A VIRIAL EXPANSION FOR DISCRETE CHARGES BURIED IN A MEMBRANE

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ABSTRACT Recent experiments (1,2) have shown that hydrophobic ions adsorbed to lipid membranes repel each other significantly at densities as low as one charge every few tens of square nanometers. This paper shows how to calculate the mutual repulsion of a population of such ions, assumed to be discrete but free to diffuse laterally in the plane of the membrane. The results fall between those for uniformly smeared charges (the "three-capacitor" model) and those for discrete charges immobilized on a periodic lattice.

Calculations of the mutual electrostatic interactions between charges adsorbed on a lipid membrane are fundamentally important in understanding many biophysical phenomena. For a recent review, see McLaughlin (3). Previous calculations have assumed either that the ions can be treated as a uniformly smeared charge density (1,2,4–6) or that the ions sit in a perfect two-dimensional crystalline lattice, usually assumed to be hexagonal or square (1,7–9). However, adsorbed ions on real membranes are more likely to be discrete charges free to diffuse in two dimensions. Any calculations on such a population of charges require ensemble averaging over the available configurations, a procedure that at first glance seems formidably difficult. The purpose of this note is to show that the necessary statistical mechanics can in fact be easily handled by a virial expansion in the ion density, closely analogous with textbook derivations for a three-dimensional perfect gas. This general method is applied here to a particular problem, the equilibrium behavior of lipid-soluble ions buried in the lipid at a fixed depth beneath an interface with aqueous saline.

Molecules adsorbed to a hydrocarbon-water interface generally behave experimentally in the limit of low adsorption density as a two-dimensional perfect gas (10), with an equation of state $\Pi A = NkT$, where Π is the two-dimensional surface pressure, A the area of the system, N the number of particles adsorbed, k Boltzmann's constant, and T the absolute temperature. At somewhat higher densities of adsorbed molecules, one may expect that the pressure can be expressed as a power series in the density (N/A),

$$\Pi/kT = (N/A) + B_2(T)(N/A)^2 + B_3(T)(N/A)^3 + B_4(T)(N/A)^4 + \cdots \qquad (1)$$

and hope that the series converges quickly enough to be useful. This is a virial expansion for the surface pressure, exactly like that for a three-dimensional gas except that Π and A replace P and V. A standard statistical-mechanical argument (11, 12) shows that

$$B_2(T) = \frac{1}{2}\int_0^{\infty}\int_0^{2\pi}[1 - \exp(-U/kT)]r\,d\theta\,dr, \tag{2}$$

where U is the potential energy for two ions separated by a distance $r \cdot B_2(T)$ has the dimensions of area; for a repulsive potential it is a measure of the circular zone of repulsion surrounding any ion into which another ion can venture only at an energy cost of kT or greater. One can see intuitively that when each molecule's share of the total area, A/N, shrinks to the order of $B_2(T)$, the pressure will have to rise steeply, in agreement with Eq. 1. $B_3(T)$ is given by (12)

$$B_3(T) = \frac{1}{3}\int\int[1 - \exp(-U_{12}/kT)][1 - \exp(-U_{13}/kT)]\cdot [1 - \exp(-U_{23}/kT)]\,d\mathbf{r}_{12}\,d\mathbf{r}_{13}, \tag{3}$$

where U_{12} is the potential energy of ions 1 and 2 in a group of three, U_{13} and U_{23} similarly, and $\mathbf{r}_{23} = \mathbf{r}_{13} - \mathbf{r}_{12}$.

A useful property of all such integrals is that once any of the repulsion energies U grows large compared to kT, the precise magnitude of that U then becomes unimportant, because $1 - \exp(-U/kT)$ remains close to 1. Thus one may choose mathematically convenient expressions for U as long as (*a*) the approximation is accurate for $U \lesssim 3kT$; (*b*) where the true U is much larger than kT, the approximate form is also large.

Although the surface pressure is not a very familiar or accessible experimental variable on real biological membranes, the other thermodynamic properties of the system can be derived from it by standard identities. For example, use of the Gibbs equation (10) yields the adsorption isotherm:

$$a = K(N/A)\exp[2B_2(T)(N/A)]\exp[1.5\,B_3(T)(N/A)^2]\ldots \tag{4}$$

where a is the aqueous bulk phase activity of the surface-active ion, and K is an arbitrary partition coefficient.

