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An adsorption isotherm for the interaction of membrane-permeable hydrophobic ions with lipid vesicles

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Abstract

An adsorption isotherm is presented which describes the association of membrane-permeable hydrophobic ions with lipid vesicles. The theory is based upon the Langmuir adsorption isotherm, but it has been significantly extended to take into account diffusion of ions across the membrane and dissociation into the intravesicular space as well as electrical effects due to the build up of boundary potentials within the membrane. The boundary potentials are calculated according to the three-capacitor model of the lipid membrane. In contrast to the Gouy–Chapman theory, wich is only applicable when the charged group is located directly at the membrane-solution interface, the theory presented here allows the charge to be located at any position within the membrane.

Keywords: Membrane-permeable hydrophobic ions; Lipid vesicles; Modified Langmuir Adsorption isotherm; Three capacitor model

1. Introduction

In recent times the interaction of hydrophobic ions (in particular anionic and cationic fluorescent dyes) with lipid membranes has been extensively investigated because of the possibility of their use as probes of membrane potential [1–6]. An important step in the understanding of the mechanism of probe response and for the comparison of different probe molecules is a quantitative description of their association with the membrane. In the case of amphipathic molecules, which have a localized charge at one end and a non-polar tail, it has been found [7–9] that the adsorption process can often be successfully described by applying the Gouy–Chapman theory [9–11], which relates the surface charge density of adsorbed ions, σ, to the electrostatic potential, ψ, in the aqueous phase at the surface of the membrane according to the following equation:

$$\frac{A\sigma}{\sqrt{c}} = \sinh\left(\frac{me_0\psi}{2kT}\right) \tag{1}$$

where for a symmetrical $m:m$ electrolyte solution c is the bulk aqueous electrolyte molar concentration, m is the absolute value of the valence of the electrolyte, e_0 is the electronic charge, k is Boltzmann's constant and T is the absolute temperature. $A = 1/(8L\varepsilon\varepsilon_0 kT)^{1/2}$ where L is Avo-

gadro's constant, ε is the dielectric constant of the aqueous solution and ε_0 is the permittivity of free space. The Gouy–Chapman theory has also been found useful in describing the association of charged peptides, such as melittin [12,13], with the lipid membrane. It is, however, only applicable to cases where the charged group is located directly at the membrane-solution interface [8].

In the case of hydrophobic ions with a delocalized charge, theoretical considerations of the various contributions to the potential energy of the ions have predicted [14–19] that they are likely to bind at a position a certain distance within the membrane itself. This is supported by experimental measurements of the zeta potential of lipid vesicles with bound hydrophobic ions and comparison with the effect of the adsorbed ions on the conductance in bilayer experiments [17,20]. These experiments have shown that even under conditions where negligible zeta potentials are observed, significant effects of the ions on membrane conductance are apparent which cannot be explained by the Gouy–Chapman theory of the diffuse double layer. If the ions are actually located within the membrane this observation can be easily explained, since the ions would produce, rather than a surface potential, a boundary potential which would drop partly over the dielectric medium of the membrane and partly over the diffuse double layer region adjacent to the membrane [9,17,18]. Thus, the Gouy–Chapman theory may not provide a good description of the association of hydrophobic ions with delocalized charges with the membrane. An alternative approach is to consider the slab of membrane between the position of the charge and the interface as a capacitor, in which case the magnitude of the boundary potential could be simply calculated [8], if one had an estimate of the dielectric constant of the boundary region and its thickness. From kinetic experiments [15,16,21,22], however, it has been found that such hydrophobic ions are able to easily diffuse across the membrane and bind to the internal lipid monolayer. Thus, both external and internal boundary regions of the membrane must be considered. The consideration of only a single capacitor is inadequate. In the present paper, therefore, the three-capacitor model of the lipid membrane [23,24,17,22] is used in order to estimate the external and internal boundary potentials produced by the adsorbed ions.

