NONLINEAR ELECTRICAL EFFECTS IN LIPID BILAYER MEMBRANES

II. INTEGRATION OF THE GENERALIZED NERNST-PLANCK EQUATIONS

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ABSTRACT In this paper the ion transport across a thin lipid membrane is treated using a generalized form of the Nernst-Planck equations. An additional term is introduced into the flux equations to account for the image force acting on the ion. As the membrane thickness is of the same order of magnitude as the range of the image forces, the potential energy of the ion in the membrane is strongly dependent on position. The integration of the flux equations leads to a general expression for the integral membrane conductance λ as a function of the voltage u. The ratio $\lambda(u)/\lambda_0$ (λ_0 = membrane conductance in the limit $u \rightarrow 0$) depends on the dielectric constant and the thickness of the membrane, but is independent of the ionic radius. When the numerical values of the potential energy function, as calculated by the method of electrical images, are inserted into the expression for $\lambda(u)/\lambda_0$, a strongly non-linear current-voltage characteristic is obtained. The theoretical current-voltage curve agrees satisfactorily with the experimental data at a low ionic strength and at low voltages; at higher voltages the observed membrane conductance exceeds the predicted value.

INTRODUCTION

In the preceding communication (Walz et al., 1969) the influence of the so called ion injection effect on the conductivity of lipid bilayer membranes was investigated. It was found that the injection of ions from the aqueous phase cannot account for the observed non-linear current-voltage characteristic of the membrane. In this second part of the paper we show that the nonlinearity can be explained, at least to a first approximation, when a realistic expression for the potential energy of an ion in the membrane is introduced. The membrane is considered again as a continuous liquid film of dielectric constant ϵ_2 interposed between two infinite continuous phases of dielectric constant ϵ_1, but the film is no longer treated as a macroscopic phase. An ion moving from the aqueous phase ($\epsilon_1 \approx 80$) across the boundary into the hydrocarbon phase ($\epsilon_2 \approx 2$) is subjected to image forces; i.e. the ion is repelled from the boundary while in the aqueous phase but attracted toward the boundary while in the lipid phase. The membrane is therefore equivalent to a symmetrical potential profile with a single peak in the middle between the two boundaries. Accordingly, the ion concentration in the membrane is no longer constant even in the equilibrium state.

Under nonequilibrium conditions, i.e. when an electrical potential difference is applied between the external solutions, the ion fluxes in the membrane are described by the Nernst-Planck equations. In the case of a thin film, however, the flux equations have to be generalized to include the variation of the potential energy of the ion across the film. This approach is similar to a recent treatment of Manning (1968), but we want to include charged particles explicitly in our considerations.

After introduction of the generalized form of the Nernst-Planck equations in the next section, we calculate the potential energy profile explicitly by the method of images. The current-voltage characteristic of the membrane is then evaluated by numerical integration of the flux equations. The calculation leads to the prediction of a strongly nonlinear current-voltage curve which, to a first approximation, agrees with the experimental observations on bimolecular lecithin membranes.

THE GENERALIZED NERNST-PLANCK EQUATIONS

We consider the membrane as a thin liquid film interposed between two aqueous solutions of an electrolyte A^+B^-, the boundaries being at $x = 0$ and $x = d$ (x is the coordinate normal to the membrane surface). As coupling effects between the fluxes of different ion species may be neglected here, the transport equation states that the flux of, say, the cation in the membrane is given by the product of the ionic concentration c_+, the mobility u_+, and the gradient of the electrochemical potential $\tilde{\mu}_+$

$$\Phi_+ = - c_+ u_+ \frac{d\tilde{\mu}_+}{dx} \tag{1}$$

The electrochemical potential $\tilde{\mu}_+$ is the sum of the chemical potential

$$\mu_+ = \mu_+^0 + kT \ln c_+ ,$$

and of the coulombic energy of the ion due to the electrical potential:

