficiently low that

$$\varepsilon^* \left| \left(\frac{d\psi^*}{dx} \right)_{x=0} \right| \ll 4\pi |\sigma| \tag{A.19}$$

Eq. (A.16) gives, with help of Eq. (12),

$$\beta = \sigma \sqrt{\frac{\pi z^2}{2RT\varepsilon\Gamma}} \quad \text{and} \quad |\beta| = \sqrt{\frac{\pi z^2 \sigma^2}{2RT\varepsilon\Gamma}}. \tag{A.20}$$

Inserting (A.20) into (A.18) we get

$$e^{\phi(0)} = \left\{ \sqrt{1 + \frac{\pi z^2 \sigma^2}{2RT\varepsilon\Gamma}} + \sqrt{\frac{\pi z^2 \sigma^2}{2RT\varepsilon\Gamma}} \right\}^{2\frac{\sigma}{|\sigma|}}. \tag{A.21}$$

To obtain Eq. (65) we must only recall that

$$\frac{zF}{RT} \psi(0) = \frac{|z|}{z} \phi(0). \tag{A.22}$$

From (A.21) and (A.22) we find, in fact,

$$\eta = e^{-\frac{zF}{RT}\psi(0)} = e^{-\frac{|z|}{z}\phi(0)} = \left\{ \sqrt{1 + \frac{\pi z^2 \sigma^2}{2RT\varepsilon\Gamma}} + \sqrt{\frac{\pi z^2 \sigma^2}{2RT\varepsilon\Gamma}} \right\}^{-2\frac{\sigma}{z}\left|\frac{z}{\sigma}\right|}. \tag{A.23 = Eq. 65}$$

The boundary ion concentration $c_i(0)$ will therefore be

$$c_i(0) = c_i e^{-\frac{zF}{RT}\psi(0)} = c_i \eta. \tag{A.24}$$

To obtain Eq. (A.23 = Eq. 65) we have made use of the approximation (A.19). To rewrite it in a more explicit form and analyze the condition for its validity, we recall relationship (A.8). Recalling that $\phi^* = zF\psi^*/RT$, we can deduce from (A.8) that

$$\varepsilon^* \left| \left(\frac{d\psi^*}{dx} \right)_{x=0} \right|_{\text{eq}} \leqq 2\pi |z| F d c_{is}^*(x_M). \tag{A.25}$$

Combining (A.25) and (A.19) we realize that condition (A.19) will be satisfied *a fortiori* if

$$2\pi |z| F d c_{is}^*(x_M) \ll 4\pi |\sigma| \tag{A.26}$$

or, simplifying and rearranging

$$c_{is}^*(x_M) \ll \frac{2|\sigma|}{|z| F d}. \tag{A.27}$$

Relation (A.27) is thus a sufficient condition for the validity of Eq. (A.23 = Eq. 65). For the charge density of one electronic charge per 1,000 Å^2 and a 70-Å thick membrane, (A.27) gives, for example,

$$c_{is}^*(x_M) \ll 4 \times 10^{-3} \text{ moles/liter}. \tag{A.28}$$

Introducing the density per unit surface, $N_{is}^* = c_{is}^* \, d/2$, condition (A.27) becomes more simply

$$N_{is}^*(x_M) \ll \frac{|\sigma|}{|z| F}. \tag{A.27$'$}$$

Relationship (A.27′) expresses the requirement that the surface density of complexes be much less than that of the fixed charges.

Appendix B

The Effect on Transmembrane Driving Forces of Rate Limitations at the Interface

We show here that, when a voltage V is applied between the two solutions, and the system is in the equilibrium domain, the total driving force between the two interfaces, defined as the difference between the interfacial values of the electrochemical potential is always equal to zFV. By contrast, in the kinetic domain rate limitations at the surface cause an ionic concentration gradient to build up across the membrane in such a direction as to oppose the applied electric component of the total driving force. The result is that the total driving force between the two interfaces in this domain is less than zFV. The amount less, for a given value of V, will be shown to be either a constant or a function of the ion concentration, according as to whether the mechanism of permeation across the interfaces is the partition of complexes (P_{is}) or the interfacial complex-forming reaction (R_{is}).