A frequently observed phenomenon [7,15,19, 22,25–36] in the interaction of hydrophobic ions with lipid membranes is that of saturation, i.e. there is a limit to the amount of the ions which can be incorporated into the membrane. There are two possible mechanisms by which this may come about. Firstly, there may be an inherent limit to the number of ions which can be bound, due to deformation of the lipid bilayer by the included ions, so that the incorporation process eventually becomes thermodynamically unfavourable. Secondly, the saturation could have an electrostatic origin. The binding of ions to the membrane causes a boundary potential to build up, which through electrostatic repulsion hinders the binding of further ions. Both of these mechanisms are likely to contribute to a greater or less extent depending upon the nature of the adsorbing ion and its position within the bilayer. In the present paper both of these possibilities are taken into account via a combination of the Langmuir adsorption isotherm, which contains a limit to the number of adsorbed ions in the absence of electrostatic effects, and the Boltzmann relation, which introduces a decrease in the magnitude of the apparent association constant of the ion for the membrane as the boundary potentials increase.

The Langmuir adsorption isotherm has previously been applied by various authors [7,22,25, 26,28–37] to the description of the binding of hydrophobic ions to lipid membranes. The data obtained are often shown in the form of a Scatchard plot [7,25,26,28,34,35], from the slope of which one can determine the association constant. In the case of some ions curvature of the Scatchard plots has been observed [7,34]. Some authors [34] have interpreted this as indicating the presence of two classes of binding sites in the membrane with different association constants for the ion. An alternative explanation [7,36] is that the magnitude of the apparent association constant varies as more ion binds due to electrostatic effects, as discussed earlier. Although both of these explanations are possible and cannot be

distinguished, since hydrophobic ions are charged, electrostatic effects must be present, and they should be considered as a cause of curvature before the concept of different classes of binding sites is introduced.

2. Theory

Let us consider the association of a hydrophobic ion with a lipid vesicle, which has a limit to the number of ions it can accept. The Langmuir adsorption isotherm relates the amount of bound ion to the equilibrium concentration of free ion as follows:

$$\bar{r} = \frac{nK\bar{c}}{1 + K\bar{c}} \tag{2}$$

where $\bar{r}$ is the equilibrium concentration of bound ion per unit concentration of vesicles, n is the total number of ions which could be bound per vesicle in the absence of electrostatic effects, $\bar{c}$ is the equilibrium concentration of free ions at the membrane-solution interface and K is the microscopic apparent association constant for the binding of an ion to a single site in the membrane [22,38]. In the case of the binding of a charged species, such as a hydrophobic ion, however, the value of the apparent association constant would be expected to vary with the concentration of bound ion due to the build-up of a boundary potential. Thus, making use of the Boltzmann relation [8–10,18] one can show that

$$K = K_i \exp\left(-\frac{zFU_b}{RT}\right) \tag{3}$$

where K_i is the microscopic association constant 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 is located and the membrane-solution interface.

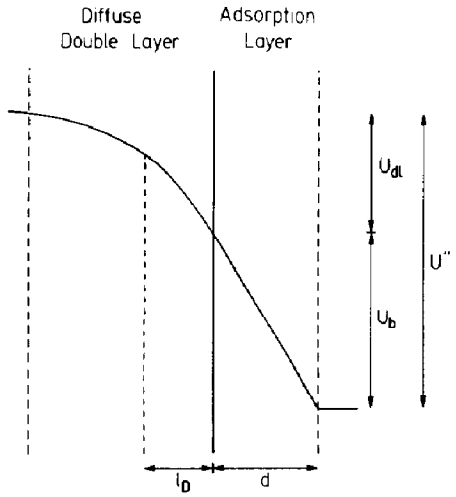

Fig. 1. Total boundary potential, U'', at the membrane-solution interface. U'' is made up of the potential drop across the adsorption layer in the membrane, U_b, and the potential drop in the diffuse double layer region of the adjacent solution, U_{dl}. The hydrophobic ions are assumed to bind to adsorption planes at a distance d from the external and internal surfaces of the vesicle. The thickness of the diffuse double layer is defined by the Debye length, l_D.

