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ELECTROSTATIC FREE ENERGY AND SHIFT OF THE PHASE TRANSITION FOR CHARGED LIPID MEMBRANES

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For a charged membrane in an electrolyte solution the electrostatic free energy is derived treating the system as a diffuse double layer. The dependence of the free energy on external parameters like surface charge density and temperature is obtained and the physical basis discussed. As an application the charges are shown to exert an electrostatic surface pressure on the lipid chain packing which leads to a shift in the phase transition of lipid membranes. The results confirm the interpretation of experimental data as given by Träuble et al. in the accompanying paper.

1. Introduction

Recently it has been shown experimentally that the behaviour of lipid membranes depends strongly on electrical charges present on the membrane and in the bordering electrolyte solution [1,2]. For lipids with ionizable groups the transition temperature T_t (between the ordered and fluid state) can be varied by changing the ionic environment, e.g., pH and mono- or divalent metal ion concentrations. It was also found that connected with the thermal phase transition there is a release or adsorption of cations at the membrane surface. The inverse effect, triggering of the phase transition by cations at constant temperature, has been observed as well. Furthermore, lipids of different charge have been shown to undergo phase separation under favourable conditions [3].

The basis for a common understanding of these effects lies in the free energy contribution of the charges. Knowing its dependence on the external parameters like temperature and charge density it is then straightforward to derive expressions for the experimentally observed electrostatic effects, i.e., the electrostatic shift in the transition temperature T_t, in the bulk pH, or in the area f per lipid molecule in the membrane plane.

In the literature, there seems to be some confusion about the electrostatic free energy to be used for a correct interpretation of the experimental findings. It is the aim of this paper to present a careful derivation of the electrostatic free energy for the charged membrane–electrolyte system. The theoretical model used is the double-layer concept of Gouy and Chapman. The charges on the plane membrane surface, smeared out homogeneously, are balanced by an ion cloud in the bordering electrolyte. The internal electrostatic energy is calculated by integrating the energy density of the electric field over the system's volume (section 3). From this internal energy the electrostatic free energy can be derived in different ways, by an integration over temperature or by a charging process (section 4). The free energy is found as a function of the surface charge density which itself depends in a definite manner on the pH and metal ion concentrations in the electrolyte solution (section 5).

As an application of the theoretical results for the electrostatic free energy we investigate the electrostatic shift of the transition temperature T_t. The latter can be influenced electrostatically in a twofold way. For both mechanisms the shift of T_t is found proportional to the electrostatic "surface pressure", i.e., the variation of the electrostatic free energy with the area f per lipid molecule. A large body of experimental results on the electrostatic shift of T_t is presented in the accompanying paper by Träuble, Teubner, Woolley and Eibl [4] (TTWE). For a comparison of these results with theory they used the same expressions for the electrostatic free energy and surface pressure as derived in the present paper, and found excellent agreement.

In order to state the physical problem clear enough we start in section 2 with the description of the electrostatic shift of the transition temperature T_t, and proceed later to the derivation of the electrostatic free energy and surface pressure.

2. Electrostatic shift of T_t

Charges can act on the transition temperature in a twofold way. On the one hand, the area f per lipid molecule in the layer plane is increased by electrostatic repulsion. From theoretical investigations of the hydrocarbon chain system it is known that for larger area f the transition temperature is lowered [5,6]. The reason for this lies in the stabilizing effect of steric hindrance which becomes less effective for larger areas f thus lowering T_t. This area-induced lowering seems to have been observed in experiments where f was varied using lipids with polar heads of different size [7].

On the other hand, at the melting transition a loosening of the packing has been found, i.e., an abrupt increase Δf of the area f per lipid molecule [8,9]. This change Δf induces, at the phase transition, a change in the electrostatic energy of the system (which depends upon f). The electric energy favors the more loosely packed state, the transition into this state happens more easily, and the transition temperature T_t is lowered. This jump-induced shift therefore acts in the same direction as the area-induced shift.

These two effects will now be treated more exactly starting with the jump-induced shift. The phase transition will always be regarded as a first order transition between two phases A and B (in the actual case with the transition smeared out we extrapolate the interesting quantities from the pure A and B phases to the hypothetical T_t). The phase transition occurs when the free energies of the two phases are equal

$$F_A(T_t, f_A) = F_B(T_t, f_B) . \tag{1}$$

F denotes the free energy per lipid molecule. In this expression the states A and B are characterized solely by the area f per lipid molecule, all other properties differing between A and B are taken as functions of the area f.