Now we specialize to the case of charges buried in a lipid at a fixed distance l from an aqueous interface. This is a model for the adsorption of large hydrophobic ions (such as tetraphenylborate or dipicrylamide) or ion complexes (such as valinomycin-K^+) onto a lipid membrane, the subject of several recent experimental studies (1, 2). The far interface of the bilayer is neglected, because many calculations have shown (13) that the electrostatic field for an ion buried, say, 0.3 nm deep in a membrane is practically the same whether the membrane is 4 nm or infinitely thick. Finally, the assumption that all adsorption is localized to a single plane is a drastic simplification of the complex compromise between hydrophobicity and image forces which causes binding just inside the membrane.

We use cylindrical coordinator r, θ, Z with the interface as the r, θ plane. A real charge q at $z = -l$ gives rise in the aqueous phase to a polarization that acts like a fictitious image charge of $-\omega q$ at $z = +l$. The parameter ω lies between 0.95 and 1.0. The lower limit comes from considering the aqueous phase to be a simple dielectric of $\epsilon = 80$ with no salt or conductivity. The upper limit is obtained if the aqueous salt solution is treated as a perfect conductor throughout. A real charge with a neighbor at a lateral distance of r feels an electrostatic potential ϕ due to its own image, its neighbor, and its neighbor's image:

$$\phi(r) = [q/4\pi\epsilon_o\epsilon_m][-\omega_a(2l)^{-1} + r^{-1} - \omega_b(r^2 + 4l^2)^{-1/2}]. \tag{5}$$

ϵ_o is the permittivity of free space, ϵ_m the dielectric constant of the membrane. Here two separate values for ω have been written, because in a real salt solution the screening effect of the salt depends on distance. At large distances r from a source ion, any potential in the aqueous phase is attenuated by a factor of roughly $\exp(-\kappa r)$; $1/\kappa$ is the Debye length, 0.3 nm for the 1.0 M NaCl solutions used in references 1 and 2. Therefore, the boundary condition on Laplace's equation in the membrane phase for $r \gtrsim 3/\kappa = 0.9$ nm will be that the potential at the interface be practically zero, whereupon $\omega_b \cong 1.0$ yields the right image potential. Conversely, for potentials at or very near the source ion, the salt will have little shielding effect, so that $\omega_a \cong 0.95$ gives the appropriate image charge. The term in $\omega_a(2l)^{-1}$, which is independent of the presence of any other adsorbed ions, is part of the self-energy needed to create the isolated doublet consisting of a charge and its image. Although it is of great significance in determining the absolute partition coefficient K in Eq. 4, one can ignore it when considering the repulsion between doublets (assumed to be preformed), just as one also ignores the fixed electrostatic energy needed to assemble the real ion from electrons and nucleons. A further discussion of this point is presented in the Appendix. Thus the potential energy U to bring two doublets together from infinite lateral separation, where they do not interact, to a finite r where they do, is simply

$$U = q\phi(r) - q\phi(\infty) = [q^2/4\pi\epsilon_o\epsilon_m][r^{-1} - \omega_b(r^2 + 4l^2)^{-1/2}]. \tag{6}$$

At $T = 293$°K and $\epsilon_m = 2$, $(q^2/4\pi\epsilon_o\epsilon_m kT)$ is about 28.5 nm. Fig. 1 plots U/kT and $[1 - \exp(-U/kT)]$ vs. r for $l = 0.3$ nm and $\omega_b = 0.95$ and 1.0. Both potentials give $[1 - \exp(-U/kT)] \approx 1$ for $r \lesssim 1.1$ nm, but for $r \gtrsim 0.9$ nm, as discussed above, the Debye shielding makes $\omega_b = 1.0$ a good approximation.[1] The further approximation:

$$U = [q^2/4\pi\epsilon_o\epsilon_m][2l^2r^{-3}] \tag{7}$$

is now made. This expression is also plotted in Fig. 1. Clearly its values differ very little from those of Eq. 6 with $\omega_b = 1.0$; moreover, the difference is in the right direction to make it an even more realistic estimate of the true U. Andersen et al. (1) also chose

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[1] At lower concentrations of salt, the repulsion between adsorbed ions does seem to get somewhat stronger (6); the present model would need some modification to be applied to such experiments.

