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RECTIFICATION PHENOMENA IN CARRIER-MEDIATED ION TRANSPORT

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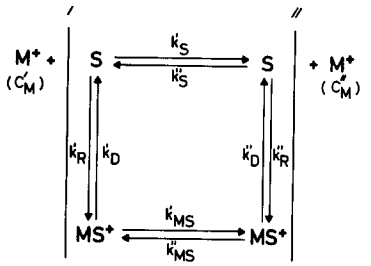
SUMMARY

Ion transport across membranes mediated by carrier molecules may show rectification phenomena either if the membrane is asymmetrical or if the aqueous solutions on both sides of the membrane are asymmetrical with respect to the transported ion. This is shown for a model based on an "Eyring treatment" of diffusion across the membrane and on a chemical reaction for charge transport across the membrane interface. The model is applied to ion transport across lipid bilayer membranes in the presence of valinomycin.

INTRODUCTION

Rectification phenomena have been frequently encountered during the study of electrical characteristics of biological membranes[1–3]. Because of their similarity to solid state effects they have been often explained on the basis of fixed charge models like semiconductor junctions[1,3,4], so that the concentration profile of the permeant ions is determined by the potential profile of the fixed charges and the current across the membrane obeys the laws of electrodiffusion. These models do not make use of special transport mechanisms. Their rectification is a result of electrostatic forces acting on the permeant ions.

On the other hand, ion transport across biological membranes is often believed to be effected by well-defined "transport channels", such as pores or carrier molecules. It is the purpose of the present paper to show that rectification can be also understood on the basis of a simple carrier model. Whereas the existence of real biological carrier molecules is still an open question, the action of some macrocyclic compounds, like valinomycin or the macrotetrolides, as carrier molecules for alkali ions in biological membranes is well established, especially through studies with artificial lipid membranes[5–10]. During the last few years we have developed a carrier transport model, which is based on an "Eyring treatment" of the membrane. This model has been successfully applied to the action of the above-mentioned antibiotics on black lipid films and has lead to a quantitative evaluation of the transport parameters[11–13]. While those studies were performed with symmetrical systems, the present paper considers asymmetrical membranes or asymmetrical aqueous solutions, and shows that rectification phenomena may be observed in these systems.



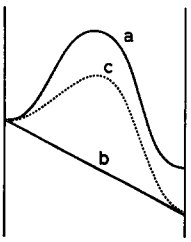


Fig. 1. Transport of a cation M^+ (concentration c'_M, c''_M) by a neutral carrier S across an asymmetrical membrane.

Fig. 2. Schematic energy profile of a carrier molecule inside an asymmetrical membrane. a, zero voltage across the membrane; b, electrostatic energy produced by a voltage (constant-field approximation) for a positively charged ion complex; c, resulting potential energy for the complex.

Description and analysis of the model

The model described below is a generalization of a previously published one (see refs 11–13).

We consider a membrane which is in contact on both sides with aqueous solutions of an univalent cation M^+ (see Fig. 1). The membrane contains neutral carrier molecules for the cations M^+ of total concentration N_0 (moles/cm^2). Free carrier molecules S from the membrane may undergo a chemical reaction at the interface with an ion M^+ from the aqueous phase. This heterogeneous reaction is described by a recombination (or association) rate constant k_R and a dissociation rate constant k_D. These rate constants may be different on both sides of the membrane, as we also consider asymmetrical membranes. We use ′ and ″ to designate concentrations and rate constants on the left- and right-hand side of the membrane, respectively. We further assume that the free carrier molecules S, and also their ion complexes MS^+, possess energy profiles which have minima near the interfaces. These minima could stem from a specific interaction of the carrier molecules with the membrane interfaces. The electrical image forces acting on the positively charged complex near the interface of two media with different dielectric constants also give rise to such an energy profile[14]. For an asymmetrical membrane the energy minima will be of different depth (Fig. 2, Curve a). Carrier molecules S may cross the membrane by jumping from one interface over the barrier to the other interface. According to Eyring[15] this process can be described by rate constants (Fig. 1). For asymmetrical membranes these rate constants have different values according to the direction of diffusion between the left-hand and right-hand side. On application of a voltage to the membrane k'_S and k''_S are unaffected, as S is a neutral molecule. The rate constants k'_{MS} and k''_{MS} for the complex, however, are enhanced and reduced by a voltage U. If the maximum of the barrier is in the middle of the membrane and if the barrier is sufficiently steep, its height is changed by $FU/2RT$ (see Fig. 2)[15,11].

