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A theoretical description of non-steady-state diffusion of hydrophobic ions across lipid vesicle membranes including effects of ion–ion interactions in the aqueous phase

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Abstract

A theoretical model of hydrophobic ion diffusion across vesicular membranes is presented, which is based upon activated rate theory. The model is applicable to the sudden addition of hydrophobic ions to a vesicle suspension, for example in a stopped-flow experiment. The time course of diffusion is calculated by numerical integration of differential rate equations for the ion concentrations and electrical potential differences across the membrane. The model utilizes the three-capacitor model of the membrane and an extended Debye–Hückel theory, taking into account non-neutrality on each side of the membrane. At low ionic strengths good agreement is found between the infinite time diffusion potential and the equilibrium Nernst potential. At large excess of inert electrolyte discrepancies are found, but under such conditions the membrane potential is negligible due to screening.

Keywords: Hydrophobic ions; Lipid vesicles; Diffusion; Three capacitor model; Debye–Hückel theory

1. Introduction

The kinetics of diffusion of hydrophobic ions across lipid membranes has previously been studied by a number of authors [1–10] as a model system for the carrier mechanism of ion transport. It has been found [2,4–10] that the kinetics can often be treated by using the activated rate theory of ion diffusion of Eyring [11–13]. Läuger has also applied the theory to facilitated diffusion through ion channels and to active transport by ion pumps [14,15]. The basis of the theory is that diffusion is assumed to proceed as a series of jumps across potential energy barriers within the membrane. The height of the potential energy barriers can further be modified by the presence of intramembrane electrical potential gradients. Equations describing steady-state diffusion across lipid membranes have been developed by equating the fluxes across each energy barrier [11–14,16].

Since hydrophobic ions generally bind readily to lipid membranes, their diffusion across the membrane is normally quite rapid and hence specialized fast reaction techniques are often necessary to study the kinetics. The most widely used techniques have been those of relaxation kinetics, including the voltage-jump, charge pulse and temperature-jump methods [2,6,8,9,17,18]. These studies have all used electical detection. In

a smaller number of cases the diffusion of some hydrophobic dye ions across lipid vesicle membranes has been studied by rapid flow methods using optical detection [19–24]. In the case of the relaxation techniques the system is initially at equilibrium and it is then perturbed by a sudden change of an environmental parameter. For sufficiently small perturbations the kinetic equations can be mathematically simplified, so that in many cases rate constants for the diffusion process can be analytically determined. Under steady-state diffusion conditions, such as in isotope flux experiments, the theory of irreversible thermodynamics [25,26] has often been applied. This involves the introduction of phenomenological coefficients relating the flux to the electrochemical potential difference across the membrane. In the case of flow methods, immediately after mixing the system is far from equilibrium. Diffusion of ions across the membrane then proceeds until the electrochemical potentials of ions on each side of the membrane are equal. Due to the movement of the ions, during the diffusion process there are also changes in the electrical potential gradients within the membrane, which result in changes of the rate constants describing the individual steps of diffusion. Under such conditions neither the simplifications of relaxation kinetics theory nor irreversible thermodynamics are applicable. Therefore, it would appear that a complete description of the time course of diffusion for a stopped-flow experiment involving the initial mixing of the diffusing species with a membrane suspension necessitates numerical simulation.

In a previous publication [24] differential rate equations were presented for the diffusion of a hydrophobic dye ion across lipid vesicle membranes. The electrical potential dependence of the rate of diffusion was explained according to activated rate theory by the effect of the intramembrane potential on the activation energy barrier for diffusion within the membrane. In the present paper the theory is extended to take into account the effect of boundary potentials on the binding reactions of ions to the membrane, and an equation based upon the Debye–Hückel theory is developed which allows the calculation of the total membrane potential at any point in time. The equation also permits the calculation of the equilibrium diffusion potential as a function of ionic strength. This allows a more exact analysis of the question of whether or not the membrane potential produced due to ion diffusion under given ionic strength conditions can be expected to affect the diffusion rate.

2. Theory

Based on the results of electrical relaxation studies [2,6,17,18] and rapid flow studies [19–24], in which distinct kinetic phases have been observed, the diffusion of a hydrophobic ion across a lipid vesicle membrane is assumed to occur in the following series of steps:

(a) diffusion of the ion through the aqueous solution and binding to the external lipid monolayer of the vesicle
(b) translocation of the ion across the membrane to the internal lipid monolayer
(c) dissociation of the ion from the internal lipid monolayer and diffusion into the intravesicular space

The mechanism is shown schematically in Fig. 1. The vesicles are assumed to have a finite number of sites on both sides of the membrane available for the binding of ions. It should be noted that the term binding site here does not imply a specific interaction as in the case of a substrate binding to the active site of an enzyme. Instead it

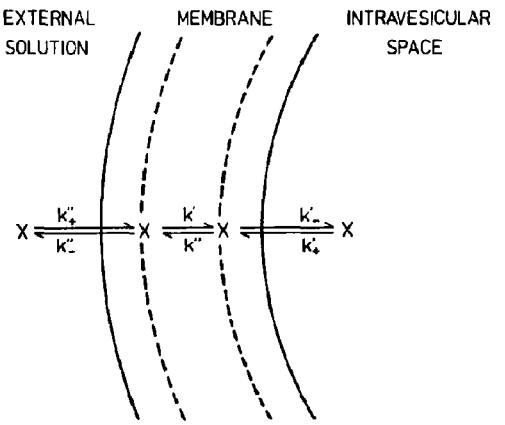

Fig. 1. Mechanism of diffusion of a hydrophobic ion, X, across a vesicle membrane.

is merely used as a convenient mathematical idea in order to set a limit to the number of ions which can bind to a single vesicle. This is based on the fact that saturation of lipid membranes by hydrophobic ions has been frequently observed [2,20,24,27–39]. As well as a limited number of binding sites, electrostatic effects [27,38,40,41] may also be a possible cause of saturation. These will be taken into account later.