Combination of eqs. (2) and (3) yields a modified Langmuir adsorption isotherm:

$$\bar{r} = \frac{nK_i \exp\left(\frac{FU_b}{RT}\right)\bar{c}}{1 + K_i \exp\left(\frac{FU_b}{RT}\right)\bar{c}} \tag{4}$$

Equation (4) is written for the case of a singly negatively charged ion, $z = -1$. For simplicity all of the following derivation will also refer to a singly charged anion, but the equations can easily be converted to the case of a cation by introducing a negative sign in the Boltzmann factor. In eqs. (2) and (4) $\bar{c}$ refers to the ion concentration at the surface of the membrane. At high ion concentrations, however, $\bar{c}$ may differ significantly from the bulk ion concentration due to the build-up of a surface potential, U_{dl}, on the membrane, which extends into the diffuse double layer region of the solution adjacent of the membrane

(see Fig. 1). However, if one treats the potential differences in the diffuse double layer region and the adsorption layer together as a total boundary potential, U'', such that

$$U'' = U_b + U_{dl} \tag{5}$$

then eq. (4) can be rewritten in terms of the bulk ion concentration in the external aqueous solution, c_x^o. Thus,

$$\bar{r} = \frac{nK_i c_x^o \exp\left(\frac{FU''}{RT}\right)}{1 + K_i c_x^o \exp\left(\frac{FU''}{RT}\right)} \tag{6}$$

Equation (6) is, however, only strictly valid for the case where the ion is membrane-impermeable. If the ion is able to diffuse across the membrane to positions near the intravesicular space (see Fig. 6 in ref. [22]), eq. (6) must be rewritten in terms of the externally bound ions alone. Before proceeding further let us make the following definitions:

$n_o \equiv$ maximum possible number of ions which can bind to the external monolayer of a single vesicle

$n_i \equiv$ maximum possible number of ions which can bind to the internal monolayer of a single vesicle

$N_o = c_x^o / c_V^* \equiv$ 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

$r_o \equiv$ number of ions bound within the external monolayer per vesicle

$r_i \equiv$ number of ions bound within the internal monolayer per vesicle

$A_0 \equiv$ external surface area of a vesicle

$A_i \equiv$ internal surface area of a vesicle.

The quantities c_x^o 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 single vesicle. The total vesicle concentration is denoted by c_V^* and L is Avogrado's constant. If one assumes that the total number of ions which can be bound to a vesicle in the absence of electrostatic effects is proportional to the membrane surface area, then n_o and n_i can be related to n by:

$$n_o = \left(\frac{A}{A_o + A_i}\right) n; \qquad n_i = \left(\frac{A_i}{A_o + A_i}\right) n \tag{7}$$

Thus, in terms of externally and internally bound ions eq. (6) can be rewritten in the form of the following two equations,

$$r_o = \frac{\left(\frac{A_o}{A_o + A_i}\right) nK_i c_x^o \exp\left(\frac{FU''}{RT}\right)}{1 + K_i c_x^o \exp\left(\frac{FU''}{RT}\right)} \tag{8}$$

$$r_i = \frac{\left(\frac{A_i}{A_o + A_i}\right) nK_i c_x^i \exp\left(-\frac{FU'}{RT}\right)}{1 + K_i c_x^i \exp\left(-\frac{FU'}{RT}\right)} \tag{9}$$

where U'' and U' are the extravesicular and intravesicular total boundary potentials, respectively. The difference in the sign of the Boltzmann factors in eqs. (8) and (9) is due to the definition of the boundary potential, i.e., inside minus outside [22]. Thus, a positive external boundary potential will favour association of anions from the external solution, but a positive internal boundary potential will hinder association of anions from the intravesicular solution.