Consider the basic flux equation

$$-J^*_{is} = \frac{D^*_{is}}{RT}\, c^*_{is}\, \frac{d\bar{\mu}^*_{is}}{dx}. \tag{B.1}$$

If a small voltage V is applied between the two aqueous phases, as must be the case when measuring zero-current conductances, the profile of the concentration $c^*_{is}(x)$ will shift from its equilibrium shape, so that in first approximation we can write

$$c^*_{is}(x) = c^*_{is}(x)_{\mathrm{eq}} + \alpha(x)\, V \tag{B.2}$$

where $\alpha(x)$ is the derivative of $c^*_{is}(x)$ with respect to the voltage, calculated at $V=0$. From the same type of considerations, and recalling that the driving force $-d\bar{\mu}^*_{is}/dx$ vanishes at equilibrium, we can write

$$\frac{d\bar{\mu}^*_{is}}{dx} = \beta(x)\, V. \tag{B.3}$$

Substituting Eqs. (B.2) and (B.3) in Eq. (B.1) and considering only the terms linear in V, we find

$$-J^*_{is} = \frac{D^*_{is}}{RT}\, c^*_{is}(x)_{\mathrm{eq}}\, \frac{d\bar{\mu}^*_{is}}{dx}. \tag{B.4}$$

Dividing both sides of Eq. (B.1) by $D^*_{is} c^*_{is}(x)_{\mathrm{eq}}/RT$, using the explicit expression of the electrochemical potential and integrating between 0 and d, we get

$$J^*_{is} = \frac{\langle D^*_{is}\, c^*_{is}(x)_{\mathrm{eq}}\rangle}{d} \left\{ RT \ln \frac{c^*_{is}(0)}{c^*_{is}(d)} + zF\left[\psi^*(0) - \psi^*(d)\right] \right\} \tag{B.5}$$

where

$$\langle D^*_{is}\, c^*_{is}(x)_{\mathrm{eq}}\rangle = \frac{d}{\displaystyle\int_0^d \frac{dx}{D^*_{is}(x)\, c^*_{is}(x)_{\mathrm{eq}}}}. \tag{B.6}$$

Eq. (B.5) shows that for small applied voltages the current can be expressed as a product of the driving force and a term which is merely a function of the profile of the diffusion coefficient and of the concentration of complexes *at equilibrium*. Since the profile of the concentration at equilibrium is by definition independent of whether the behavior of the conductance pertains to the equilibrium or the kinetic domains, the different expectations for these domains must be accounted for in terms of the dependence of the driving force on concentrations and rate constants.

The total driving force, given in the parenthesized term of Eq. (B.5) is the sum of a chemical and an electrical component, namely

$$f^{*T}=f^{*\,\mathrm{ch.}}+f^{*\,\mathrm{el.}} \tag{B.7}$$

where

$$f^{*\,\mathrm{ch.}}=RT\ln\frac{c^*_{is}(0)}{c^*_{is}(d)} \tag{B.8}$$

and

$$f^{*\,\mathrm{el.}}=zF[\psi^*(0)-\psi^*(d)]=zFV^*. \tag{B.9}$$

If we define V' and V'' according to

$$V'=\psi'-\psi^*(0); \qquad V''=\psi''-\psi^*(d) \tag{B.10}$$

Eq. (B.9) becomes, in terms of V', V'' and the total potential V,

$$f^{*\,\mathrm{el.}}=zF[V-(V'-V'')]. \tag{B.11}$$

Considering Eqs. (45), (46) and (60), and following the procedure shown in the footnote[13], the "chemical" driving force for a small applied potential is found to be given by

$$f^{*\,\mathrm{ch.}}=-f^{*\,\mathrm{el.}}+zFV\left[1+\frac{2RT}{zF}\,\frac{\Delta^{0*}_{is}[0]}{c^*_{is}(0)_{\mathrm{eq}}}\,G_0(i)\right]. \tag{B.12}$$