Before proceeding let us make the following definitions:

$n_0 \equiv$ number of binding sites in the external monolayer per vesicle

$n_i \equiv$ number of binding sites in the internal monolayer per vesicle

$N_0 = c_x^0/c_v^* \equiv$ number of ions in the extravesicular solution per vesicle

$N_i = c_x^i V_i L \equiv$ number of ions in the intravesicular solution per vesicle

$r_0 \equiv$ number of external binding sites occupied per vesicle

$r_i \equiv$ number of internal binding sites occupied per vesicle

The quantities c_x^0 and c_x^i refer to the molar concentrations of ion in the external and internal solution, respectively, V_i being the volume of the intravesicular space of a single vesicle. The total vesicle concentration is denoted by c_v^* and L is Avogadro's constant.

The rates of change of the numbers of ions in each of the four possible aqueous or lipid environments are described by the following series of coupled differential equations.

$$\frac{dN_0}{dt} = -n_0 k''_+ N_0 c_v^* + (k''_+ N_0 c_v^* + k''_-) r_0 \qquad (1)$$

$$\frac{dr_0}{dt} = n_0 k''_+ N_0 c_v^* - (k''_+ N_0 c_v^* + k''_-) r_0 + k'' \left(\frac{n_0 - r_0}{n_i}\right) r_i - k' \left(\frac{n_i - r_i}{n_i}\right) r_0 \qquad (2)$$

$$\frac{dr_i}{dt} = n_i k'_+ \frac{N_i}{LV_i} - \left(k'_+ \frac{N_i}{LV_i} + k'_-\right) r_i + k' \left(\frac{n_i - r_i}{n_0}\right) r_0 - k'' \left(\frac{n_0 - r_0}{n_0}\right) r_i \qquad (3)$$

$$\frac{dN_i}{dt} = -n_i k'_+ \frac{N_i}{LV_i} + \left(k'_+ \frac{N_i}{LV_i} + k'_-\right) r_i \qquad (4)$$

The rate constants k''_+, k'_+, k''_-, k'_-, k'' and k' are as defined in Fig. 1, where the binding and dissociation rate constants refer to the interaction with a binding site rather than a vesicle as a whole. The derivation of eqs. (1)–(4) has been described previously [24]. The only difference between these equations and the ones previously presented, eqs. (25)–(28) in ref. [24], is that here the possible effect of saturation on the translocation rate has been included. This has been done according to Ketterer et al. [2] by multiplying the terms describing the translocation steps by the probability that a free site is available in the opposite monolayer. Thus, the probability that a given binding site in the internal layer is free is given by $1 - r_i/n_i$, and the probability that a given binding site in the external layer is free is given by $1 - r_0/n_0$. At large excesses of binding sites over ions these probabilities approach unity and eqs. (1)–(4) then reduce to equations of identical form to those presented in ref. [24]. Integration of eqs. (1)–(4) allows the calculation of the numbers of the various species at any point in time. The calculation can be simplified, however, i.e., one differential equation can be omitted, by utilizing the mass conservation law,

$$\frac{c_x^*}{c_v^*} = N_0 + r_0 + r_i + N_i \qquad (5)$$

where c_x^* is the total ion concentration.

Before the time course of diffusion can be calculated, however, electrostatic effects must be taken into account. As stated in the introduction, because of the charge of the ions electrical potential gradients will be created across the membrane by the diffusion process. As diffusion proceeds and the concentrations of ions in each of the four environments changes, the electrical potential gradients are also altered. The consequence of this is that some of the rate constants in eqs. (1)–(4) may become time-dependent, because their magnitudes are likely to depend on the instantaneous value of the electrical potential difference between the two ion environments that the reaction separates.

First let us consider the binding and dissociation steps (steps (a) and (c)). The binding of an ion to or its dissociation from the vesicle membrane can be further divided into the following two steps:

$$X + V \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} XV_{EC} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} XV \tag{6}$$

where X and V represent the ion and the vesicle, respectively. The first step involves the diffusion of the ion to the vesicle surface and the formation of an encounter complex, XV_{EC}. The second step involves the incorporation of the ion into the vesicle membrane, resulting in a bound ion, XV. The rate constants here refer to binding to or dissociation from a vesicle as a whole, and V refers to a vesicle with initially all binding sites free. Because XV_{EC} is an intermediate state which will rapidly be converted into XV, in this case we can apply the steady-state approximation [42–44] to the concentration of XV_{EC}, i.e., $d[XV_{EC}]/dt = 0$, which leads to the following equation for the overall rate of the binding reaction:

$$\frac{d[XV]}{dt} = \frac{k_1 k_2}{k_{-1} + k_2}[X][V] - \frac{k_{-1} k_{-2}}{k_{-1} + k_2}[XV] \tag{7}$$

Experimentally it has been found in many cases [23,42,45–50] that the association of small hydrophobic ions or molecules to lipid membranes is a diffusion-controlled reaction. In terms of the two step binding reaction (6), this means that $k_2 \gg k_{-1}$. Thus, in the case of diffusion-controlled formation of XV eq. (7) reduces to,

$$\frac{d[XV]}{dt} = k_1[X][V] - \frac{k_{-1}}{K_2}[XV] \tag{8}$$

where $K_2 = k_2/k_{-2}$ is an equilibrium constant describing the incorporation step. If the equation is now compared to the corresponding reaction scheme written in terms of binding sites (scheme (4) in ref. [24]), it can be seen that the rate constants are related by,

$$k_1 = nk_+ \tag{9}$$

$$k_{-1}/K_2 = k_- \tag{10}$$

where n, k_+ and k_- refer to the values for either the external or internal monolayer, depending on which is being considered. At this stage we can introduce the electrical potential dependence of the binding reaction. This is done by considering the variation of the equilibrium constant, K_2, with the electrical boundary potential at the surface of the vesicle. Thus, making use of the Boltzmann relation [41,51–54] one can show that