$$\tilde{\mu}_+ = \mu_+^0 + kT \ln c_+ + e_0 \psi \tag{2}$$

(k = Boltzmann constant, T = absolute temperature, e_0 = elementary charge). For simplicity, the ionic activity coefficients in the aqueous phase are assumed to be unity. In contrast to the situation in a macroscopic phase, the standard chemical potential μ_+^0 is here a function of position. Apart from an additive constant, μ_+^0 is equal to the work W_+ required to transfer the ion from a distant point in the aqueous phase to the point x in (or near) the membrane:

$$\mu_+^0(x) = W_+(x) + C \tag{3}$$

The potential energy $W_+(x)$ can be calculated by consideration of the image forces acting on the ion (see the next section). The mobility u_+ is related to the diffusion coefficient, D_+, of the cation in the membrane by the Einstein equation

$$u_+ = \frac{D_+}{kT} \tag{4}$$

Introducing the dimensionless variables

$$w_+(x) = \frac{W_+(x)}{kT}\,;\ \varphi(x) = \frac{\psi(x)}{kT/e_0}$$

we obtain from equations 1–4:

$$\Phi_+ = -D_+\left(\frac{dc_+}{dx} + c_+\frac{d\varphi}{dx} + c_+\frac{dw_+}{dx}\right) \tag{5}$$

Replacement of e_0 by $-e_0$ in equation 2 leads to the relation for the anion flux:

$$\Phi_- = -D_-\left(\frac{dc_-}{dx} - c_-\frac{d\varphi}{dx} + c_-\frac{dw_-}{dx}\right) \tag{6}$$

Equations 5 and 6 are the generalized Nernst-Planck equations; for constant potential energies w_+ and w_- they reduce to the usual form of the Nernst-Planck equations. The boundary conditions for the integration of equations 5 and 6 are:

$$\begin{gathered} c_+(-\infty) = c_-(-\infty) = c' \\ c_+(\infty) = c_-(\infty) = c'' \\ w_+(-\infty) = w_+(\infty) = o \\ w_-(-\infty) = w_-(\infty) = o \\ \varphi(-\infty) = o; \qquad \varphi(\infty) = u. \end{gathered} \tag{7}$$

These boundary conditions can be simplified somewhat for the purpose of numerical computation. As will be shown later, the potential energies $w_+(x)$ and $w_-(x)$ rapidly fall to zero near the boundary of the membrane so that $w_\pm(-\infty) \approx w_\pm(o) = o$ and $w_\pm(\infty) \approx w_\pm(d) = o$. This means that also the ion concentrations rapidly approach their constant values a few Angstroms outside the membrane, if we assume sufficient stirring of the aqueous phases. We may therefore approximate

$c_\pm(o)$ by c' and $c_\pm(d)$ by c''. This approximation is confirmed by the result of the numerical analysis which shows that the shape of the conductance function (equation 19) is insensitive to the change of the integration limits. Finally, as the conductivity of the membrane is very low compared with the conductivity of the aqueous solutions, practically the total drop of the electric potential occurs in the membrane itself.[1] We may therefore replace the integration limits $x = -\infty$ and $x = \infty$ by $x = 0$ and $x = d$, respectively. The stationary state solution of equation 5 can be obtained by standard methods and is given by

$$c_+(x) = e^{-(w_+ + \varphi)} \left[c' + (c'' e^u - c') \frac{\int_0^x e^{w_+ + \varphi}\, dx}{\int_0^d e^{w_+ + \varphi}\, dx} \right] \tag{8}$$

with

$$\Phi_+ = - D_+ \frac{c'' e^u - c'}{\int_0^d e^{w_+ + \varphi}\, dx} \tag{9}$$

The corresponding equations for the anion are obtained from equations 8 and 9 by replacing φ by $-\varphi$ and u by $-u$.