Combining eqs. (8) and (9) one arrives at the following complete adsorption isotherm for the total amount of bound ions:

$$r = \frac{\left(\frac{A_o}{A_o + A_i}\right) nK_i c_x^o \exp\left(\frac{FU''}{RT}\right)}{1 + K_i c_x^o \exp\left(\frac{FU''}{RT}\right)} + \frac{\left(\frac{A_i}{A_o + A_i}\right) nK_i c_x^i \exp\left(-\frac{FU'}{RT}\right)}{1 + K_i c_x^i \exp\left(-\frac{FU'}{RT}\right)} \tag{10}$$

Before one can use eq. (10) to simulate data or to fit experimental data, however, one needs to know how the magnitudes of U'' and U' depend upon r.

In order to obtain the magnitudes of U'' and U' let us apply the three-capacitor model of the lipid membrane [17,22–24]. The hydrophobic ions are assumed to bind to adsorption planes located symmetrically with respect to the centre of the membrane (see Fig. 8 in ref. [22]). Thus, the membrane can be considered to be analogous to a system of three capacitors in series, where C_o 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_o is taken to include the capacitance of the electrical double layer of the adjacent aqueous solution. As previously reported [22], on the basis of this model the external and internal boundary potentials, U'' and U', are related to the charge densities of ions in the external and internal adsorption planes, q_i'' and q_i', according to the Gauss law by

$$q_i' = C_i U_i - C_o U' \tag{11}$$

$$q_i'' = C_o U'' - C_i U_i \tag{12}$$

where U_i is the potential difference in the membrane interior between the two adsorption planes. For the three capacitors in series the total membrane capacitance, C_m, is related to C_o and C_i by:

$$\frac{1}{C_m} = \frac{2}{C_o} + \frac{1}{C_i} \tag{13}$$

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

$$U_m = U' + U'' + U_i \tag{14}$$

Combination of eqs. (11)–(14) then leads to

$$U_i = \frac{U_m - \frac{1}{C_o}(q_i'' - q_i')}{\left(1 + \frac{2C_i}{C_o}\right)} \tag{15}$$

It should be noted that the equation previously reported for U_i (eq. 43 in ref. [22]) is only valid for circumstances under which the capacitance of the adsorption layer, C_o, greatly exceeds that of the membrane interior region, C_i. This would be the case if the dielectric constant of the adsorption layer is much greater than that of the membrane interior. It would also be the case if the thickness of the adsorption layer is small in comparison to the distance within the membrane between the adsorption planes. Since for hydrophobic ions both of these conditions are likely to be met, the equation for U_i given in ref. [22] is probably a good approximation.

An expression for the external boundary potential, U'', can now be obtained by combination of eqs. (12), (13) and (15). Similarly, an expression for the internal boundary potential, U', can be obtained by combination of eqs. (11), (13) and (15). Thus,

$$U'' = \frac{q_i''}{C_o} + \frac{C_m}{C_o - 2C_m}\left[\frac{U_m - \frac{1}{C_o}(q_i'' - q_i')}{\left(1 + \frac{2C_i}{C_o}\right)}\right] \tag{16}$$

$$U' = \frac{C_m}{C_o - 2C_m}\left[\frac{U_m - \frac{1}{C_o}(q_i'' - q_i')}{\left(1 + \frac{2C_i}{C_o}\right)}\right] - \frac{q_i'}{C_o} \tag{17}$$

The charge densities can be estimated from the number of ions bound to the internal and external monolayers and the internal and external surface areas of the vesicle, i.e.,

$$q_i'' = -\frac{e_0 r_o}{A_o}, \qquad q_i' = -\frac{e_0 r_i}{A_i} \tag{18}$$

Let us also define, as in the previous paper [22], the quantity $\alpha \equiv C_m/C_o$, whose value is such that $0 < \alpha \leq 0.5$. Substituting $C_m C_o/(C_o - 2C_m)$ for C_i from eq. (13) as well as C_m/α for C_o and the above expressions for q into eqs. (16) and (17) yields

$$U'' = \alpha U_m + \frac{\alpha^2 e_o}{C_m}\left(\frac{r_o}{A_o} - \frac{r_i}{A_i}\right) - \frac{\alpha e_o}{C_m}\frac{r_o}{A_o} \tag{19}$$

$$U' = \alpha U_m + \frac{\alpha^2 e_o}{C_m}\left(\frac{r_o}{A_o} - \frac{r_i}{A_i}\right) + \frac{\alpha e_o}{C_m}\frac{r_i}{A_i} \qquad (20)$$