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[13] With the help of Eqs. (45) and (46) and by straightforward rearrangements, Eq. (B.8) can be rewritten in the form

$$f^{*\,\mathrm{ch.}}=RT\ln\frac{c_i(0)}{c_i(d)}+RT\ln\left[1+\frac{\Delta^{0*}_{is}[I]I}{\bar{K}_i k_s c_s c_i(0)}\right]-RT\ln\left[1+\frac{\Delta^{0*}_{is}[-I](-I)}{\bar{K}_i k_s c_s c_i(d)}\right]. \tag{a}$$

Recalling Eq. (8), the definitions of V' and V'' given in Eq. (B.10), as well as Eqs. (15) and (50), we find

$$c_i(0)=\omega c_i e^{\frac{zF}{RT}V'} \quad \text{and} \quad c_i(d)=\omega c_i e^{\frac{zF}{RT}V''} \tag{b}$$

so that the first term on the right-hand side of Eq. (B.1) becomes

$$RT\ln\frac{c_i(0)}{c_i(d)}=zF(V'-V''). \tag{c}$$

On the other hand for currents sufficiently small that

$$\left|\frac{\Delta^{0*}_{is}[I]I}{\bar{K}_i k_s c_s c_i(0)}\right|<1 \quad \text{and} \quad \left|\frac{\Delta^{0*}_{is}[-I](-I)}{\bar{K}_i k_s c_s c_i(d)}\right|<1 \tag{d}$$

we can expand the last two terms of Eq. (a) in power series of I near $I=0$. Considering Δ^{0*}_{is}, $c_i(0)$ and $c_i(d)$ as functions of I, keeping only the terms linear in I, and recalling that $c^*_{is}(0)_{\mathrm{eq}}=c^*_{is}(d)_{\mathrm{eq}}=\bar{K}_i k_s c_s c_i(0)_{\mathrm{eq}}$, and that, for small currents, $I=G_0(i)\,V$, we find

$$\begin{aligned} &RT\ln\left[1+\frac{\Delta^{0*}_{is}[I]I}{\bar{K}_i k_s c_s c_i(0)}\right]-RT\ln\left[1+\frac{\Delta^{0*}_{is}[-I](-I)}{\bar{K}_i k_s c_s c_i(d)}\right] \\ &\simeq 2RT\frac{\Delta^{0*}_{is}[0]}{c^*_{is}(0)_{\mathrm{eq}}}\,G_0(i)\,V. \end{aligned} \tag{e}$$

Substituting (c) and (e) into (a) and using Eq. (B.11) to express $V'-V''$ in terms of V and $f^{*\,\mathrm{el.}}$, we obtain finally Eq. (B.12).

The total driving force between the internal sides of the interfaces will then be

$$f^{*T}=f^{*\,\mathrm{ch.}}+f^{*\,\mathrm{el.}}=zFV\left[1+\frac{2RT}{zF}\,\frac{\Delta_{is}^{0*}[0]}{c_{is}^{*}(0)_{\mathrm{eq}}}\,G_0(i)\right]. \tag{B.13}$$

Observing from Eqs. (60) and (64) that

$$-1\leqq\frac{2RT}{zF}\cdot\frac{\Delta_{is}^{0*}[0]}{c_{is}^{*}(0)_{\mathrm{eq}}}\,G_0(i)\leqq 0 \tag{B.14}$$

Eqs. (B.13) and (B.14) show that the value of the total driving force between the two interfaces is always either smaller or, at most, equal to *zFV*. In the equilibrium domain the total driving force is given by Eq. (B.13), where the second term in the brackets is negligible compared to one; and therefore the driving force is equal to *zFV*. By contrast, in the kinetic domain the total driving force is given by Eq. (B.13), where the second term in brackets is not negligible compared to one; and therefore the driving force is less than *zFV*. It is instructive in this situation to analyze separately the particular cases when either (P_{is}) mechanism or (R_{is}) mechanism are the only routes of permeation across the interfaces.