In the following we restrict ourselves to the case that the ion concentrations on both sides of the membrane are equal ($c' = c'' = c$). The electric current $J = e_0 \cdot (\Phi_+ - \Phi_-)$ is then given by

$$J(u) = - e_0 c \left[D_+ \frac{e^u - 1}{\int_0^d e^{w_+ + \varphi}\, dx} - D_- \frac{e^u - 1}{\int_0^d e^{w_- - \varphi}\, dx} \right] \tag{10}$$

In the preceding paper it was shown that the ionic concentrations in the membrane are so low that space charge effects may be neglected and that the potential is an almost linear function of x:

$$\varphi(x) \approx \frac{ux}{d} \qquad (o \leqq x \leqq d) \tag{11}$$

In our model, the potential energies $w_+(x)$ and $w_-(x)$ are symmetrical with respect

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[1] This is no longer true if the ionic strength of the aqueous solutions is very low ($<10^{-3}$M). Then the potential drop in the diffuse space charge layers and the concomitant phenomenon of ion injection has to be taken into account (see part I of this series).

to the middle of the membrane:

$$w_{\pm}(d - x) = w_{\pm}(x) \tag{12}$$

Equation 10 can therefore be written in the form

$$J(u) = - e_0 c(e^u - 1)\left[\frac{D_+}{\int_0^d e^{w_+ + ux/d}\, dx} + \frac{D_-}{\int_0^d e^{w_- + ux/d}\, dx}\right] \tag{13}$$

We may define the integral membrane conductivity λ by

$$\lambda = -\frac{e_0}{kT}\frac{J}{u} \tag{14}$$

and the limiting (ohmic) conductivity by

$$\lambda_0 = -\frac{e_0}{kT}\left(\frac{dJ}{du}\right)_{u=0} \tag{15}$$

From equation 13

$$\lambda_0 = \frac{e_0^2 c}{kT}\left[\frac{D^+}{\int_0^d e^{w_+(x)}\, dx} + \frac{D_-}{\int_0^d e^{w_-(x)}\, dx}\right] \tag{16}$$

These results are valid for arbitrary symmetrical potentials. In the next section it will be shown that for the assumed model the functions $w_+(x)$ and $w_-(x)$ are of the general form

$$w_+(x) = w_0(x) + a_+ \tag{17}$$

$$w_-(x) = w_0(x) + a_- \tag{18}$$

where a_+ and a_- are independent of x. With equations 17 and 18 the ratio λ/λ_0 is obtained as

$$\frac{\lambda(u)}{\lambda_0} = \frac{e^u - 1}{u} \cdot \frac{\int_0^d e^{w_0(x)}\, dx}{\int_0^d e^{w_0(x) + ux/d}\, dx} \tag{19}$$

It is easily seen from equation 19 that the current-voltage curve is ohmic ($\lambda/\lambda_0 \equiv 1$) if the potential energy w_0 is a constant. This assumption is characteristic for membrane theories based on macroscopic models. We want to derive a more realistic expression for $w_0(x)$ in the following section.

CALCULATION OF THE POTENTIAL ENERGY PROFILE BY THE METHOD OF ELECTRICAL IMAGES

Before considering the thin membrane, we begin with a much simpler case, namely two semi-infinite phases which are in contact at the plane $x = 0$. ϵ_1 and ϵ_2 are the dielectric constants of phase 1 ($x < 0$) and phase 2 ($x > 0$), respectively. We set the potential energy of an ion at $x = -\infty$ equal to zero. The potential energy at an arbitrary point x is then equal to the work done in transferring the ion from $-\infty$ to x. The main part of this energy arises from the dielectric polarisation of the medium surrounding the ion. We may therefore consider the ion simply as a conducting sphere of radius r and charge $\pm e_0$ embedded in a dielectric. The potential energy, $w(\infty)$, of the ion at a point located in medium 2 far from the boundary is then the sum of the energies w_i associated with the following three processes: (1) discharging of the sphere in medium 1; (2) transfer of the uncharged sphere across the boundary ($w_2 = 0$); (3) recharging of the sphere in medium 2. If the energies are expressed in units of kT, then, after Born (1920):