According to the Nernst equation the total membrane potential, U_m, is given by:

$$U_m = -\frac{RT}{F}\ln\frac{V_i L c_x^\circ}{N_i} \qquad (21)$$

where A is the surface area at the centre of the membrane. Thus, substituting for U_m into eqs. (19) and (20) yields:

$$U'' = -\frac{RT}{F}\ln\frac{V_i L c_x^\circ}{N_i} + \frac{\alpha^2 e_o}{C_m}\left(\frac{r_o}{A_o} - \frac{r_i}{A_i}\right) - \frac{\alpha e_o}{C_m}\frac{r_o}{A_o} \qquad (22)$$

$$U' = -\frac{RT}{F}\ln\frac{V_i L c_x^\circ}{N_i} + \frac{\alpha^2 e_o}{C_m}\left(\frac{r_o}{A_o} - \frac{r_i}{A_i}\right) + \frac{\alpha e_o}{C_m}\frac{r_i}{A_i} \qquad (23)$$

In a similar way by using the definitions of α, q and U_m above as well as eq. (13) one can derive the following expression relating U_i to the concentrations of ion in the various positions from eq. (15),

$$U_i = -\frac{(1-2\alpha)RT}{F}\ln\frac{V_i L c_x^\circ}{N_i} + \frac{\alpha(1-2\alpha)e_o}{C_m}\left(\frac{r_o}{A_o} - \frac{r_i}{A_i}\right) \qquad (24)$$

Combination of eq. (22) with eq. (8) and eq. (23) with eq. (9) provides two simultaneous equations. However, there are four variables, namely N_o, N_i, r_o and r_i. Therefore, for the simulation or fitting of data a further two equations are necessary. Firstly, let us use the idea that at equilibrium the electrochemical potentials of ions bound to the external, μ_i'', and to the internal monolayer, μ_i', must be equal. For the external monolayer,

$$\mu_i'' = \mu_o' + RT\ln\frac{r_o}{A_o} + zF\Psi_i'' \qquad (25)$$

For the internal monolayer

$$\mu_i' = \mu_o' + RT\ln\frac{r_i}{A_i} + zF\Psi_i' \qquad (26)$$

where the electrochemical potentials per mole of ions are on a concentration scale of ions bound per unit area of membrane. The electrical potentials of ions located in the external and internal monolayers are denoted by Ψ_i'' and Ψ_i', respectively, and z represents the valence of the ion. If it is assumed that the chemical environment of an ion in the external monolayer is identical to that of one in the internal monolayer, i.e., $\mu_o'' = \mu_o'$, then equating eqs. (25) and (26) and substituting U_i for $(\Psi_i' - \Psi_i'')$ yields that for a monovalent anion ($z = 1$),

$$U_i = -\frac{RT}{F}\ln\frac{r_o A_i}{r_i A_o} \qquad (27)$$

Now equating the two expressions for U_i, eqs. (24) and (27), gives the following third simultaneous equation,

$$-\frac{(1-2\alpha)RT}{F}\ln\frac{V_i L c_x^\circ}{N_i} + \frac{\alpha(1-2\alpha)e_o}{C_m}\left(\frac{r_o}{A_o} - \frac{r_i}{A_i}\right) + \frac{RT}{F}\ln\frac{r_o A_i}{r_i A_o} = 0 \qquad (28)$$

The final simultaneous equation is simply obtained by making use of the mass conservation law. Thus,

$$\frac{c_x^*}{c_V^*} = N_o + r_o + r_i + N_i \qquad (29)$$

where c_x^* and c_V^* are the total concentrations of hydrophobic ion and vesicle, respectively. Solving of the set of eqs. (8), (9), (22), (23), (28) and (29) for given values of the parameters K_i, n and α is now sufficient to simulate data or fit data to the adsorption isotherm. For the purposes of the calculation it should be noted that c_x° and c_x^i are better substituted in all equations by $c_V^* N_o$ and $N_i/V_i L$, respectively, as given in the definitions earlier. The fitting of experimental data to obtain values of K_i, n and α can be carried out by using the least squares method, in which N_o, r_o, r_i and N_i are determined from the set of simultaneous eqs. (8), (9), (22), (23), (28) and (29).