$$K_2 = K_2^i \exp\left(-\frac{zFU_b}{RT}\right) \tag{11}$$

where K_2^i is the value of K_2 in the absence of a boundary potential, z is the valence of the ion, F is Faraday's constant, R is the ideal gas constant, T is the absolute temperature and U_b is the potential difference between the position in the membrane where the ion binds and the membrane-solution interface. Substituting eq. (11) into eq. (10) for K_2 yields that the dissociation rate constant, k_-, in the general case of any value of U_b is given by

$$k_- = k_-^i \exp\left(\frac{zFU_b}{RT}\right) \tag{12}$$

where k_-^i refers to the value of k_- in the absence of a boundary potential. The consequence of diffusion control is, thus, that the total electrical potential dependence of the binding process can be explained by the variation of the dissociation rate constant alone. The binding rate constant, k_+, as shown in eq. (9), is independent of the magnitude of the boundary potential. Because there are two membrane-solution interfaces whose boundary potentials will not necessarily be equal, there are rate constants describing dissociation from each of the interfaces, which are given by:

$$k_-'' = k_-^i \exp\left(\frac{zFU''}{RT}\right) \tag{13}$$

$$k_-' = k_-^i \exp\left(-\frac{zFU'}{RT}\right) \tag{14}$$

where U'' and U' are the extravesicular and intravesicular boundary potentials, respectively.

The boundary potentials are taken to include the diffuse double layer region adjacent to the membrane [41]. They are defined in the same direction across the vesicle membrane, i.e., inside minus outside, which accounts for the difference in sign of the two exponential terms [24,41].

Now let us consider the electrical potential dependence of the translocation step (step b)). If one assumes a symmetrical energy barrier to translocation in the absence of an electrical potential difference, then according to activated rate theory the effect of a potential difference on the rate constants in each direction can be described [24] by the following two equations.

$$k' = k_0 \exp\left(-\frac{zFU_i}{2RT}\right) \tag{15}$$

$$k'' = k_0 \exp\left(\frac{zFU_i}{2RT}\right) \tag{16}$$

where U_i is the potential difference in the membrane interior between the ions bound to the inner and outer lipid monolayers (inside minus outside), and k_0 is the translocation rate constant at zero voltage.

The electrical potential dependence of all the rate constants appearing in eqs. (1)–(4) have now been defined. However, as diffusion across the membrane proceeds the values of the electrical potential differences will be continually changing. Therefore, we still require a method to calculate the values of U'', U' and U_i at any point in time. In order to do this we apply the three-capacitor model of the lipid membrane [24,41,55–57]. The basis of this model is that the hydrophobic ions are assumed to bind to adsorption planes located symmetrically with respect to the centre of the membrane (see Fig. 1). Thus, the membrane can be considered to be analogous to a system of three capacitors in series, where C_0 is the electrical capacitance of the two regions between the adsorption planes and the adjacent aqueous solution and C_i that between the two adsorption planes in the membrane interior. The capacitance C_0 is taken to include the capacitance of the electrical double layer of the adjacent aqueous solution. If one assumes a homogeneous electric field in each of the membrane regions it can be shown that the electrical potential differences U'', U' and U_i are related to the charge densities of ions in the external and internal adsorption planes, q_i'' and q_i', according to Gauss' law for the electric field discontinuity between two dielectric media [58,59] by

$$q_i' = C_i U_i - C_0 U' \tag{17}$$

$$q_i'' = C_0 U'' - C_i U_i \tag{18}$$

The assumption of homogeneous electric fields which has been made in the derivation of eqs. (17) and (18) is equivalent to ignoring the membrane curvature of the vesicle. The limits of the validity of this approximation will be discussed later. For three parallel-plate capacitors in series the total membrane capacitance, C_m, is related to C_0 and C_i by

$$\frac{1}{C_m} = \frac{2}{C_0} + \frac{1}{C_i} \tag{19}$$

and the total membrane potential, U_m, is simply given by the sum of the potential differences of the individual regions, i.e.,

$$U_m = U' + U'' + U_i \tag{20}$$

As has been shown previously [41], combination of eqs. (17)–(20) allows one to derive the following three equations relating the electrical potential differences U'', U' and U_i to the concentrations of ions bound to the external and internal monolayers:

$$U'' = \alpha U_m - \frac{z\alpha^2 e_0}{C_m}\left(\frac{r_0}{A_0} - \frac{r_i}{A_i}\right) + \frac{z\alpha e_0}{C_m}\frac{r_0}{A_0} \tag{21}$$

$$U' = \alpha U_m - \frac{z\alpha^2 e_0}{C_m}\left(\frac{r_0}{A_0} - \frac{r_i}{A_i}\right) - \frac{z\alpha e_0}{C_m}\frac{r_i}{A_i} \tag{22}$$

$$U_i = (1-2\alpha)U_m - \frac{z\alpha(1-2\alpha)e_0}{C_m}\left(\frac{r_0}{A_0} - \frac{r_i}{A_i}\right) \tag{23}$$

where $\alpha \equiv C_m/C_0$, A_0 and A_i are the external and internal surface areas of a vesicle, respectively, and e_0 is the elementary charge. The quan-

tity α may have values varying between zero and 0.5. Its significance has been discussed in more detail elsewhere [41]. It is an approximate measure of the distance of the adsorption planes from the adjacent membrane-solution interface. Differentiating eqs. (21)–(23) with respect to time one obtains:

$$\frac{dU''}{dt} = \alpha\frac{dU_m}{dt} - \frac{z\alpha^2 e_0}{C_m}\left(\frac{1}{A_0}\frac{dr_0}{dt} - \frac{1}{A_i}\frac{dr_i}{dt}\right) + \frac{z\alpha e_0}{C_m}\cdot\frac{1}{A_0}\frac{dr_0}{dt} \quad (24)$$