$$w(\infty) = \frac{q_0}{r}\left(\frac{1}{\epsilon_2} - \frac{1}{\epsilon_1}\right)$$

$$q_0 \equiv \frac{e_0^2}{2kT} = 282A \ (25°C) \qquad (20)$$

In the vicinity of the interface the electric field of the ion is no longer a Coulomb field because the induced charges in the other medium disturb the original field. The true potential of an ion of charge e_0 located at a point $P(x, y, z)$ in medium 2 may be obtained by introducing a fictitious charge $e = -\vartheta e_0$ at the image point $P'(-x, y, z)$ in medium 1. The electric potential of the ion is then given by the relations

$$\psi_1 = \frac{(1 + \vartheta)e_0}{\epsilon_1 \rho} \quad (x \leqq o) \qquad (21)$$

$$\psi_2 = \frac{e_0}{\epsilon_2 \rho} - \frac{\vartheta e_0}{\epsilon_2 \rho'} \quad (x \geqq o) \qquad (22)$$

in which ρ and ρ' are the distances measured from P and P', respectively. If ϑ is taken to be

$$\vartheta = \frac{\epsilon_1 - \epsilon_2}{\epsilon_1 + \epsilon_2} \qquad (23)$$

the boundary conditions

$$\psi_1 = \psi_2 \qquad (24)$$

$$\epsilon_1 \frac{\partial \psi_1}{\partial x} = \epsilon_2 \frac{\partial \psi_2}{\partial x} \tag{25}$$

$$\frac{\partial \psi_1}{\partial y} = \frac{\partial \psi_2}{\partial y}; \qquad \frac{\partial \psi_1}{\partial z} = \frac{\partial \psi_2}{\partial z} \tag{26}$$

at the interface $x = 0$ are fulfilled by equations 21 and 22.

The coulomb force between two charged spheres is the same as that between two point charges of the same magnitude which are located at the center of the spheres. The force $F(x)$ between the ion and its image therefore is equal to

$$F(x) = -\frac{\vartheta e_0^2}{\epsilon_2 (2x)^2} \tag{27}$$

The force is attractive ($F < 0$) when the medium 2 in which the ion is located has the smaller dielectric constant. For the reduced potential energy $w(x)$, of the ion at a point $x > 0$ in phase 2 the following relation holds

$$w(\infty) - w(x) = -\int_x^{\infty} \frac{F(x)}{kT}\, dx = \frac{q_0 \vartheta}{2\epsilon_2 x} \tag{28}$$

in which $w(\infty)$ is given by equation 20. The expression in equation 28 is valid as long as the ion of radius r is located completely in the medium 2, i.e., for $x > r$. The potential energy in phase 1 is obtained in an analogous fashion by integration from $x = -\infty$ to $x = -r$. Hence

$$w(x) = -\frac{q_0 \vartheta}{2\epsilon_1 x} \qquad (-\infty < x < -r) \tag{29a}$$

$$w(x) = \frac{q_0}{r}\left(\frac{1}{\epsilon_2} - \frac{1}{\epsilon_1}\right) - \frac{q_0 \vartheta}{2\epsilon_2 x} \qquad (r < x < \infty) \tag{29b}$$

In the intermediate region $-r \leqq x \leqq r$ where the ion is partly in the medium 1 and partly in the medium 2, the function $w(x)$ may be approximated by a smooth connection between the two branches of expression in equation 29, for example by a polynomial of the third degree.