3. Computer simulations

In the case of the simulation of experimental data the theoretical value of r of given values of K_i, n and α and given values of the total vesicle and hydrophobic ion concentrations can be calculated by solving the set of simultaneous equations presented in the previous section. The total free ion concentration, c_x, can be simply calculated according to the mass conservation law,

$$c_x = c_x^* - c_v^*(r_o + r_i) \qquad (30)$$

In Figs. 2–4 results of simulations are shown in which K_i, n and α are independently varied. The values of the parameters were chosen so as to be of the same order of magnitude as values previously experimentally determined [22]. The simulated data were calculated using a constant total vesicle concentration of 0.2 nM and a wide range of total hydrophobic ion concentrations, i.e., 1–1000 nM. The results are plotted in the form of Scatchard diagrams. The simulations were performed by solving the simultaneous equations using a modification of the Powell hybrid method [39] within a subroutine of the Numerical Algorithms Group (NAG) Fortran Library.

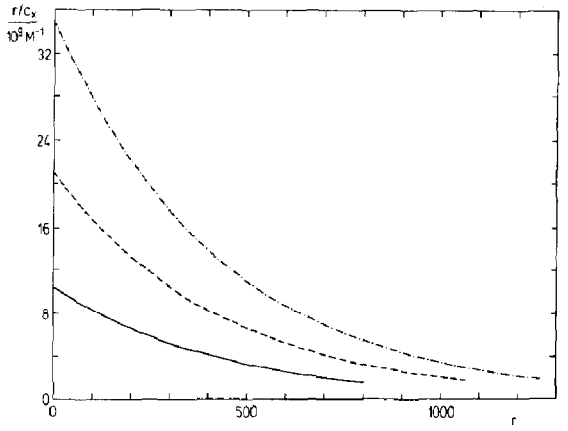

Fig. 2. Computer simulations in the form of Scatchard diagrams at a constant total vesicle concentration of 0.2 nM, $n = 7\times10^3$, $\alpha = 0.1$ and varying values of the association constant, K_i: (—•—) $K_i = 5\times10^6$ M^{-1}, (— — —) $K_i = 3\times10^6$ M^{-1}, and (———) $K_i = 1.5\times10^6$ M^{-1}. The values of the various constants used were: $A_o = 1.63\times10^{-14}$ m^2, $A = 1.29\times10^{-14}$ m^2, $V_i = 1.37\times10^{-19}$ dm^3, $C_m = 1\times10^{-2}$ F m^{-2} and $T = 295$ K.

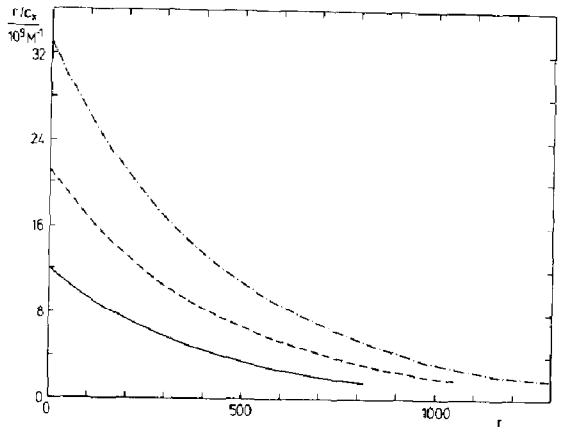

Fig. 3. Computer simulations in the form of Scatchard diagrams at a constant total vesicle concentration of 0.2 nM, $K_i = 3\times10^6$ M^{-1}, $\alpha = 0.1$ and varying values of the maximum number of ions which can be bound per vesicle, n: (—•—) $n = 11\times10^3$, (— — —) $n = 7\times10^3$, and (———) $n = 4\times10^3$. The values of the constants used are as given in Fig. 2.