Working at constant pressure we would have to use the free enthalpy G instead of F. For a discussion of electrostatic effects the difference between F^{el} and G^{el} is negligible [10].*

Using the symbol Δ for differences between states B and A, e.g.,

$$\Delta F(T, f_A, f_B) = F_B(T, f_B) - F_A(T, f_A) , \tag{2}$$

and remembering $F = U - TS$ we can write eq. (1) in the form

$$\Delta U - T_t \Delta S = 0 . \tag{3}$$

This relation has the following meaning: If B has a higher energy than A, then A is stable at low temperatures. If, on the other hand, B has a higher entropy than A clearly B will be stable at high temperatures. The transition between A and B occurs when the energy difference ΔU is compensated by the difference in thermal energy $T\Delta S$.

Eq. (3) may be used with some advantage instead of eq. (1) if ΔU and ΔS depend only weakly on temperature. Being interested in the charges' influence on T_t we split off the electrostatic parts in the energy and entropy, $\Delta U = \Delta U^* + \Delta U^{el}$ and $\Delta S = \Delta S^* + \Delta S^{el}$, to get

$$T_t = (\Delta U^* + \Delta U^{el})/(\Delta S^* + \Delta S^{el}) . \tag{4}$$

According to the signs of ΔU^{el} and ΔS^{el} and to which of both dominates, the transition temperature is shifted up or down by the electrostatic. This behavior is equally well represented, using $\Delta F^{el} = \Delta U^{el} - T_t \Delta S^{el}$, by

$$T_t = T_t^* + \Delta F^{el}/\Delta S^* , \tag{5}$$

with $T_t^* = \Delta U^*/\Delta S^*$. As discussed above the change ΔF^{el} of the electrostatic free energy at the phase transition can be traced back to the jump Δf in the packing density. Expanding in lowest order yields

$$\Delta F^{el} = (\partial F^{el}/\partial f)\,\Delta f , \tag{6}$$

and

$$T_t - T_t^* = \Delta T_t = (\Delta f/\Delta S^*)\,\partial F^{el}/\partial f . \tag{7}$$

This relation has already been used by Träuble and

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* This has to be noted on comparing with TTWE where the free enthalpy per mole is denoted by G, so that $G^{el} = LF^{el}$, L being the Avogadro number.

Eibl [1,2] in the same context and represents the jump-induced shift ΔT_t of the transition temperature.

The area-induced shift δT_t in principle also follows from the free energy of the system. The areas f_A and f_B entering eq. (1) are the equilibrium values of f in phase A or B obtained by minimizing the total free energy with respect to f

$$\partial F_i^*/\partial f_i + \partial F_i^{el}/\partial f_i = 0 , \tag{8}$$

for i = A or B. Due to the electrostatic term the areas in A and B differ from the areas f_i^* of the uncharged system, $\delta f_i = f_i - f_i^*$. The shift δf_i being small compared to f_i the first term in eq. (8) can be expanded in lowest order as

$$\partial F_i^*/\partial f = (\partial^2 F_i^*/\partial f^2)_{f_i^*}\, \delta f_i . \tag{9}$$

This relation inserted into eq. (8) leads to the result

$$\delta f_i = (1/\chi_i^*)\, \Pi_i^{el*} , \tag{10}$$

with the electrostatic surface pressure defined by

$$\Pi_i^{el*} = -(\partial F_i^{el}/\partial f)_{f_i^*} , \tag{11}$$

and the "stiffness" of the uncharged system by

$$\chi_i^* = (\partial^2 F_i^*/\partial f^2)_{f_i^*} , \tag{12}$$

(all derivatives taken at constant temperature). This shift of the area of a lipid molecule may be observed far away from the phase transition.

To evaluate the area-induced shift δT_t in lowest order we neglect the jump effects, ΔT_t or Δf, being small themselves. Expanding T_t^* in terms of $\delta f_A = \delta f_B = \delta f$ we obtain

$$T_t^* = T_t^{**} + (\partial T_t^{**}/\partial f^*)\, \delta f . \tag{13}$$

T_t^{**} is the transition temperature of the uncharged system. Inserting eq. (10) yields for the area-induced shift

$$T_t^* - T_t^{**} = \delta T_t = (1/\chi^*)(\partial T_t^{**}/\partial f^*)\, \Pi^{el*} . \tag{14}$$

We remember that $\partial T_t^{**}/\partial f^*$ was found to be negative [5–7].

Combining the two shift effects it is clear that the linear approximation used in the derivation of δT_t, eq. (14) has also to be introduced in eq. (7) for ΔT_t, i.e., the values of the uncharged system have to be inserted for Δf and ΔS^*. The result for the total charge-induced shift of the transition temperature is then obtained as

$$T_t - T_t^{**} = -\left(\frac{1}{\chi^*}\left|\frac{\partial T_t^{**}}{\partial f^*}\right| + \frac{\Delta f^*}{\Delta S^{**}}\right)\Pi^{el*} . \tag{15}$$

This shift is proportional to the electrostatic surface pressure, the proportionality factor being given solely by quantities of the uncharged system. These quantities are not very well known. They are all positive which shows explicitly that the two shift effects act in the same direction. Since Π^{el} will be shown to be positive the transition temperature T_t is lowered.