Therefore,

$$k'_{MS} = k'_{MS}(0)\, e^{-u/2}$$
$$k''_{MS} = k''_{MS}(0)\, e^{u/2} \qquad (1)$$

with F, Faraday constant; R, gas constant; $u=FU/RT$, reduced voltage; k'_{MS} (0), k''_{MS} (0), rate constants in the absence of voltage.

Eqn 1 contains the complete voltage dependence of the model, since we assume that the other rate constants are independent of voltage. If we denote the interfacial concentrations of S and MS^+ by N'_S, N''_S, N'_{MS} and N''_{MS}, respectively, the sum of the net chemical production and of the flux across the membrane for each species must disappear in the stationary state[11,13]:

$$\frac{dN'_S}{dt} = -k'_R c'_M N'_S + k'_D N'_{MS} - k'_S N'_S + k''_S N''_S = 0$$

$$\frac{dN''_S}{dt} = -k''_R c''_M N''_S + k''_D N''_{MS} - k''_S N''_S + k'_S N'_S = 0$$

$$\frac{dN'_{MS}}{dt} = k'_R c'_M N'_S - k'_D N'_{MS} - k'_{MS} N'_{MS} + k''_{MS} N''_{MS} = 0$$

$$\frac{dN''_{MS}}{dt} = k''_R c''_M N''_S - k''_D N''_{MS} - k''_{MS} N''_{MS} + k'_{MS} N'_{MS} = 0 \qquad (2)$$

Eqns 2 are consistent with the fact that the total number of carrier molecules per unit area of membrane N_0 is given by

$$N_0 = N'_S + N''_S + N'_{MS} + N''_{MS} \qquad (3)$$

If we assume MS^+ to be the only charge carrier in the membrane, the stationary electrical current density J is

$$J = F(k'_{MS} N'_{MS} - k''_{MS} N''_{MS}) \qquad (4)$$

When the solutions of Eqns 2 are inserted into Eqn 4 one obtains

$$J = \frac{FN_0(a'k'_{MS} - a''k''_{MS})}{b(k'_{MS} + k''_{MS}) + c'k'_{MS} + c''k''_{MS} + h} \qquad (5)$$

with the coefficients a', a'', b, c', c'' and h being voltage independent:

$$a' = k'_R c'_M k''_D k''_S \qquad c' = k''_D(k'_R c'_M + k'_S + k''_S)$$

$$a'' = k''_R c''_M k'_D k'_S \qquad c'' = k'_D(k''_R c''_M + k'_S + k''_S)$$

$$b = (k'_R c'_M k''_S + k''_R c''_M k'_S + k'_R c'_M k''_R c''_M)$$

$$h = k''_S k''_D(k'_R c'_M + k'_D) + k'_S k'_D(k''_R c''_M + k''_D)$$

The rate constants describing the transport model have been considered as being independent of one another. In reality there is a coupling caused by the principle of microscopic reversibility. This principle can be simplified here in the following way. If there is no difference in the electrochemical potential between the aqueous phases on both sides of the membrane (*i.e.* if $c'_M = c''_M$ and $U=0$), the electrical current

must be zero, since there is no driving force (we do not consider temperature and pressure gradients). This is equivalent to (see Eqns 5 and 1):

$$\frac{k'_R k'_{MS}(0) k''_D k''_S}{k''_R k''_{MS}(0) k'_D k'_S} = 1 \tag{6}$$

Rectification

The explicit voltage dependence of the current is obtained by inserting Eqn 1 into Eqn 5. The reduced voltage at zero current, u_0, is calculated from the disappearing nominator of Eqn 5. Regarding Eqn 6 one obtains the Nernst potential

$$\frac{c'_M}{c''_M} = e^{u_0} \tag{7}$$

We define v as the reduced voltage relative to u_0:

$$v = u - u_0 \tag{8}$$

Inserting Eqns 1, 6 and 8 into Eqn 5 yields the current as a function of voltage relative to zero current voltage:

$$J(v) = \frac{-2FN_0 \sinh(v/2)}{\frac{(b+c')}{a'} e^{-v/2} + \frac{(b+c'')}{a''} e^{v/2} + \frac{h}{[a'a''k'_{MS}(0)k''_{MS}(0)]^{\frac{1}{2}}}} = \frac{R(v)}{S(v)} \tag{9}$$

We define a rectification ratio r by

$$r = |J(+v)| / |J(-v)| \tag{10}$$

From Eqn 9 one derives:

(a) $|R(v)| = |R(-v)|$ in general

(b) $|S(v)| = |S(-v)|$ only if $a' = a''$ and $c' = c''$

Therefore, we conclude that the model shows rectification (*i.e.* a rectification ratio r different from one) if either $a' \neq a''$ or $c' \neq c''$, or both.