From figs. 2–4 it can be seen that the magnitude of the y-intercept is dependent on the values of K_i and n, but is independent of the value of α. This can be easily understood, since at $r = 0$, i.e., finite dilution of the hydrophobic ions, no electrostatic potentials are present and hence the y-intercept is simply given, as in the case of a straightforward Scatchard plot [37], by nK_i.

The effect of varying α can be seen in Figs. 4 and 5. As α increases, the capacitance of the adsorption layer decreases and hence the magni-

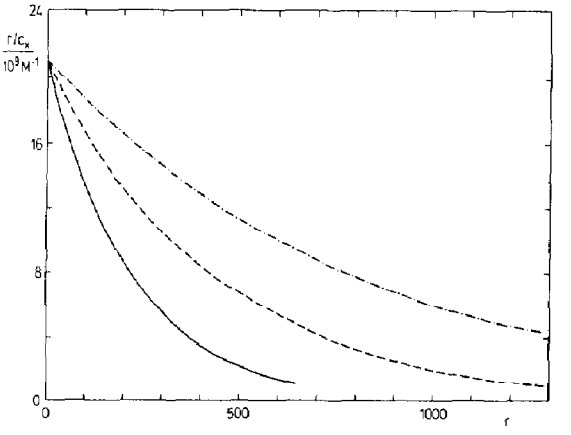

Fig. 4. Computer simulations in the form of Scatchard diagrams at a constant total vesicle concentration of 0.2 nM, $K_i = 3\times10^6$ M^{-1}, $n = 7\times10^3$, and varying values of α: (—•—) $\alpha = 0.05$, (— — —) $\alpha = 0.1$, and (———) $\alpha = 0.2$. The values of the constants used are as given in Fig. 2.

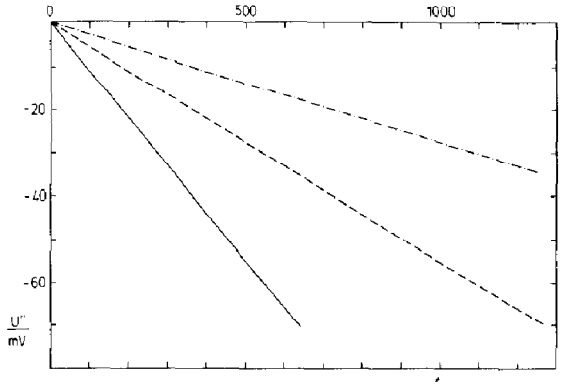

Fig. 5. Computer simulations of the variation of the external boundary potential, U'', with the total number of bound ions per vesicle, r, at varying values of α. The simulations refer to a constant total vesicle concentration of 0.2 nM, $K_i = 3 \times 10^6$ M^{-1}, and $n = 7 \times 10^3$ (--●—) $\alpha = 0.05$, (— — —) $\alpha = 0.1$, and (———) $\alpha = 0.2$. The values of the constants used are as given in Fig. 2. The internal boundary potential, U', shows similar behaviour to U'', but because of the definition the sign is reversed.

tude of the boundary potential produced by a given amount of adsorbed ion increases (see Fig. 5). As more ion binds the increased rate of change of the boundary potential is then responsible for the greater degree of curvature of the Scatchard plot at higher α values (see Fig. 4). The downward curvature of the Scatchard plots is typical of negative cooperativity in the binding process [37], i.e., the binding of one ion hinders the binding of further ions, in this case due to electrostatic repulsion.

4. Discussion

The simulations have shown that the adsorption isotherm presented here can adequately reproduce experimentally observed behaviour for the association of membrane-permeable hydrophobic ions with lipid vesicles without having to introduce the concept of different classes of binding sites with different affinities for the ions. It is, however, necessary for the fitting of experimental data to the adsorption isotherm that the Scatchard plot shows significant curvature, i.e., significant electrostatic effects should be evident. This is necessary so that a best fit value of the parameter α can be determined. If no curvature at all is apparent, then it may be assumed that the boundary potentials produced are negligible, in which case electrostatic effects can be ignored and the simple Langmuir adsorption isotherm could be applied, although the number of ions moving across the membrane and dissociating into the intravesicular space would still have to be taken into account. This could be done by using eqs. (8) and (9) of the theory section and setting $U'' = U' = 0$.