$$\frac{dU'}{dt} = \alpha\frac{dU_m}{dt} - \frac{z\alpha^2 e_0}{C_m}\left(\frac{1}{A_0}\frac{dr_0}{dt} - \frac{1}{A_i}\frac{dr_i}{dt}\right) - \frac{z\alpha e_0}{C_m}\cdot\frac{1}{A_i}\frac{dr_i}{dt} \quad (25)$$

$$\frac{dU_i}{dt} = (1-2\alpha)\frac{dU_m}{dt} - \frac{z\alpha(1-2\alpha)e_0}{C_m} \times\left(\frac{1}{A_0}\frac{dr_0}{dt} - \frac{1}{A_i}\frac{dr_i}{dt}\right) \quad (26)$$

Now that ratc cquations have been found for U'', U' and U_i, it just remains to find an equation for the rate of change of the total membrane potential, U_m, which appears in the first terms on the right hand side of eqs. (24)–(26). In a previous publication [24] the total membrane potential was estimated from the total ion current across the membrane and the total membrane capacitance according to the following equation,

$$U_m = \frac{z\Delta N_i e_0}{C_m A} \quad (27)$$

where A is the surface area at the centre of the membrane. However, eq. (27) assumes that the electrical potentials of ions in the extravesicular and intravesicular solution always change by equal and opposite amounts, which for hydrophobic ions is not true because of the high concentrations of ions which bind to the membrane. Therefore, eq. (27) is strictly only valid for hydrophilic ions which have negligible membrane-bound concentrations. At equilibrium the membrane potential can be calculated according to the Nernst equation, which can be written in the form:

$$U_m = \frac{RT}{zF}\ln\frac{V_i L c^\circ_x}{N_i} \quad (28)$$

At any other point in time, however, a different expression is required.

Let us consider the electrochemical potentials, μ, of ions in the external and internal solution. For the external solution,

$$\mu'' = \mu_0 + RT\ \ln\ c^\circ_x + \mu''_{i-I} \quad (29)$$

For the internal solution,

$$\mu' = \mu_0 + RT\ \ln\ c^i_x + \mu'_{i-I} \quad (30)$$

where μ_0 is the standard chemical potential on the molar scale and μ_{i-I} represents the chemical potential due to electrostatic ion–ion interactions in the solution. Assuming that the electrical potential in solution is completely due to ionic interactions, μ''_{i-I} and u'_{i-I} are given by

$$\mu''_{i-I} = zF\Psi'' \quad (31)$$

$$\mu'_{i-I} = zF\Psi' \quad (32)$$

where Ψ'' and Ψ' are the electrical potentials in the external and internal solutions, respectively. Subtracting eq. (31) from eq. (32) and rearranging, yields that the total membrane potential is given by

$$U_m = \frac{\mu'_{i-I} - \mu''_{i-I}}{zF} = \frac{\Delta\mu}{zF} \quad (33)$$

This equation is valid at any point in time. Thus, differentiating with respect to time yields

$$\frac{dU_m}{dt} = \frac{1}{zF}\frac{d\ \Delta\mu}{dt} \quad (34)$$

The problem is now how to calculate the chemical potential of an ion due to ion–ion interactions. However, this problem has been treated for electrolyte solutions by Debye and Hückel [60–62]. The difference here is that we have two electrolyte solutions separated by a membrane and, becuase of ion diffusion, electrical neutrality does not hold in either of the solutions. Under

these circumstances the linearized Poisson–Boltzmann equation obtains the form:

$$\frac{1}{r^2}\frac{\mathrm{d}}{\mathrm{d}r}\left(r^2\frac{\mathrm{d}\Psi_r}{\mathrm{d}r}\right) = -\frac{e_0}{\varepsilon_0\varepsilon}\sum\nu_i z_i + \frac{e_0^2\Psi_r}{\varepsilon_0\varepsilon kT}\sum\nu_i z_i^2 \tag{35}$$

Where Ψ_r is the electrical potential at a distance, r, from the reference ion, ε_0 is the permittivity of a vacuum, ε is the dielectric constant of the aqueous medium, k is Boltzmann's constant and ν_i is the number of an ionic species per unit volume in the bulk solution. In the classical Debye–Hückel theory the first term on the right hand side of eq. (35) equals zero because of electroneutrality. Solving this differential equation yields that Ψ_r is given by

$$\Psi_r = \frac{ze_0}{4\pi\varepsilon_0\varepsilon}\cdot\frac{\mathrm{e}^{\kappa a}}{1+\kappa a}\cdot\frac{e^{-\kappa r}}{r} - \frac{b}{\kappa^2} \tag{36}$$

where

$$\kappa = \left(\frac{e_0^2}{\varepsilon_0\varepsilon kT}\sum\nu_i z_i^2\right)^{1/2} \tag{37}$$

$$b = -\frac{e_0}{\varepsilon_0\varepsilon}\sum\nu_i z_i \tag{38}$$

The hydrophobic ion is here assumed to be spherical with a radius, a. The constants of integration which appear in the solution of eq. (35) have been evaluated by considering boundary conditions and the special case of electroneutrality as in the case of the classical Debye–Hückel theory. The quantity κ is the reciprocal Debye length. The electrical potential, Ψ_i, due to an ion itself is simply given by

$$\Psi_i = \frac{1}{4\pi\varepsilon_0\varepsilon}\cdot\frac{ze_0}{r} \tag{39}$$

Therefore, subtracting eq. (39) from eq. (36) yields that the potential, Ψ_{cloud}, due to the ionic cloud surrounding a reference ion is

$$\Psi_{cloud} = \frac{ze_0}{4\pi\varepsilon_0\varepsilon r}\cdot\left(\frac{\mathrm{e}^{\kappa a}}{1+\kappa a}\cdot\frac{\mathrm{e}^{-\kappa r}}{r} - 1\right) - \frac{b}{\kappa^2} \tag{40}$$