This rectification behaviour will become clearer by considering two special cases.

Asymmetrical solutions but symmetrical membrane

These conditions are equivalent to:

$$k'_R = k''_R = k_R$$

$$k'_D = k''_D = k_D$$

$$k'_S = k''_S = k_S$$

$$k'_{MS}(0) = k''_{MS}(0) = k_{MS}$$

$$c'_M \neq c''_M \tag{11}$$

The result becomes especially simple if we make the additional assumptions:

$$\begin{aligned} k_R c'_M &\ll k_D \qquad k_R c''_M \ll k_D \\ k_R c'_M &\ll k_S \qquad k_R c''_M \ll k_S \end{aligned} \tag{12}$$

The conditions of Eqn 12 can be easily met by using sufficiently small concentrations c'_M and c''_M.

With the definitions of Eqn 5 and Eqn 12 it follows:

$$\begin{aligned} c &= 2k_D k_S \\ h &= 2k_D^2 k_S \\ b &\ll c \end{aligned} \tag{13}$$

Inserting Eqn 13 into Eqn 9 yields the result

$$g \equiv \frac{-J}{FN_0 k_R \sqrt{c'_M c''_M}} = \frac{\frac{k_{MS}}{k_D} \sinh(v/2)}{1 + \frac{k_{MS}}{k_D} \sqrt{\frac{c'_M}{c''_M}} \left(e^{v/2} + \frac{c''_M}{c'_M} e^{-v/2} \right)} \tag{14}$$

Using Eqns 10 and 14 one derives a rectification ratio

$$r = \frac{1 + \frac{k_{MS}}{k_D} \sqrt{\frac{c'_M}{c''_M}} \left(e^{-v/2} + \frac{c''_M}{c'_M} e^{v/2} \right)}{1 + \frac{k_{MS}}{k_D} \sqrt{\frac{c'_M}{c''_M}} \left(e^{v/2} + \frac{c''_M}{c'_M} e^{-v/2} \right)} \tag{15}$$

The rectification ratio is a monotonic function of voltage. Its maximum value reached at high voltages is

$$r_{max} = \lim_{v \to \infty} r(v) = \frac{c''_M}{c'_M} \tag{16}$$

In Fig. 3, r is plotted as a function of reduced voltage v for different values of k_{MS}/k_D (Eqn 15). The higher the value of k_{MS}/k_D the lower is the voltage at which r_{max} is reached. The basis of rectification is the current–voltage relationship. Fig. 4 shows current–voltage curves at a given value k_{MS}/k_D for symmetrical and asymmetrical aqueous solutions (Eqn 14). The symmetries are reflected in the g–v curves. The limited value of r_{max} given by Eqn 16 is a result of the saturating current. At sufficient high voltages each complex formed at the positive side of the membrane is transported to the negative side, whereas those complexes formed at the negative side have no chance to cross the membrane. Thus J_{max} is given by the formation rate of complexes at the positive interface. As this formation rate is proportional to the ion concentration, the ratio of maximum currents in opposite directions reflects the corresponding ion concentrations (see Eqn 16).