Let us now briefly consider the significance of the value of α. If the lipid membrane could be considered as a completely homogeneous dielectric medium, then the value of α would be approximately equal to the ratio of the distance of an adsorption plane in the membrane from its adjacent aqueous interface to the thickness of the membrane. Thus, α has a range of $0 < \alpha \leq 0.5$. If α has a very small value, this means that the charge of the ion is located close to the membrane-solution interface. Because of the high dielectric constant of water, only a small boundary potential would then be produced, which could be shielded by the presence of counter-ions in the adjacent solution. Thus, in this case electrostatic effects would be minimised. If, however, the value of α is closer to 0.5, the charge of the ion would be located further towards the centre of the membrane. In this case there are no counter-ions present and because of the low dielectric constant of the membrane, large boundary potentials would be expected to be produced and thus significant curvature of the Scatchard plot should be apparent.

Let us consider again the case where the charge is located directly at the membrane-solution interface. As introduced in the theory section, the boundary region is taken to include the region of the membrane between the adsorption plane and the membrane-solution interface as well as the adjacent diffuse double layer region (see Fig. 1). Thus, the external capacitance, C_o, can be considered to be made up of a capacitance due to the double layer, C_{dl}, and a capacitance due to the membrane, C_b. For two capacitors in series, the capacitances are then related by [17]:

$$\frac{1}{C_o} = \frac{1}{C_{dl}} + \frac{1}{C_b} \tag{31}$$

The capacitances of the two regions are given by:

$$C_{dl} = \frac{\varepsilon_o \varepsilon_{aq}}{l_D}, \qquad C_b = \frac{\varepsilon_o \varepsilon_b}{d} \tag{32}$$

where ε_o is the permittivity of a vacuum, ε_{aq} and ε_b are the dielectric constants of water and membrane in the adsorption layer, respectively, l_D is the Debye length and d is the thickness of the adsorption layer. If the charge of the ion is located directly at the interface, then d is infinitely small. Thus, C_b would be infinitely large, and according to eq. (31) the capacitance of the boundary region, C_o, would simply equal the capacitance of the electrical double layer, C_{dl}. The Debye length can be estimated from the following equation [40],

$$l_D = \frac{1}{F}\sqrt{\frac{\varepsilon_o \varepsilon_{aq} RT}{2I}} \tag{33}$$

where I is the ionic strength of the solution in mol m^{-3}. For an ionic strength of 250 mol m^{-3}, a temperature of 295 K and using a value of 80 for the dielectric constant of water, the Debye length can be estimated from eq. (33) to have a value of 0.61 nm, which according to eq. (32) corresponds to a capacitance of the double layer of 1.16×10^{-4} F cm^{-2}. Assuming a typical total membrane capacitance, C_m, of 1×10^{-6} F cm^{-2} [40], a value of α for these circumstances can then be estimated. Thus,

$$\alpha = C_m/C_o$$
$$= 1 \times 10^{-6}/1.16 \times 10^{-4} = 0.0086$$

At the particular ionic strength and temperature stated, this would therefore be the minimum possible theoretical value of α.

Because of the application of the three-capacitor model of the lipid membrane and the inclusion of the diffuse double layer regions with the external capacitors, the theory presented here should be applicable to the treatment of the association of hydrophobic ions to lipid vesicles irrespective of the position within the membrane that the ions bind. Thus, cases where the Gouy–Chapman theory has previously been successfully used should also be able to be described by the theory presented as well as situations where the Gouy–Chapman theory is not applicable. It is hoped in the near future to demonstrate the application of the theory on experimental data for the association of potential-sensitive dyes to lipid vesicles.

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