At $r = a$, i.e., at the surface of the ion, the potential becomes

$$\Psi_{cloud}^{r=a} = -\frac{ze_0}{4\pi\varepsilon_0\varepsilon}\cdot\left(\frac{\kappa}{1+\kappa a}\right) - \frac{b}{\kappa^2} \tag{41}$$

According to the Born theory the potential energy of a reference ion due to electrostatic interactions with its ion cloud can be calculated from the work done, W, in charging the ion from a hypothetical uncharged state to its final charge of ze_0. Thus,

$$W = \int_0^{ze_0}\Psi_{cloud}^{r=a}\,\mathrm{d}q \tag{42}$$

Substituting eq. (41) into eq. (42) for $\Psi_{cloud}^{r=a}$, carrying out the integration, and then multiplying by Avogadro's constant, L, shows that the chemical potential of an ion at its surface due to electrostatic interactions with all other ions in the solution is given by

$$\mu_{i-I} = \frac{L}{\varepsilon_0\varepsilon}\frac{ze_0^2}{\kappa^2}\sum\nu_i z_i - \frac{L}{8\pi\varepsilon_0\varepsilon}\frac{z^2e_0^2\kappa}{1+\kappa a} \tag{43}$$

The summation in the first term of eq. (43) is equal to zero for all other ions in the solution apart from the diffusing ion and its counterion. Thus, for the external solution the excess charge comes from the excess of counterions over hydrophobic ions left behind, whereas for the internal solution it comes from the hydrophobic ions which traverse the membrane. Accordingly, it can be shown from eq. (43) that the chemical potentials of the hydrophobic ions in the external and internal solutions due to ion–ion interactions are given by,

$$\mu''_{i-I} = -\frac{F^2z^2}{\varepsilon_0\varepsilon\kappa_0^2}c_v^*(r_0 + r_i + N_i) - \frac{L}{8\pi\varepsilon_0\varepsilon}\frac{z^2e_0^2\kappa_0}{1+\kappa_0 a} \tag{44}$$

$$\mu'_{i-I} = \frac{F^2z^2}{\varepsilon_0\varepsilon\kappa_i^2}\frac{N_i}{V_iL} - \frac{L}{8\pi\varepsilon_0\varepsilon}\frac{z^2e_0^2\kappa_i}{1+\kappa_i a} \tag{45}$$

where κ_0 and κ_i represent the reciprocal Debye lengths in the external and internal solutions,

respectively. Now subtracting eq. (44) from eq. (45) yields,

$$\Delta\mu = \frac{F^2z^2}{\varepsilon_0\varepsilon}\left[\frac{N_i}{V_iL}\frac{1}{\kappa_i^2} + \frac{c_v^*}{\kappa_0^2}(r_0 + r_i + N_i)\right] - \frac{Lz^2e_0^2}{8\pi\varepsilon_0\varepsilon}\left[\frac{\kappa_i}{1+\kappa_i a} - \frac{\kappa_0}{1+\kappa_0 a}\right] \quad (46)$$

Differentiating eq. (46) with respect to time yields,

$$\frac{\mathrm{d}\,\Delta\mu}{\mathrm{d}t} = \frac{F^2z^2}{\varepsilon_0\varepsilon}\left[\frac{1}{V_iL}\frac{1}{\kappa_i^2}\frac{\mathrm{d}N_i}{\mathrm{d}t} - \frac{N_i}{V_iL}\frac{2}{\kappa_i^3}\frac{\mathrm{d}\kappa_i}{\mathrm{d}t} - \frac{2c_v^*}{\kappa_0^2}\frac{\mathrm{d}\kappa_0}{\mathrm{d}t}(r_0 + r_i + N_i) + \frac{c_v^*}{\kappa_0^2}\left(\frac{\mathrm{d}r_0}{\mathrm{d}t} + \frac{\mathrm{d}r_i}{\mathrm{d}t} + \frac{\mathrm{d}N_i}{\mathrm{d}t}\right)\right] - \frac{Lz^2e_0^2}{8\pi\varepsilon_0\varepsilon}\left[\frac{1}{(1+\kappa_i a)^2}\frac{\mathrm{d}\kappa_i}{\mathrm{d}t} - \frac{1}{(1+\kappa_0 a)^2}\frac{\mathrm{d}\kappa_0}{\mathrm{d}t}\right] \quad (47)$$

Equations for the rate of change of the reciprocal Debye lengths can be derived from the definition given in eq. (37). This is best achieved, as in the case of $\Delta\mu$, if the ionic strength due to inert electrolyte is treated separately from that of the hydrophobic ions and its counterions. Thus, one obtains

$$\kappa_0 = e_o\sqrt{\frac{L}{\varepsilon_0\varepsilon kT}}\left[\left(c_x^*\frac{1+m}{m} - c_v^*(r_0 + r_i + N_i)\right)z^2 + 2I\right]^{1/2} \quad (48)$$

$$\kappa_i = e_0\sqrt{\frac{L}{\varepsilon_0\varepsilon kT}}\left(\frac{N_i}{V_iL}z^2 + 2I\right)^{1/2} \quad (49)$$

where m is the number of counterions per hydrophobic ion, i.e., for a 1:1 electrolyte $m = 1$, and $I = \frac{1}{2}\Sigma c_i z_i^2$ represents the ionic strength due to inert electrolyte alone, excluding the hydrophobic ions and their counterions. Differentiating eq. (48) and eq. (49) with respect to time gives