For the (P_{is}) mechanism, $(\overline{K}_i^F c_i=K_i^B=0)$, so that the conductance is given by Eq. (74), Eqs. (B.11), (B.12) and (B.13) become, with the help of Eqs. (60) and (74)

$$f^{*\,\mathrm{el.}}=zF[V-(V'-V'')];\quad f^{*\,\mathrm{ch.}}=zF\left[V'-V''-\frac{2A_{is}^{*}/k_{is}^{B}}{1+2A_{is}^{*}/k_{is}^{B}}\,V\right] \tag{B.15}$$

and

$$f^{*T}=zFV\left[1-\frac{2A_{is}^{*}/k_{is}^{B}}{1+2A_{is}^{*}/k_{is}^{B}}\right]. \tag{B.16}$$

From the second Eq. (B.15) we realize that the effect of rate limitation at the interfaces is that of producing a gradient of concentration of complexes in the opposite direction of *zFV*. If the transport is completely surface rate limited, $k_{is}^{B}\ll A_{is}^{*}$, the countergradient of complexes will balance exactly the electric driving force, so that f^{*T} [*see* Eq. (B.16)] will vanish. Note that f^{*T} is a constant fraction of *zFV* independent of the permeant ion concentration, so that the dependence of the conductance on concentrations is determined by that of the term $\langle D_{is}^{*}c_{is}^{*}(\mathrm{eq})\rangle$, as in the equilibrium domain. This is why the behavior of $G_0(i)$ in the presence of (P_{is}) mechanism alone presents the same functional dependence on c_i as in the equilibrium domain, (*see* Fig. 2), although its value at any permeant ion concentration is reduced by a constant factor.

For the (R_{is}) mechanism $(k_{is}^{B}=0)$, the driving forces (B.11)–(B.13) become, with the help of Eqs. (60), (77), (66) and (68)

$$f^{*\,\mathrm{el.}}=zF[V-(V'-V'')];$$

$$f^{*\,\mathrm{ch.}}=zF\left[V'-V''-\frac{2A_{is}^{*}[\overline{K}_i^F c_i\eta+k_s^B+2A_s^{*}]}{2A_{is}^{*}[\overline{K}_i^F c_i\eta+k_s^B+2A_s^{*}]+(k_s^B+2A_s^{*})\,\overline{K}_i^B}\,V\right] \tag{B.17}$$

and

$$f^{*T}=zFV\left[\frac{(k_s^B+2A_s^{*})\,\overline{K}_i^B}{2A_{is}^{*}[\overline{K}_i^F c_i\eta+k_s^B+2A_s^{*}]+(k_s^B+2A_s^{*})\,\overline{K}_i^B}\right]. \tag{B.18}$$

In contrast to the case of (P_{is}) mechanism, $f^{*\,\mathrm{ch.}}$ and $f^{*\,T}$ are now dependent on the concentration c_i. In particular, $f^{*\,T}$ is a decreasing function of c_i, and tends to vanish asymptotically at the same rate as $1/c_i$, regardless of whether association does or does not occur in the aqueous solutions. This implies that, when aqueous ion-carrier complexation does not occur, so that $\langle D_{is}^* c_{is}^*(\mathrm{eq})\rangle$ increases proportionally with the concentration c_i, the product $\langle D_{is}^* c_{is}^*(\mathrm{eq})\rangle f^{*\,T}$ and, therefore, the conductance will approach to a constant nonzero value, as indicated in Eqs. (79) and (80) and illustrated in Fig. 3 (portion *bc*) and curve 1 of Fig. 4 (portion *bc*). However, if, in addition, ion-carrier complexation occurs also in the aqueous phase, the term $\langle D_{is}^* c_{is}^*(\mathrm{eq})\rangle$ remains finite, and the conductance will tend to vanish at high concentrations. This accounts for the expectations of Eq. (77), illustrated in Fig. 3 (portion *de*) and in curve 1 of Fig. 4 (portion $d_1 e_1$).

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