Now let us extend the previous results to a thin film of dielectric constant ϵ_2 which is in contact at $x = 0$ and $x = d$ with media of dielectric constant ϵ_1. If the ion is located at the point x in the membrane, an infinite set of image charges with coordinates $x_\nu = 2\nu d \pm x$ has to be introduced in order to fulfill the boundary conditions. One has to place the charges

$$e_{-\nu} = \vartheta^{2\nu} e_0 \text{ at } x_\nu = -2\nu d + x \qquad (\nu = 1, 2, 3, \ldots)$$

$$e_{\nu} = \vartheta^{2\nu} e_0 \text{ at } x_\nu = 2\nu d + x \qquad (\nu = 1, 2, 3, \ldots)$$

$$e'_{-\nu} = -\vartheta^{2\nu+1} e_0 \text{ at } x_\nu = -2\nu d - x \qquad (\nu = 0, 1, 2, \ldots)$$

$$e'_{\nu} = -\vartheta^{2\nu-1} e_0 \text{ at } x_\nu = 2\nu d - x \qquad (\nu = 1, 2, 3, \ldots) \tag{30}$$

The image charges $e_{-\nu}$ and e_ν are located symmetrically with respect to the ion and therefore give no contribution to the total force $F(x)$ acting on the ion. By summation over the forces due to $e'_{-\nu}$ and e'_ν we obtain after some rearrangement:

$$F(x) = -\frac{\vartheta e_0^2}{4\epsilon_2}\left\{\frac{1}{x^2} + \frac{1}{d^2}\sum_{\nu=1}^{\infty}\left[\frac{\vartheta^{2\nu}}{(\nu + x/d)^2} - \frac{\vartheta^{2\nu-2}}{(\nu - x/d)^2}\right]\right\} \qquad (31)$$

For an infinitely thick membrane ($d \rightarrow \infty$), this relation reduces to equation 27. The potential energy $w(x)$ of the ion in the membrane is then given by

$$\begin{aligned} w(x) &= w(r) - \int_r^x \frac{F(x)}{kT}\,dx \qquad\qquad (r < x < d - r) \\ &= w(r) + \frac{q_0\vartheta}{2\epsilon_2}\left\{\frac{1}{r} - \frac{1}{x}\right. \\ &\left. + \frac{1}{d}\left[S\left(\vartheta, \frac{r}{d}\right) - S\left(\vartheta, \frac{x}{d}\right)\right] + \frac{1}{\vartheta^2 d}\left[S\left(\vartheta, -\frac{r}{d}\right) - S\left(\vartheta, -\frac{x}{d}\right)\right]\right\} \qquad (32) \end{aligned}$$

where $S(\vartheta, y)$ is an abbreviation for the convergent sum

$$S(\vartheta, y) \equiv \sum_{\nu=1}^{\infty} \frac{\vartheta^{2\nu}}{\nu + y} \qquad (-1 < y < 1) \qquad (33)$$

The energy $w(x)$ has the general form

$$w(x) = w_0(x) + a(r)$$

in which $w_0(x)$ is independent of the ionic radius r and $a(r)$ is independent of the coordinate x. This relation has already been used in the preceding section. Near the membrane boundary at $x = 0$ the ion is sufficiently far from the second boundary at $x = d$, so that the potential energy will be given approximately by expression in equation 29 *b* which was derived for a single interface. We may therefore insert $w(r)$, as given by equation 29 *b*, into equation 32 and thereby obtain:

$$w(r) \approx \frac{q_0}{r}\left(\frac{1}{\epsilon_2} - \frac{1}{\epsilon_1}\right) - \frac{q_0\vartheta}{2\epsilon_2 r} \qquad (34)$$