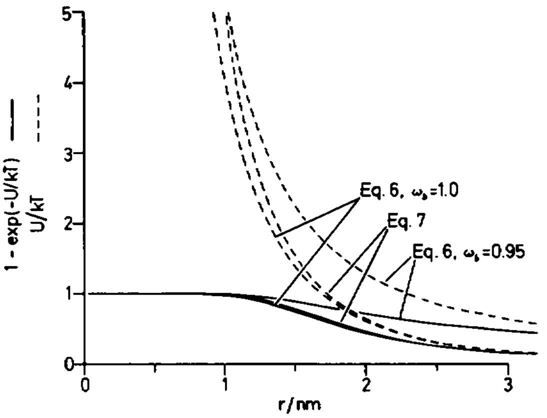

FIGURE 1 Normalized repulsion energies U/kT (----) from Eqs. 6 and 7 and corresponding functions $[1 - \exp(-U/kT)]$ (———) as functions of ion separation r. The parameter values assumed are $q = 1.602 \times 10^{-19}$ C, $T = 293°$K, $l = 0.3$ nm, $\epsilon_m = 2$. The true U should begin at small r like the curve marked $\omega_b = 0.95$ and converge at larger r to the curve $\omega_b = 1.0$. Fortuitously, the curve from Eq. 7 does just that.

this formulation for U in their hexagonal lattice model, though they considered it to be an intuitively obvious postulate not requiring detailed justification. We now substitute Eq. 7 into Eq. 2, integrate over θ, and define $q^2l^2/(2\pi\epsilon_0\epsilon_m kT) \equiv w^3$ and $w^3/r^3 \equiv x$. Then

$$B_2(T) = (\pi w^2/3) \int_0^\infty [1 - \exp(-x)]x^{-5/3}\mathrm{d}x = (\pi w^2/2)\Gamma(\tfrac{1}{3}) \approx 4.2\,w^2.$$

$B_3(T)$ may be evaluated numerically to be approximately 5.0 w^4. Its calculation requires the potential energy for a cluster of three ions to be the sum of the three pairwise repulsions. This is true if the water acts as a perfect conductor, or a simple dielectric, or if it supports a linearized Poisson-Boltzmann law. Superposition is only likely to fail when the charges are very close to each other. But as argued above, U_{ij} will then be large enough anyway for its detailed magnitude not to matter.

The virial expansion, Eq. 1, then becomes

$$\Pi/kT = (N/A) + 4.2\,w^2(N/A)^2 + 5.0\,w^4(N/A)^3 + \cdots. \tag{8}$$

For $q = 1.6 \times 10^{-19}$ C, $T = 293°$K, $l = 0.3$ nm, $\epsilon_m = 2$, w^2 is 2.98 (nm)^2. For these parameters, as long as there is <1 molecule per 20 (nm)^2, it seems reasonably safe to neglect the $B_3(T)$ term and the higher terms, which should be correspondingly smaller still.

For comparison with the "three-capacitor" model of references 1 and 2, if the Nq coulombs per area A were smeared into a uniform sheet of charge at the same depth l, the electrostatic energy would correspond (10) to an equation of state

$$\Pi = NkT/A + [q^2l/(2\epsilon_m\epsilon_o)](N/A)^2. \tag{9}$$

The quadratic term in this equation is $[q^2\pi^2/(2l\epsilon_m\epsilon_o kT)]^{1/3}/4.2 = 4.29$ times larger than the corresponding term in Eq. 8 for the above parameters. It should not be surprising that discrete mobile charges have a much weaker repulsion than smeared charges, as the former can arrange to avoid each other most of the time. However, if we imagine heating the membrane, at some temperature the particles should acquire a kinetic energy so much greater than the electrostatic potential energies discussed here that they can no longer be bothered to dodge each other. Thus, as $T \rightarrow \infty$, $kTB_2(T)$ should approach $q^2l/2\epsilon_m\epsilon_o$. This limit can be analytically verified from Eqs. 2 and 6; Eq. 7 is no longer a valid approximation here. Actual numerical integration of Eqs. 2 and 6 shows that $kTB_2(T)$ reaches 80% of its limiting smeared charge value at a temperature of 46,400°K.