To estimate the relative importance of the two shift effects we compare in eq. (15) Δf^* with $\chi^{*-1}|\partial T_t^{**}/\partial f^*|\, \Delta S^{**}$, which is also of the dimension of an area per particle. Using for the stiffness [11] $\chi^* \approx 1$ dyn cm^{-1}/Å^2, for the variation of the transition temperature [6] $|\partial T_t^{**}/\partial f^*| \approx 1$ deg/Å^2, and for the entropy change [12] $\Delta S^{**} \approx 20$ cal/mole deg $\approx$ 15×10^{-16} erg/deg, we find

$$\frac{1}{\chi^*}\left|\frac{\partial T_t^{**}}{\partial f^*}\right| \Delta S^{**} \approx 15 \text{ Å}^2 .$$

This value entering δT_t has to be compared with Δf^* entering ΔT_t. Exact values of Δf^* are not known, one may expect [8,9]

$$\Delta f^* \approx 10 \text{ Å}^2 .$$

According to this estimate the area-induced and jump-induced shifts of T_t are of comparable magnitude. TTWE did not take into account the area-induced shift but showed that quantitative agreement between theory and experiment is obtained using the value $\Delta f^* = 15$ Å^2, for the lipid MPA under investigation. This value may then be regarded as an effective one representing both shift effects.

The electrostatic free energy and surface pressure entering eq. (15) will be investigated in the following sections.

3. The electrostatic internal energy

In this section the electrostatic internal energy of the charged lipid–electrolyte system will be derived. This is most easily done by integrating the energy density $(\epsilon/8\pi)E^2$ of the electrostatic field E over the volume, ϵ being the dielectric constant.

We describe our system electrostatically as a diffuse electric double layer, usually called after Gouy and

Chapman. The charges on a plane surface are balanced by mobile charges in the electrolyte solution on one side of it. The distribution of the mobile ions is governed by electrostatic interaction with the surface charges and by thermal motion. In directions parallel to the plane surface all charges are smeared out homogeneously and boundary effects are neglected. This model is directly applicable to lipid monolayers on an electrolyte surface. In the case of bilayers in a lipid–electrolyte solution further assumptions are necessary in order to apply the model: (i) the interaction between bilayers facing each other is negligible (this holds for highly dilute solutions), and (ii) the interaction between the two layers of the same bilayer is negligible (because of the small dielectric constant in the hydrocarbon region).

The Gouy–Chapman double layer has been discussed at length by Verwey and Overbeek [13]. Mainly for definitions we repeat the most important relations. The space charge density is given by

$$\rho(z) = \sum_i \nu_i e\, n_i(z)\,, \tag{16}$$

where $z > 0$ is the coordinate normal to the surface, e the positive unit charge, ν_i and n_i the valency and particle density of the ion kind i. The density n_i depends itself on the electrostatic potential $\psi(z)$ via the Boltzmann distribution

$$n_i(z) = n_i \exp\,[-\nu_i e\, \psi(z)/kT]\,, \tag{17}$$

T being the temperature and k Boltzmann's constant. For simplicity we specialize to a 1 : 1 electrolyte, i.e., $\nu_1 = -\nu_2 = 1$ and $n_1 = n_2 = n$ (because of electrical neutrality far away from the charged surface). Then the Poisson equation reads

$$\mathrm{d}^2\psi(z)/\mathrm{d}z^2 = (4\pi n e/\epsilon)\{\exp\,[e\,\psi(z)/kT] - \exp\,[-e\,\psi(z)/kT]\}\,. \tag{18}$$

The first integration of this equation can be done on multiplying both sides with $2\,\mathrm{d}\psi/\mathrm{d}z\,\mathrm{d}z$, the integration constant being fixed by the boundary conditions $\psi(\infty) = 0$ and $\mathrm{d}\psi(\infty)/\mathrm{d}z = 0$. The result is

$$\mathrm{d}\psi(z)/\mathrm{d}z = \{(8\pi n kT/\epsilon)\,\{\exp\,[e\,\psi(z)/2kT] + \exp\,[-e\,\psi(z)/2kT] - 2\}\}^{1/2}. \tag{19}$$

In our case of a symmetric electrolyte the square root can be evaluated and introducing

$$\kappa = (8\pi n e^2/\epsilon kT)^{1/2} \tag{20}$$

we find

$$\mathrm{d}\,\psi(z)/\mathrm{d}z = -2\kappa(kT/e)\sinh\,[e\,\psi(z)/2kT]\,. \tag{21}$$

The sign is chosen for physical reasons; in our case of negatively charged lipids the electric potential $\psi(z)$ is negative, then $\mathrm{d}\psi/\mathrm{d}z$ has to be positive. The solution $\psi(z)$ of this equation can be obtained analytically only in the limiting cases of low and high potential. For $|e\,\psi(z)/kT| \ll 1$ one gets

$$\psi(z) = \psi_0\, \mathrm{e}^{-\kappa z}\,, \tag{22}$$

leading to the interpretation of $\kappa^{-1} = \lambda_D$ as the screening length. For $|e\,\psi(z)/kT| \gg 1$ the potential $\psi(z)$ falls off more quickly than in eq. (22).