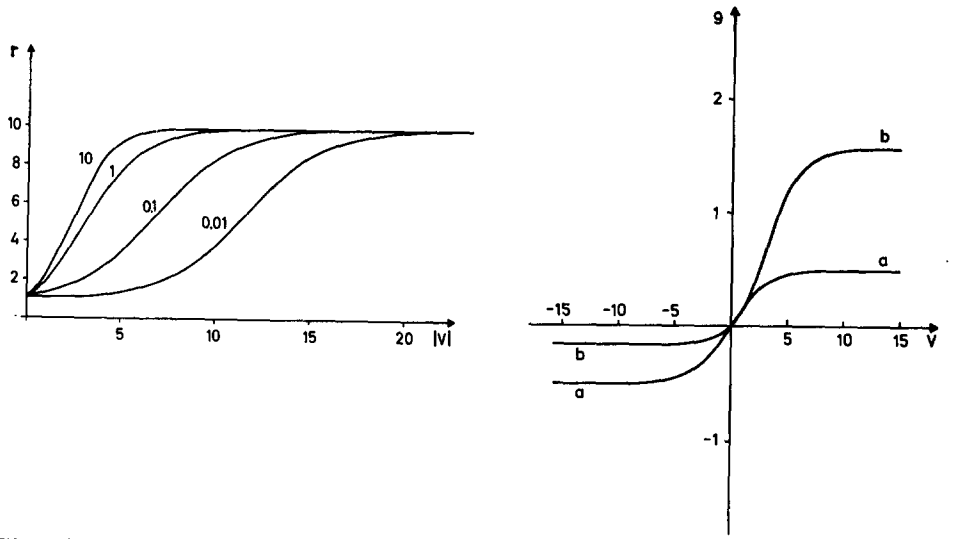


Fig. 3. Rectification ratio r as a function of reduced voltage v (in units $RT/F = 25.6$ mV at 25 °C) for different values of k_{MS}/k_D according Eqn 15 with $c''_M/c'_M = 10$.

Fig. 4. Current–voltage curve according Eqn 14 with $k_{MS}/k_D = 1$. g = dimensionless current, v = reduced voltage (in units $RT/F = 25.6$ mV at 25 °C) relative to zero current voltage. a) $c'_M = c''_M$; b) $c''_M = 10c'_M$.

Symmetrical aqueous solutions and surface reactions but asymmetrical barrier shapes

This means

$$\begin{aligned} &c'_M = c''_M \\ &k'_R = k''_R \qquad\qquad k'_{MS}(0) \neq k''_{MS}(0) \\ &\qquad\text{but} \\ &k'_D = k''_D \qquad\qquad k'_S \neq k''_S \end{aligned} \tag{17}$$

Assumptions 17 together with Eqn 6 show that the barrier shapes for the free carrier molecules and the complexes are coupled:

$$k'_{MS}(0)/k''_{MS}(0) = k'_S/k''_S \tag{18}$$

From Eqns 9, 10 and 18 one calculates

$$r = \frac{1 + f(e^{v/2}/k''_S + e^{-v/2}/k'_S)}{1 + f(e^{-v/2}/k''_S + e^{v/2}/k'_S)} \tag{19}$$

with

$$f = \frac{k'_{MS}(0)k''_S}{k_D}\left(1 + \frac{k_R c_M}{k'_S + k''_S}\right)$$

From Eqn 19 we obtain a maximum rectification ratio at high voltages

$$r_{max} = \frac{k'_S}{k''_S} \tag{20}$$

Eqns 19 and 20 show that, even for symmetrical aqueous solutions, a carrier model may show rectification. The asymmetries in the rate constants k_S (or k_{MS}) could stem from a different interaction of the carrier molecules with both membrane interfaces, which is equivalent to a different activation energy for diffusion in opposite directions. From Eqns 9 and 18 one further derives that the zero current potential is equal to zero (*i.e.* $v=u$). Nevertheless, high rectification ratios are possible and appear as a consequence of an asymmetrical membrane.

We have shown previously that the carrier transport model presented above adequately describes the transport of alkali metal ions, such as K^+ or Rb^+ mediated by valinomycin across artificial lipid membranes[12,13]. In the following sections experiments are described which were designed to measure the rectification characteristic of that transport system and to compare it with theoretical expectations.

EXPERIMENTAL

Membranes were formed from a 0.5% solution of phosphatidylserine (Koch-Light Laboratories) in *n*-decane. The procedure and experimental set up was similar to that described by Läuger *et al.*[16] (membrane area 0.1 cm^2). Two current and two voltage electrodes (platinized platinum) were used. Valinomycin was added as an ethanolic solution to the lipid–decane phase and the ethanol was then evaporated[12]. The valinomycin concentration had to be kept rather low to avoid diffusion polarization generated by concentration changes of the charge carrier near the membrane. The influence of diffusion polarization, which leads to erroneous results, is much stronger with asymmetrical aqueous solutions than with symmetrical solutions (see Appendix). On the other hand, the conductance induced by valinomycin should be at least one order of magnitude higher than the conductance of the unmodified membrane (without valinomycin), in order to get a well-defined system, which can be compared with the theory. A concentration of 10^{-5} M in the lipid–decane phase met both conditions.