The total potential profile is then obtained by combination of equations 29 *a*, 32, and 34:

$$\begin{aligned} w(x) &= -\frac{q_0\vartheta}{2\epsilon_1 x} \qquad\qquad (-\infty < x < -r) \\ w(x) &= \frac{q_0}{r}\left(\frac{1}{\epsilon_2} - \frac{1}{\epsilon_1}\right) + \frac{q_0\vartheta}{2\epsilon_2 d}\left[-\frac{d}{x} + S\left(\vartheta, \frac{r}{d}\right)\right. \\ &\left. - S\left(\vartheta, \frac{x}{d}\right) + \frac{1}{\vartheta^2} S\left(\vartheta, -\frac{r}{d}\right) - \frac{1}{\vartheta^2} S\left(\vartheta - \frac{x}{d}\right)\right] \qquad (r < x < d - r) \\ w(x) &= \frac{q_0\vartheta}{2\epsilon_1(x - d)} \qquad\qquad (d + r < x < \infty) \qquad (35) \end{aligned}$$

In the regions $-r \leqq x \leqq r$ and $d - r \leqq x \leqq d + r$ we use the same approximation function that would be used to connect the two branches of equation 29. The function $w(x)$, as may be shown from equation (35), is symmetrical with respect to the middle of the membrane, i.e., the relation $w(d - x) = w(x)$ holds.

RESULTS AND DISCUSSION

In the following we give some numerical data for the general relations derived in section 2 and 3. This will enable us to compare the theoretical predictions of the model with the observed current-voltage characteristics of lipid bilayer membranes. As may be seen from equations 19 and 35, only three parameters of the model enter into the theoretical conductance function $\lambda(u)/\lambda_0$, namely the dielectric constants of the aqueous and the lipid phase (ϵ_1 and ϵ_2, respectively), and the film thickness d. In particular, $\lambda(u)/\lambda_0$ is independent of the ionic radius.

The thickness of bimolecular membranes formed from egg lecithin was determined by optical methods to be about 70 A (Huang and Thompson, 1965, 1966). For the resistance of the membrane, however, only the central hydrocarbon-like part seems to be essential. We therefore adopt as the effective film thickness a somewhat lower value, $d = 50$ A. For the dielectric constant of water the value $\epsilon_1 = 78.5$ (25°C) is used. The dielectric constant of the lipid film is not precisely known; from measurements of the electric capacitance of the film (Hanai et al., 1964) in connection with the optically determined film thickness, one obtains $\epsilon_2 \approx 2$, the dielectric constant of a hydrocarbon. As the dielectric constant of the membrane may be increased by uptake of solutes from the aqueous phase, numerical computations were also performed with $\epsilon_2 = 3$ and $\epsilon_2 = 4$.

In Fig. 1 the potential energy, $w(x)$, of an ion of radius $r = 2$ A, as obtained by numerical evaluation of equation 35, is plotted against the coordinate x for different values of the dielectric constant ϵ_2. It is seen that $w(x)$ is strongly dependent upon ϵ_2, but falls rapidly to zero at the membrane boundaries in all cases. Especially at

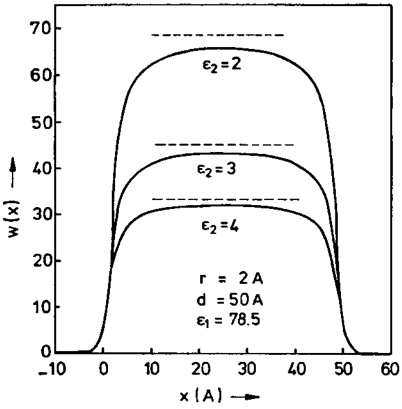

FIGURE 1 Potential energy $w(x)$ of an ion in the membrane, expressed in units of kT (k = Boltzmann constant, T = absolute temperature) after equation 35. Ion radius $r = 2$ A. The external phase is assumed to be an aqueous solution of dielectric constant $\epsilon_1 = 78.5$ (25°C); ϵ_2 = dielectric constant of the membrane; d = membrane thickness. The dashed lines indicate the Born energy of the ion in a macroscopic phase ($d \rightarrow \infty$) of dielectric constant ϵ_2.