To derive the electrostatic energy U^{el} the relation eq. (21) for the electric field $E_z = -\partial\psi/\partial z$ is sufficient. We integrate the energy density of the electric field over the volume

$$U^{\mathrm{el}} = (\epsilon/8\pi)\int E_z^2\,\mathrm{d}V\,. \tag{23}$$

We normalize the extensive qualities U^{el} and V per lipid molecule in the surface. It is convenient to introduce also the surface energy density $u^{\mathrm{el}} = U^{\mathrm{el}}/f$, for which we find using eq. (21)

$$u^{\mathrm{el}} = \frac{\epsilon}{8\pi}\int_0^\infty \left(\frac{\mathrm{d}\psi}{\mathrm{d}z}\right)^2 \mathrm{d}z = \frac{\epsilon}{8\pi}\int_{\psi_0}^{0} \frac{\mathrm{d}\psi}{\mathrm{d}z}\,\mathrm{d}\psi = \frac{\epsilon\kappa}{2\pi}\left(\frac{kT}{e}\right)^2\left(\cosh\frac{e\psi_0}{2kT} - 1\right). \tag{24}$$

In our problem the quantity given beforehand is the surface charge density σ. It is determined by the charge q per lipid molecule (the relation between q and the ionic environment will be treated later in section 5) and the area f

$$\sigma = q/f\,. \tag{25}$$

On the other hand, there is a simple relation between σ and the surface potential ψ_0. Electroneutrality yields, using eq. (21)

$$\sigma = -\int_0^\infty \rho(z)\,\mathrm{d}z = -(\epsilon/4\pi)\,\mathrm{d}\psi_0/\mathrm{d}z = c\sinh(e\psi_0/2kT), \tag{26}$$

with the constant

$$c = (\epsilon\kappa/2\pi)kT/e\ , \tag{27}$$

defined as in TTWE. Eq. (26) allows to express u^{el}, eq. (24), directly in terms of σ,

$$u^{el} = \frac{\epsilon\kappa}{2\pi}\left(\frac{kT}{e}\right)^2 \left\{\left[1+\left(\frac{\sigma}{c}\right)^2\right]^{1/2} - 1\right\}\ . \tag{28}$$

This is our result used later. An important point to note is that the electrostatic energy is positive. Since the reference state of vanishing energy is uncharged, $\sigma = 0$, each state with a finite σ has a higher energy [*].

Concerning this reference state the following remark should be added. Vanishing surface charge corresponds to the case $T = 0$ where the counterions are not able to diffuse around thermally but are adsorbed at the surface. In Stern's picture of a double layer [14] the counterions approach the surface up to dimensions of the ionic radius, for $T = 0$, building up a molecular condenser. In the Gouy–Chapman model the ionic radius vanishes and with it $\sigma = 0$ for $T = 0$.

To gain more insight in the energy u^{el}, eqs. (24) and (28), let us consider the simple case $|e\psi_0/kT| \ll 1$, which means low potential and high temperature with a well established ion cloud. From eqs. (26) and (24) one finds

$$\sigma = (\epsilon\kappa/4\pi)\psi_0\ , \tag{29}$$

and

$$u^{el} = \tfrac{1}{4}\sigma\psi_0\ , \tag{30}$$

or

$$U^{el} = (\pi\lambda_D/\epsilon f)q^2 = (\epsilon f/16\pi\lambda_D)\psi_0^2\ . \tag{31}$$

This result corresponds to a condenser with the distance $d = \lambda_D/2$ between the plates. It is well known that by changing the geometry of a condenser its energy varies quite differently according to whether the charge or the voltage is held constant. In our case of constant charge q we find if the area f is varied

$$(\partial U^{el}/\partial f)_q = -(\pi\lambda_D/\epsilon f^2)q^2 < 0\ . \tag{32}$$

This means that for increasing f, i.e., separating the charges further, the energy decreases [‡]. This leads to a positive electrostatic surface pressure Π^{el}, if the internal energy is dominant in the free energy. In this case the simple picture of a condenser explains qualitatively the positive Π^{el}, and according to eq. (15) the decrease in the transition temperature induced by the charges. The other case where the electrostatic surface pressure arises mainly from the entropy term in the free energy will be discussed in the following section.