Rubidium was used as transported ion. An experiment was initiated with a 10^{-3} M concentration of RbCl on both sides of the membrane. After measuring the current–voltage curve for this symmetrical system, an appropriate amount of a 10^{-1} M RbCl solution was added to one side of the membrane, under stirring, up to a final concentration of 10^{-2} M. The hydrostatic pressure across the membrane was kept constant by adding the same amount of a 10^{-3} M RbCl solution to the other side of the membrane. By this procedure a 10:1 concentration gradient of RbCl across the membrane was generated and again the J–U curve measured. In all solutions the ionic strength was kept constant at 1 M by adding LiCl, which is not transported by valinomycin. This was necessary because of the negative surface charge of phosphatidylserine membranes[12]. The pH was kept at 5.6, the temperature at 25 °C.

RESULTS AND DISCUSSION

Fig. 5 shows current–voltage curves for symmetrical and asymmetrical aqueous solutions. The full lines were drawn according to Eqn 14. This equation is only applicable if the conditions of Eqn 12 are fulfilled. For the system of phosphatidyl-

inositol membranes with valinomycin and potassium, we determined the absolute values of the rate constants by using relaxation methods. The conditions of Eqn 12 are met by using the values given in ref. 13 (for concentrations $c_M \leq 10^{-2}$ M). We found very similar values for the rate constants of the system used during this study (Benz, R. and Stark, G., unpublished). The zero current voltage of curve b is equal to the Nernst potential for a rubidium concentration ratio of 1:10, which means that Rb^+ is the only charge carrier contributing to current.

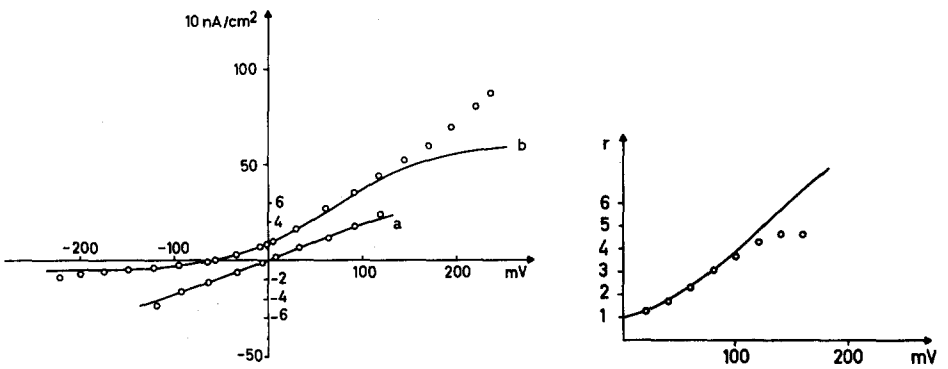


Fig. 5. Current–voltage curves for a phosphatidylserine membrane with 10^{-5} M valinomycin in the membrane-forming solution. Full lines according Eqn 14 with $k_{MS}/k_D = 0.25$; $N_0 k_R = 1.25 \cdot 10^{-6}$ cm/s. a) $c'_M = c''_M = 10^{-3}$ M RbCl, right-hand ordinate scale; b) $c'_M = 10^{-3}$ M, $c''_M = 10^{-2}$ M RbCl, left-hand ordinate scale. Ionic strength 1 M by adding LiCl. Abscissa: Absolute voltage across the membrane in mV.

Fig. 6. Rectification of a phosphatidylserine membrane induced by valinomycin/rubidium. Full line according Eqn 15. Abscissa: Voltage (in mV) relative to zero current voltage. For further details see legend of Fig. 5 (Curve b).

Apart from experimental quantities, the theoretical curves according to Eqn 14 are determined by k_{MS}/k_D and $N_0 k_R$. It has been shown previously for symmetrical systems (and can be also concluded from Eqn 14 for the generalized case) that k_{MS}/k_D is responsible for the form of the J–U curve under these conditions (which can be super- or sublinear), whereas $N_0 k_R$ can be derived from the absolute value of the current at a given voltage (or from the zero voltage conductivity $\lambda_0 = (J/U)_{U \approx 0}$) (ref. 12). The full lines of Fig. 5 were calculated by using the same values of parameters for Curves a and b. The behaviour of systems with asymmetrical aqueous solutions can therefore be predicted from studies with symmetrical systems. The agreement between theory and experiment is satisfactory for voltages up to at least 100 mV. Above this voltage deviations occur, which probably result from structural changes of the membrane caused by the Maxwell pressure of the charged membrane capacity[17]. These deviations are also reflected in the voltage dependence of the rectification ratio (Fig. 6). The maximum rectification ratio (with the given experimental conditions $r_{max} = 10$) is not reached, because of the limited breakdown voltage of bilayer membranes.