$$\frac{\mathrm{d}\kappa_0}{\mathrm{d}t} = -\frac{e_0z^2c_v^*}{2}\left(\frac{L}{\varepsilon_0\varepsilon kT}\right)^{1/2} \times\left(\frac{\mathrm{d}r_0}{\mathrm{d}t} + \frac{\mathrm{d}r_i}{\mathrm{d}t} + \frac{\mathrm{d}N_i}{\mathrm{d}t}\right) \times\left[\left(c_m^*\frac{1+m}{m} - c_v^*(r_0 + r_i + N_i)\right)z^2 + 2I\right]^{-1/2} \quad (50)$$

$$\frac{\mathrm{d}\kappa_i}{\mathrm{d}t} = \frac{e_0z^2}{2V_iL}\left(\frac{L}{\varepsilon_0\varepsilon kT}\right)^{1/2}\left(\frac{N_iz^2}{V_iL} + 2I\right)^{-1/2}\frac{\mathrm{d}N_i}{\mathrm{d}t} \quad (51)$$

We now have in eqs. (2)–(4), (24)–(26), (34), (47), (50) and (51) a complete set of coupled differential equations, which by integration will allow the calculation of r_0, r_i, N_i, U'', U', U_i, U_m, $\Delta\mu$, κ_0 and κ_i at any point in time. The changing values of the rate constants can be simultaneously calculated using eqs. (13)–(16) and N_0 can be determined from the mass conservation law, eq. (5).

3. Computer simulations

Using the equations derived in the previous section the time course of ion binding to the vesicle membrane and translocation across the membrane can be simulated. This has been carried out using a Digital VAX computer. The coupled differential rate equations were integrated using backward differentation formulae [63] within a subroutine of the Numerical Algorithms Group (NAG) Fortran Library. The initial values of r_0, r_i and N_i before any ions have bound were set to zero. The initial values of κ_0, κ_i, $\Delta\mu$, U_m, U_i, U'' and U' have been calculated according to eqs. (48), (49), (46), (33), (23), (21) and (22), respectively. Stopped-flow kinetic transients of a hundred seconds can thus be simulated within a few seconds of computer time.

In a previous publication [24] the variation with time of the concentration of ions in each of the four environments as well as of the intramembrane potential, U_i, has been presented. Therefore, here we shall restrict ourselves to a discussion of the effect of the total membrane potential, U_m, and the boundary potentials, U'' and U', on the diffusions process.

In Table 1 infinite time values of the potentials U_m, U_i, U'' and U' are listed as a function of the ionic strength of inert electrolyte. The values have been obtained by simulation until no further changes occurred; in this case after 100 seconds. The concentrations of hydrophobic ions and vesicles as well as all other values of parameters necessary for the calculation have been chosen based upon previously published experimental data [23,24]. Also listed in Table 1 are values of the equilibrium Nernst potential, U_m^N, which have been calculated from the infinite time values of c_x^o and N_i according to eq. (28). At low ionic strengths it can be seen that there is good agreement between U_m^N and U_m^∞. However, as the ionic strength increases a discrepancy between the two values arises, and at ionic strengths greater than or equal to 15 mM the magnitude of the theoretical Nernst potential is significantly greater than that of U_m^∞. Such a breakdown of the theory at increasing ionic strength is also observed for the Debye–Hückel theory of electrolyte solutions. In this case attempts have been made to extend the range of application of the theory by using the full non-linearized Poisson–Boltzmann equation [62]. If one looks again at Table 1, however, it is also apparent that the values of U_m^∞ and U_m^N decrease dramatically at increasing ionic strength. At an ionic strength of 0.015 mM the values of U_m^∞ and U_m^N are already almost negligible in comparison to the values of U_i^∞, U_∞'' and U_∞'. Therefore, it would appear that as long as the ionic strength is approximately two orders of magnitude greater than the total concentration of the diffusing ion the membrane potential produced by diffusion is so small that it has no effect on the diffusion rate and can thus be neglected. The time variation of the total membrane potential at three different values of the ionic strength is shown in Fig. 2. In each case the kinetic behaviour predicted is very similar, but the final value of the membrane potential is markedly decreased at increasing ionic strength. The two kinetic phases are due to the initial rapid binding of hydrophobic ions to the membrane surface, followed by the slower diffusion across the membrane to the inner lipid monolayer.

The reason for the dramatic reduction in the membrane potential at increasing ionic strength can be attributed to the screening of the electrostatic potentials of the diffusing ion on each side of the membrane by the inert electrolyte. In contrast, the values of U_i^∞, U_∞'' and U_∞' are relatively insensitive to the ionic strength, because the inert electrolyte is assumed not to be able to penetrate the membrane and hence no screening can occur. Changes are merely observed at low ionic strengths because of the coupling of the boundary and intramembrane potentials to the total membrane potential.

Table 1

Effect of the ionic strength, I, on the infinite time values of the potentials U_m, U_i, U'' and U' and on the equilibrium Nernst potential, U_m^N. The values of all the parameters used, except for the ionic strength, are as given in Fig. 3

I (mM)	U_m^N (mV)	U_m^∞ (mV)	U_i^∞ (mV)	U_∞'' (mV)	U_∞' (mV)
150×10^{-6}	−4.70	−4.70	−2.96	−29.6	27.9
150×10^{-5}	−0.613	−0.613	−1.50	−29.1	30.0
150×10^{-4}	−0.0636	−0.0635	−1.30	−29.0	30.2
150×10^{-3}	-6.71×10^{-3}	-6.39×10^{-3}	−1.28	−29.0	30.3
150×10^{-2}	-7.38×10^{-4}	-6.48×10^{-4}	−1.28	−29.0	30.3
150×10^{-1}	-2.03×10^{-4}	-6.65×10^{-5}	−1.28	−29.0	30.3
150	-1.44×10^{-4}	-6.82×10^{-6}	−1.28	−29.0	30.3