4. The electrostatic free energy

The free energy of our electrostatic model, the double layer of Gouy and Chapman, will be derived in two different ways. The first way is a straightforward application of the thermodynamic relation

$$U = \partial\beta F/\partial\beta\ , \tag{33}$$

$\beta = (kT)^{-1}$, which inverted yields the free energy F by an integration of the internal energy U over the inverse temperature β. This procedure has been applied by Debye and Hückel [10] in the problem of point-charge screening. A second way was introduced later by Debye [15] using a charging process. In connection with the Gouy–Chapman model this charging process has been discussed by Verwey and Overbeek [13]. It has also been applied to polyelectrolytes by Lifson and Katchalsky [16].

4.1. Temperature integration

The thermodynamic relation eq. (33) is incomplete insofar as the variables to be held constant at the differentiation have not been specified. In general these are the quantities by an external change of which work is done on the system, e.g., the volume and the particle number for an ideal gas. In our case of a charged inhomogeneous system the charges have to be held constant, not only their number or magnitude (q) but also the area per charge (f), in short the surface charge density σ (this will be generalized in section 5

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[*] In contrast to this the reference state of Debye and Hückel was the state with bare ions. Allowing an ion cloud to build up around each ion the counterions approach the originally bare ion and electrostatic energy is gained.

[‡] Considering point charges on a plane, cubic order with $f = r^2$, r the distance between nearest neighbours, we find $U^{el} \sim 1/r \sim 1/f^{1/2}$, which varies more weakly than $U^{el} \sim 1/f$ for a condenser. In a condenser the screening of charges by countercharges on the other plate becomes more effective with increasing f, leading to a faster decrease of U^{el}.

when dissociation effects are taken into account). The electrostatic free energy F^{el} is given then by an indefinite integral, $\beta F^{\mathrm{el}} = \int U^{\mathrm{el}}(\beta, \sigma)\,\mathrm{d}\beta$, or

$$F^{\mathrm{el}} = \beta^{-1} \int_0^{\beta} U^{\mathrm{el}}(\beta', \sigma)\,\mathrm{d}\beta' + \beta^{-1} C(\sigma)\,. \tag{34}$$

The integration constant $C(\sigma)$ cannot be determined by means of the reference state $\sigma = 0$. Instead we fix it by the specification that in the high temperature limit $T \to \infty$ the entropy $S^{\mathrm{el}} = -(\partial F^{\mathrm{el}}/\partial T)_{\sigma}$ should not depend on the presence of surface charges, i.e., $S^{\mathrm{el}} \to 0$. Since the first term in eq. (34) leads to a vanishing entropy for $T \to \infty$, see eq. (41), we have to choose $C = 0$. Then eq. (34) yields, after division by f to get the electrostatic free energy per area f^{el} and insertion of u^{el} from eq. (28),

$$f^{\mathrm{el}} = (\epsilon\kappa_0/2\pi e^2 \beta) \int_0^{\beta} \mathrm{d}\beta' (\beta')^{-3/2} \times \{[1 + (\sigma/c_0)^2 \beta']^{1/2} - 1\}\,, \tag{35}$$

with the β-independent quantities κ_0 and c_0 defined by $\kappa = \kappa_0 \beta^{1/2}$ and $c = c_0 \beta^{-1/2}$. The integration is more easily carried out after the substitution $(\sigma/c_0)^2 \beta' = x^2$:

$$f^{\mathrm{el}} = \frac{\epsilon\kappa}{\pi} \left(\frac{kT}{e}\right)^2 \frac{\sigma}{c} \int_0^{\sigma/c} \frac{\mathrm{d}x}{x^2}\, [(1 + x^2)^{1/2} - 1]\,, \tag{36}$$

and yields

$$f^{\mathrm{el}} = \sigma\psi_0 - \frac{\epsilon\kappa}{\pi} \left(\frac{kT}{e}\right)^2 \left(\cosh \frac{e\psi_0}{2kT} - 1\right). \tag{37}$$

The same result has been found by TTWE. From this expression the electrostatic surface pressure Π^{el} is derived according to eq. (11)

$$\Pi^{\mathrm{el}} = -(\partial F^{\mathrm{el}}/\partial f)_{T,q}\,, \tag{38}$$

where the quantities held constant have been specified. The general result for Π^{el} is found below.