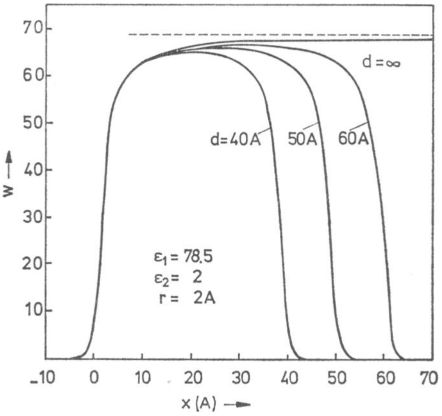

Figure 2 Potential energy $w(x)$ at fixed values of ϵ_1, ϵ_2, and r for three different membrane thicknesses d. The dashed line is the asymptote for $x \rightarrow \infty$ of the energy curve at infinite membrane thickness.

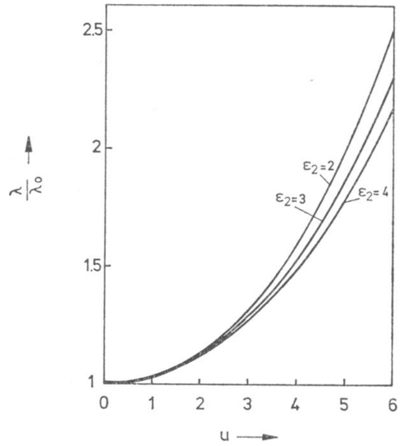

Figure 3 Membrane conductivity λ as a function of the voltage u. u is expressed in units of $kT/e_0 = 25.6$ mv (25°C). λ_0 = ohmic membrane conductivity in the limit $u \rightarrow 0$. $\lambda/\lambda_0$is independent of the ionic radius r. The values of the other parameters are the same as in Fig. 1.

a low value of ϵ_2, $w(x)$ deviates appreciably from the Born energy (dashed line) even in the middle of the membrane.

Fig. 2 shows the potential energy $w(x)$ at fixed ϵ_1, ϵ_2, and r, but for three different values of the membrane thickness d. Up to about $x = d/4$ the three curves almost coincide with the curve for $d = \infty$. This confirms the quality of the approximation introduced by equation 34.

In a second series of calculations, the numerical values of $w(x)$ were inserted into the expression for the conductance function $\lambda(u)/\lambda_0$ (equation 19). From the result which is given in Fig. 3 a strongly nonlinear current-voltage characteristic is predicted for the thin lipid membrane. The non-linearity is most pronounced at the lowest value of ϵ_2; this is plausible because the membrane should approach ohmic behavior in the limit $\epsilon_2 \rightarrow \epsilon_1$. The conductance functions for different membrane thicknesses at $\epsilon_2 = 2$ almost coincide; a graphical representation is therefore omitted here. For instance, the values of λ/λ_0 at $u = 6$ are 2.63, 2.52, and 2.46 for $d = 40$, 50, and 60 A, respectively. The experimental uncertainty about the exact value of d has thus only a small influence on the shape of the conductance function.

As experimental data on the current-voltage behavior of lipid membranes are still scanty, a comparison between theory and experiment can be made only in a preliminary way. Inspection of Fig. 4 of the preceding paper shows that the observed current-voltage curve of a dioleoyl lecithin membrane in (10^{-4}M 2,4-dinitrophenol + 10^{-4}M KCl) is described in a satisfactory manner by the theoretical curve with $\epsilon_2 = 2$ up to about $u = 3$. At higher voltages the experimental values of λ/λ_0 increase more rapidly than expected from the theoretical curve. This deviation is even more pronounced at higher ionic strength (10^{-1}M KCl). We may therefore conclude that the current-voltage characteristic of lecithin membrane under the abovementioned conditions is described to a first approximation by the generalized Nernst-Planck equations, but that the nonlinearity is enhanced by a second process. This second process is likely to be the field dissociation of ion pairs in the membrane (Wien effect). The influence of the Wien effect on the membrane conductivity will be treated in a subsequent paper.

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