Now let us consider the time course of the boundary potentials, U'' and U' (see Fig. 3). The external boundary potential, U'', shows a rapid decrease from zero volts to its equilibrium value of -29.0 mV in a single kinetic phase. This is due to the rapid binding of hydrophobic ions to the external vesicle monolayer. The internal boundary potential, U', on the other hand shows an initial rapid increase which is followed by a slower increase. The reason for the two phases is again the coupling of the boundary and intramembrane potentials to the total membrane potential. For example, at high ionic strengths such that the total membrane potential is zero, a sudden change in U'' must be accompanied by an equal and opposite change in the sum of U_i and U'. The initial rapid rate of change of U'', U' and U_i under these conditions can easily be derived by setting dU_m/dt and dr_i/dt equal to zero in eqs. (24)–(26). Thus,

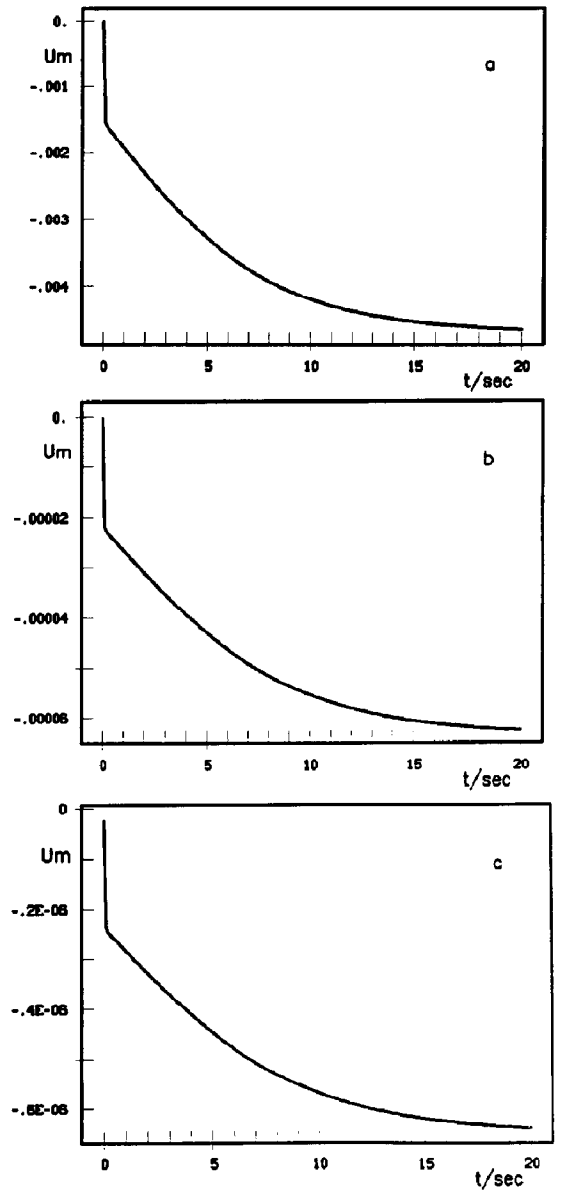

Fig. 2. Computer simulations of the variation with time of the total membrane potential, U_m, at ionic strengths of (a) 150×10^{-6} mM, (b) 150×10^{-4} mM, and (c) 150×10^{-2} mM. The values of all the parameters used, except for the ionic strength, are as given in Fig. 3.

$$\left(\frac{dU''}{dt}\right)_{t=0} = \frac{z\alpha e_0}{C_m A_0}(1-\alpha)\left(\frac{dr_0}{dt}\right)_{t=0} \tag{52}$$

$$\left(\frac{dU'}{dt}\right)_{t=0} = -\frac{z\alpha^2 e_0}{C_m A_0}\left(\frac{dr_0}{dt}\right)_{t=0} \tag{53}$$

$$\left(\frac{dU_i}{dt}\right)_{t=0} = -\frac{z\alpha(1-2\alpha)e_0}{C_m A_0}\left(\frac{dr_0}{dt}\right)_{t=0} \tag{54}$$

As discussed in the theory section, if binding of the ion to the vesicle is diffusion controlled, the boundary potential dependence of the rates of the binding and dissociation steps can be explained by the variation of the dissociation rate constants, k''_- and k'_-. For the calculation we have assumed that in the absence of boundary potentials both rate constants equal 4.9 s^{-1}. Using the infinite time values of U'' and U' given in Table 1 we can now calculate from eqs. (13) and (14) the final values of both rate constants. Thus, in the limit of high ionic strength it is found that k''_- increases to a value of 15.3 s^{-1} and k'_- to 17.7 s^{-1}. These values indicate that as time goes on and the boundary potentials build up, binding of ions to the external monolayer is hindered and dissociation of ions from the internal monolayer is facilited.

Experimentally it has been found that the time scale of the binding step is sometimes much faster than the translocation step [20,23,24]. Under such conditions the binding step can be considered independently, since it is always in equilibrium on the time scale of translocation. If one further

considers the situation of a large excess of vesicle binding sites over hydrophobic ions, it has been shown previously [24] that the reciprocal relaxation time is given by

$$\frac{1}{\tau} = n_0 k''_+ c_v^* + k''_- \qquad (55)$$

Since the effect of the boundary potential is to increase the value of k''_- this would mean that the relaxation would become non-exponential. As time goes on the relaxation rate would increase. Whether or not non-exponential kinetics are actually observed, however, depends on the relative magnitudes of $n_0 k''_+ c_v^*$ and k''_-.

4. Discussion

A theoretical description of the diffusion of hydrophobic ions across lipid vesicle membranes has been presented in which the electrical potential dependence of the rates of each step of the diffusion process are taken into account. The theory is based on a combination of the activated rate theory of Eyring, the three-capacitor model of the lipid membrane and an extension of the Debye-Hückel theory.