To see the relative importance of the internal energy and the entropy in f^{el} we also give the result for the entropy. Noting from eqs. (37) and (28) that

$$f^{\mathrm{el}} = \sigma\psi_0 - 2u^{\mathrm{el}}\,, \tag{39}$$

and knowing $f^{\mathrm{el}} = u^{\mathrm{el}} - Ts^{\mathrm{el}}$ we find on comparison

$$Ts^{\mathrm{el}} = -\sigma\psi_0 + 3u^{\mathrm{el}}\,. \tag{40}$$

In the low potential limit $|e\psi_0/kT| \ll 1$ this relation reduces, with eq. (30), to

$$Ts^{\mathrm{el}} = -u^{\mathrm{el}}\,. \tag{41}$$

The entropy term is of the order of u^{el} and because of the negative sign shifts the transition temperature, eq. (4), in the same direction as u^{el}. This doubling of the effect can also be seen directly from

$$f^{\mathrm{el}} = (2\pi/\epsilon\kappa)\,\sigma^2 = 2u^{\mathrm{el}}\,. \tag{42}$$

This relation, on the other hand, leads easily to the electrostatic surface pressure

$$\Pi^{\mathrm{el}} = (2\pi/\epsilon\kappa)\,\sigma^2\,, \tag{43}$$

in the low potential limit.

In the high potential limit $|e\psi_0/kT| \gg 1$ the internal energy

$$u^{\mathrm{el}} = (kT/e)\,|\sigma| \tag{44}$$

is negligibly small compared to $\sigma\psi_0$ in eqs. (39) and (40) and therefore

$$f^{\mathrm{el}} = -Ts^{\mathrm{el}} = \sigma\psi_0\,. \tag{45}$$

With ψ_0 from eq. (26) one gets

$$F^{\mathrm{el}} = -2q(kT/e)\ln(-\sigma/c)\,, \tag{46}$$

and with eq. (25) the surface pressure results as

$$\Pi^{\mathrm{el}} = -2(kT/e)\,\sigma\,, \tag{47}$$

in the high potential limit. Since $\sigma < 0$ we again find a positive surface pressure as in eq. (43).

The result eq. (43) for Π^{el} in the limit of low potential and high temperature can be understood by analogy to the simple condenser discussed earlier, eq. (33). In this limit the distribution of charges in the z direction is dominated by thermal diffusion. If the area f is increased this distribution remains unchanged [$\rho(z) \sim \psi(z) \sim \exp\{-\kappa z\}$ in this limit] corresponding to the condenser case. All what happens under increasing f is that charges of equal sign become separated further, in directions parallel to the surface, on the surface as well as in the ion cloud. The electrostatic energy is decreased and the entropy, eq. (41), increased leading to a positive electrostatic surface pressure Π^{el}.

In the other limit of high potential and low temperature the electrostatic surface pressure, eq. (47), has a quite different origin. In this case the distribution of the counterions is no more determined by thermal diffusion but instead by the electrostatic attraction to the charged surface. If f is increased the attraction is weakened and the counterions become more smeared out in the z direction [$\rho(z) \sim \exp\{-e\,\psi(z)/kT\}$ with $\psi(z)$ falling off faster than $\exp\{-\kappa z\}$, but increasing f tends to weaken this sharp falling off]. This smearing out gives rise to an increase in entropy which leads to the positive electrostatic surface pressure in this case.

The increase in entropy is actually large enough to overcompensate the energy needed for the counterions to diffuse away from the charged surface. This energy neglected in eq. (46) because of $|e\psi_0/kT| \gg 1$ has the tendency to weaken the surface pressure eq. (47) arising from the entropy. As a small effect it may be observed superimposed on a large entropic effect under special conditions [4]. Since the energy increase will be relatively large for small screening length $\lambda_D \sim 1/\sqrt{n}$ a high salt concentration is needed (e.g., monovalent metal ions which do not bind to the lipids).

Having derived explicit expressions for Π^{el} we are now in the position to give an estimate for the order of magnitude of the charge-induced shift in the transition temperature T_t. Our results apply to the case where q is constant; to compare with experimental results let us simply take $q = -e$, i.e., maximal charge in the case of lipids with one dissociable charge like MPA. The measurements of TTWE were done mainly under conditions where $|e\psi_0/kT| \gg 1$ holds. Inserting eq. (47) in eq. (15) we obtain for the relative shift in this case

$$(T_t - T_t^{**})/T_t^{**} = -2(\Delta f/f)kT/T\Delta S\,, \qquad (48)$$

where the stars on the r.h.s. have been omitted. Δf should be regarded as an effective jump in area in order to take into account also the area-induced shift effect. The quantity $kT/T\Delta S$ is the ratio of thermal energy to the jump in thermal energy. Using $kT/T\Delta S \approx 1/10$ and $\Delta f/f \approx 1/4$ we find

$$(T_t - T_t^{**})/T_t^{**} \approx -\tfrac{1}{20}\,, \qquad (49)$$

or $T_t - T_t^{**} \approx -15$ deg for the charge-induced shift of the transition temperature of a singly charged lipid. This result agrees well with the experimental finding for MPA [4]. Furthermore, the smallness of the effect, eq. (49), may be taken as a justification of the linear approximation used in section 2.