Rectification of the carrier model for a symmetrical membrane and asymmetrical aqueous solutions finally stems from the influence of the surface reaction.

If the rate-determining step of carrier transport is the translocation across the membrane interior (*i.e.* $k_{MS}/k_D \ll 1$), rectification at low voltages is insignificant (see Fig. 3). In the limiting case of complete dominance of diffusion across the membrane barrier and extremely fast chemical reaction (*i.e.* $k_D \to \infty$, however finite k_R/k_D), rectification disappears and the J–U curve becomes symmetrical within the frame of our model, which assumes a steep maximum of the energy barrier in the middle of the membrane (Eqn 21).

From Eqns 14 and 15:

$$J = -FN_0 \frac{k_R}{k_D} \sqrt{c'_M c''_M}\, k_{MS} \sinh(v/2) \qquad (21)$$

$$r = 1$$

As Hall *et al.*[18] have shown, even in this case of barrier-limited transport, one obtains a rectification ratio different from one by taking into account the specific shape of the energy barrier. The Eyring treatment represents only an approximative description of the latter. The experiments and their analysis presented here, however, show that, at least for valinomycin, the rectification of the asymmetrical system can be completely predicted from the study of the symmetrical system without considering the detailled shape of the energy barrier. A comparison with the data of Hall *et al.*[18] shows that this also seems to be true for the rectification of the nonactin system. The observed rectifications may therefore be explained as a result of the influence of the surface chemical reaction, which has been neglected by Hall *et al.*[18]. The question of a contribution of a specific barrier shape has to be left open.

The carrier model presented here was applied to artificial membranes. However, its fundamental statement with regard to rectification should also be valid for carrier transport in biological membranes. Different fluxes in opposite directions have been observed for amino acids coupled to sodium transport. These phenomena have also been interpreted on the basis of a carrier model by Heinz *et al.*[19].

APPENDIX

Influence of diffusion polarization on the current–voltage characteristic

We assume that the membrane resistance is very much higher than the resistance of the aqueous solutions. Then the total voltage drops across the membrane, and the charge carriers M^+ in the aqueous solutions are not driven by an electric field (as in the membrane) but must diffuse across the unstirred layers on both sides of the membrane (thickness δ). Therefore an electrical current across the membrane will generate concentration gradients at the membrane surface, which depend on the absolute value of the current and drive the flux of M^+ corresponding to the current across the unstirred layers. If the current is very high, the concentration changes near the membrane will assume appreciable values and influence the current, which depends on the surface concentrations of M^+ (see Fig. 1 and Eqn 14). We will permit maximum concentration changes across the unstirred layers of 10% (*i.e.* $\Delta c_M(\max) = 0.1\, c_M$). With this condition one calculates a maximum flow of M^+ across the unstirred layer (D = diffusion coefficient for M^+ in the aqueous phase):

$$\Phi_{max} = \frac{D}{\delta} \Delta c_M(\max) = 0.1 \frac{D}{\delta} c_M \qquad (A1)$$

The maximum stationary current across the membrane is equal to the maximum current across the unstirred layer. With Eqn A1

$$J_{max} = F \cdot \phi_{max} = 0.1 \frac{FD}{\delta} c_M \tag{A2}$$

With the numerical values: $D = 10^{-5}$ cm^2/sec; $\delta \approx 3 \cdot 10^{-2}$ cm (unpublished experimental result), one obtains from Eqn A2 for the maximum current without an influence of diffusion polarization:

$$J_{max} \approx 3 c_M \; (c_M \text{ in moles/cm}^3, J \text{ in A/cm}^2) \tag{A3}$$

Diffusion polarization is especially easy to observe with asymmetrical aqueous solutions. If $c'_M > c''_M$, and if we denote the positive current from the left-hand to the right-hand side of the membrane by $\vec{J}$, then $\vec{J}$ at high voltages mainly depends on c'_M (see Eqn 14). In contrast, diffusion polarization first occurs at the side of smaller concentration c''_M. One therefore has to insert c''_M into Eqn A3. The problems arising from diffusion polarization increase with the concentration gradient across the membrane.

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