Finally, let us consider the limitations of the theory. First of all it has been found by comparing the calculated equilibrium Nernst potential

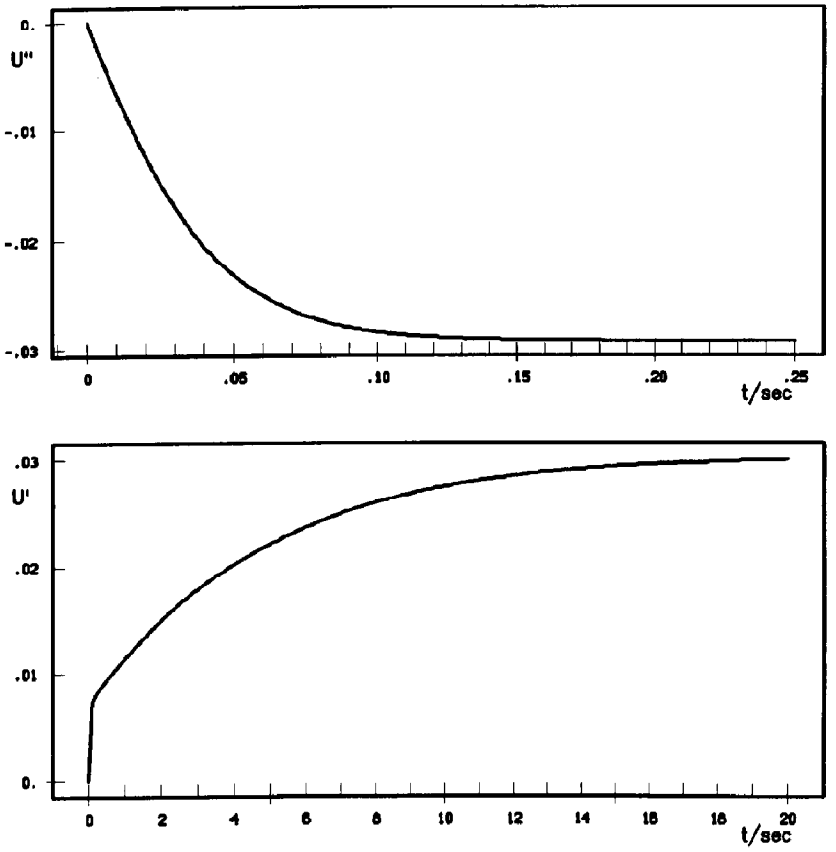

Fig. 3. Computer simulations of the variation with time of the external boundary potential, U'', (volts), and of the internal boundary potential, U', (volts). Values of the parameters used are: $c_x^* = 7.5\times10^{-8}$ M, $c_v^* = 1.4\times10^{-10}$ M, $k''_+ = k'_+ = 1.6\times10^{7}$ M^{-1} s^{-1}, $k^i_- = 4.9$ s^{-1}, $k_0 = 0.1$ s^{-1}, $n_0 = 3.86\times10^3$ external sites per vesicle, $n_i = 3.44\times10^3$ internal sites per vesicle, $V_i \times L = 8.27\times10^4$ dm^3 mol^{-1}, $C_m = 1\times10^{-2}$ F m^{-2}, $A_0 = 1.63\times10^{-14}$ m^2, $A_i = 1.29\times10^{-14}$ m^2, $\alpha = 0.2$, $z = -1$, $T = 295$ K, $a = 0.6\times10^{-9}$ m, $m = 1$ and $I = 150$ mM.

with the infinite time membrane potential that the complete theory in only strictly applicable at relatively low ionic strengths. However, it has also been found that at high ionic strengths negligible total membrane potentials are produced. Therefore, under these conditions a significantly simplified form of the theory can be applied, in which U_m is held constant and dU_m/dt equals zero. The equations (34), (47), (50) and (51) are then no longer necessary.

The calculation of the intramembrane potential and the boundary potentials is based on the three-capacitor model of the membrane. As stated in the theory section, this model assumes homogeneous electric fields in each membrane region and therefore neglects membrane curvature. Whether or not this is a valid approximation depends on the size of the vesicles being used. Let us consider a unilamellar vesicle of internal radius R_i and external radius R_0 with an electric field extending radially outward from the internal surface. Since the number of field lines leaving the internal interface must equal the number arriving at the external interface, the difference in electric field strength at the two surfaces just depends on the difference in reciprocal internal and external surface areas. Accordingly, it can be shown that the relative field inhomogeneity is given by

$$\frac{\Delta E}{E_i} = \left(\frac{R_i^2}{R_0^2} - 1 \right) \tag{56}$$

where E_i is the field strength at the internal interface and ΔE is the change in field strength on crossing the membrane from the internal to the external interface. In Table 2 the percentage field inhomogeneities are listed for vesicles of varying radii. A constant membrane thickness of 4 nm has been assumed. Since in the three-capacitor model the membrane is divided into three separate regions, the inhomogeneity in each region could be significantly less than the values listed in Table 2. Nevertheless, the values represent upper limits, which enable one to assess whether or not the membrane curvature can be neglected Small unilamellar vessicles prepared by the sonication method, for example, have typical external radii in the order of 10 nm [64]. In this case the three-capacitor model as presented here is probably not a very good approximation, since percentage field inhomogeneities of up to fifty percent could be expected. In the case of large unilamellar vesicles such as those prepared by detergent dialysis, extrusion or other methods, however, typical radii are ≥ 30 nm [64], so that here the model could be applied without significant error.

Table 2

Effect of the vesicle radius on the percentage field inhomogeneity, $(\Delta E/E_i) \times 100$

R_i (nm)	R_0 (nm)	$\frac{\Delta E}{E_i} \times 100$
10	14	−49.0
20	24	−30.6
30	34	−22.1
40	44	−17.4
50	54	−14.3
70	74	−10.5
100	104	−7.5
200	204	−3.9

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