4.2. Charging process

The results obtained above for the electrostatic free energy f^{el} and the surface pressure Π^{el} will be rederived in this chapter by means of a hypothetical charging process. We will follow closely the formulation of this method by Lifson and Katchalsky [16] for the case of charged macromolecules.

The free energy of a certain state is the amount of work which has been performed isothermally on the system to reach this state. In our case the electrostatic free energy is obtained by charging the system up to its actual state, the reference state being uncharged as earlier. The charging is carried out simultaneously on the surface and on the ions in the solution, electroneutrality being maintained throughout the process. We denote by a prime the values of the varying quantities.

The work performed by increasing the charge of an ion by de' is $\psi' de'$, ψ' being a function of the position of the ion and of its variable charge e'. The number of charges per unit volume is ρ'/e', and is also a function of position and of degree of charging. The work performed by charging all the ions in a volume element dV by de' is $\psi' de'\,(\rho'/e')\,dV$. Similarly the work performed by charging a surface element dF by de' is $\psi_0' de'\,(\sigma'/e')\,dF$. Integrating both terms, the electrostatic free energy (normalized again per lipid molecule) is obtained as

$$F^{el} = \int_0^e \frac{de'}{e'}\left(\int_V \psi'\rho'\,dV + \int_F \psi_0'\sigma'\,dF\right). \qquad (50)$$

From the electrostatic relation

$$\int_V \psi\rho\,dV + \int_F \psi_0\sigma\,dF = \frac{\epsilon}{4\pi}\int_V (\mathrm{grad}\,\psi)^2\,dV\,, \qquad (51)$$

and eq. (23) it is seen that the bracketed expression in eq. (50) is twice the internal energy $U^{el\prime}$. Introducing the charging parameter λ by $e' = \lambda e$ one finds the result [16]

$$F^{el} = \int_0^1 2\,U^{el\prime}(\lambda)\,\frac{d\lambda}{\lambda}\,. \qquad (52)$$

Inserting $u^{el\prime}(\lambda)$ from eq. (28) with $\sigma'(\lambda) = \lambda\sigma$ and $\kappa'(\lambda) = \lambda\kappa$ we obtain

$$f^{el} = \frac{\epsilon\kappa}{\pi}\left(\frac{kT}{e}\right)^2 \int_0^1 \frac{1}{\lambda}\left\{\left[1+\left(\frac{\sigma\lambda}{c}\right)^2\right]^{1/2} - 1\right\}\frac{d\lambda}{\lambda}. \qquad (53)$$

After substituting $\sigma\lambda/c = x$ this relation reduces exactly to our former result, eq. (36).

The formulation eq. (52) of the charging process can easily be transcribed to yield directly the electrostatic surface pressure. For this aim it has to be noted that $U^{el\prime}(\lambda) = fu^{el\prime}(\lambda)$ with eqs. (28) and (25) depends on λ only in the combination λ/f. Therefore the λ-integration at constant f can be replaced by an integration over f at constant $\lambda = 1$ (with $d\lambda/\lambda = -df/f$)

$$F^{el} = -\int_\infty^f 2\,U^{el}(f')\,\frac{df'}{f'}, \qquad (54)$$

where the integration is meant at constant temperature T and charge q per lipid molecule. From this expression the surface pressure Π^{el}, eq. (38), is directly read off to be

$$\Pi^{el} = 2u^{el}. \qquad (55)$$

This relation together with eq. (28) for u^{el} is our general result. The internal energy u^{el} being positive per definition, eq. (23), the electrostatic surface pressure is found positive. The earlier results for Π^{el} in the special cases of low and high potential, eqs. (43) and (47), are easily shown to fulfil the general relation eq. (55).

We may go one step further to make contact with a slightly different formulation of the charging process due to Verwey and Overbeek [13]. Our treatment of the double layer can be described thermodynamically as

$$dF^{el} = -S^{el}dT - \Pi^{el}df, \qquad (56)$$

with Π^{el} given by eq. (55). From this relation a similar one for the free energy f^{el} per unit area may be derived. Considering

$$df^{el} = (1/f)\,dF^{el} - F^{el}\,d(1/f), \qquad (57)$$

and our earlier result eq. (39), $F^{el} = q\psi_0 - 2U^{el}$, we obtain

$$df^{el} = -s^{el}dT - \psi_0\,d\sigma. \qquad (58)$$

This relation allows to determine f^{el} by an alternative charging process

$$f^{el} = \int_0^\sigma \psi_0(\sigma')\,d\sigma', \qquad (59)$$

which has been discussed by Verwey and Overbeek. In this form it was the theoretical starting point of TTWE.