Having compared the discrete mobile ion model to its infinite temperature limit, let us consider its relation to the other common model of ion adsorption, in which charges occupy fixed lattice points in a two-dimensional crystal. This model would seem most appropriate at very high densities and low absolute temperatures. Using Eq. 7, a hexagonal array of buried charges with images has an electrostatic potential energy just one-half that of a hexagonal array of real dipoles as given by Topping (14); the standard conversion (10) then yields the corresponding equation of state, $\Pi = NkT/A + 0.849[q^2l^2/(\epsilon_0\epsilon_m)](N/A)^{5/2}$. There is no easy way to prove that the discrete mobile ion model actually converges toward a crystalline array as $T \rightarrow 0$. At low temperatures, the higher order terms $B_3(T)$, $B_4(T)$, etc., become more and more significant in any virial expansion, making the power series increasingly unwieldy and perhaps even nonconvergent.

For a visual comparison, surface pressure Π vs. surface density (N/A) are plotted

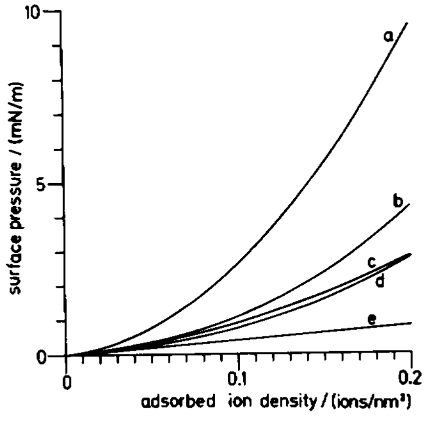

FIGURE 2

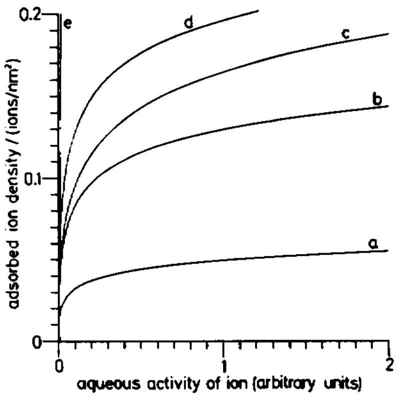

FIGURE 3

FIGURE 2 Surface pressures predicted by various models, as functions of adsorbed ion density. Parameter values are the same as in Fig. 1. (a) smeared charge model; (b) virial expansion up to the $B_3(T)$ term; (c) virial expansion up to the $B_2(T)$ term; (d) hexagonal array; (e) ideal gas without repulsion between particles.

FIGURE 3 Adsorption isotherms corresponding to the curves of Fig. 2.

in Fig. 2 for five different formulas: smeared charge, virial expansion truncated after $B_3(T)$, virial expansion truncated after $B_2(T)$, hexagonal array, and ideal gas without repulsion. The corresponding adsorption isotherms are shown in Fig. 3.

A future paper[2] will discuss the detailed application of the present method to explain the experimental data of Andersen et al. (1)

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APPENDIX

The dropping of the self-energy term $(-q^2\omega_a/4\pi\epsilon_o\epsilon_m)(2l)^{-1}$ in going from Eq. 5 to Eq. 6 may also be formally justified as follows: The virial coefficients are really just arithmetical combinations (see reference 12, pp. 262–264) of certain Z_Ns, which are ratios of partition functions Q_N:

$$Z_N(A, T) = N!\, Q_N(A, T) A^N [Q_1(A, T)]^{-N},$$

where

$$Q_N(A, T) = \Sigma \exp\,[-E_j(N, A)/kT],$$

E_j is the energy of the jth microstate with N particles, and the summations are taken over all such microstates. Now suppose that E_j is the sum of $E_j'(N, A)$, a function of the relative positions of the particles which goes to zero when all particles are infinitely remote from each other, plus N constant self-energy terms E_o, one for each particle, independent of the particle positions. Then for all j, $\exp[-E_j(N, A)/kT] = \exp(-NE_0/kT)[\exp(-E_j'(N, A)/kT]$, so $Q_N(A, T) = \exp(-NE_0/kT)\ \Sigma \exp\,[-E_j'(N, A)/kT]$. Since $(Q_1(A, T)]^{-N} = \exp(+NE_0/kT)\{\Sigma \exp\{-E_j'(1, A)/kT]\}^{-N}$, the terms with E_o cancel each other exactly in the expression for Z_N. Thus the virial coefficients and the equation of state depend only on the mutual interactions E_j', not at all on the particle self-energies E_o.

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