To avoid misunderstanding on comparing our results with the work of Verwey and Overbeek [13] it should be noted that they applied the double layer concept of Gouy and Chapman to an electrode–electrolyte system. In this case the surface potential ψ_0 is fixed electrochemically and has to be considered as an external variable in a thermodynamic relation like eq. (58). The appropriate thermodynamic potential φ^{el} is then obtained by making a Legendre transformation *

$$\varphi^{el} = f^{el} - \sigma\psi_0. \qquad (60)$$

With our result eq. (37) we find

$$\varphi^{el} = -\frac{\epsilon\kappa}{\pi}\left(\frac{kT}{e}\right)^2\left(\cosh\frac{e\psi_0}{2kT} - 1\right), \qquad (61)$$

which is exactly the result of Verwey and Overbeek.

In the low potential limit their system may again be interpreted as a condenser. The area f being increased under constant voltage ψ_0 the electrostatic energy U^{el}, eq. (31), increases, in contrast to the result eq. (32) for our lipid system.

5. Dissociation changes

In all the calculations up to this point we have been restricting ourselves to the case of a constant charge q per lipid molecule. This may equally well be expressed by a constant degree of dissociation α according to

$$q = -\alpha e, \qquad (62)$$

for lipids with one dissociable charge, $0 \leqslant \alpha \leqslant 1$. As an example for a constant α we discussed the case

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* In the same way one passes from the internal energy U to the free energy $F = U - TS$ when the temperature is fixed externally.

$\alpha = 1$, i.e., complete dissociation. The shift in T_t is then maximal and given by the simple eq. (48), for the high potential limit. In general it is well conceivable that α changes with the area f. With increasing f the electrostatic binding of positive ions at the lipid surface charges is weakened, some of the ions are released, leading to a larger α. The electrostatic free energy of a state reached by a charging process with varying α differs from that of a state reached under constant α. The electrostatic surface pressure being the derivative of the free energy with respect to the area f may be influenced by a dissociation change as well. It is the aim of this final section to determine the surface pressure for the more general situation of varying surface charge q. As an example of binding counterions we choose protons, the treatment being applicable to divalent metal ions as well.

In equilibrium α is given by

$$\alpha = K/(K + n_{\mathrm{H}0}) , \tag{63}$$

with K being the dissociation constant and $n_{\mathrm{H}0}$ the proton concentration at the surface. As in eq. (17) the latter may be found from

$$n_{\mathrm{H}0} = n_{\mathrm{H}} \exp(-e\psi_0/kT) , \tag{64}$$

where n_{H} is the bulk proton concentration. The surface potential ψ_0 being negative the proton concentration is enhanced at the surface and α lowered. This description of dissociation is equivalent to Stern's treatment [14] of adsorption at a charged surface.

In the limit of low potential $|e\psi_0/kT| \ll 1$ the electrostatic adsorption is negligible, our earlier result for Π^{el} can be used, α being given by eq. (63) with $n_{\mathrm{H}0} = n_{\mathrm{H}}$.

In the high potential limit $|e\psi_0/kT| \gg 1$ eq. (64) becomes, using eq. (26),

$$n_{\mathrm{H}0} = n_{\mathrm{H}}(2\sigma/c)^2 , \tag{65}$$

and therefore we obtain for the surface charge density with eq. (62)

$$\sigma = -\frac{e}{f}\,\frac{1}{1 + (4n_{\mathrm{H}}/Kc^2)\sigma^2} . \tag{66}$$

The solution for σ of this equation has to be inserted in u^{el}, eq. (28). If we were again to derive the electrostatic free energy we must take account of the more complicated dependencies of u^{el} on temperature T or charging parameter λ which lead to a different result for f^{el} as the one derived earlier, eq. (37). Being interested mainly in the electrostatic surface pressure the charging process eq. (52) again allows to circumvent the explicit integration. Noting that the solution for σ', eq. (66), is a function of λ/f as before the λ integration can still be replaced by an integration over f, now at constant temperature and constant proton concentration n_{H}.* Since the electrostatic surface pressure by analogy to eq. (38) is given by

$$\Pi^{\mathrm{el}} = -(\partial F^{\mathrm{el}}/\partial f)_{T,n_{\mathrm{H}}} , \tag{67}$$

we find our earlier result, eq. (55),

$$\Pi^{\mathrm{el}} = 2u^{\mathrm{el}} . \tag{68}$$

In the limit $|e\psi_0/kT| \gg 1$ under consideration this yields explicitly, as in eq. (47)

$$\Pi^{\mathrm{el}} = 2(kT/e)|\sigma| , \tag{69}$$

with σ now determined from eq. (66).

For completeness we add the thermodynamic relation corresponding to eq. (67), without restriction to one of the limiting cases of low or high potential. In contrast to eq. (56) where the charge q was considered to be constant, work can now be performed on the system by changing q. Actually q is accessible externally by the proton concentration n_{H} (and the temperature T), and in the high potential limit also by the area f. So in addition to the external variables T and f of eq. (56) the proton concentration n_{H} enters the thermodynamic relation which reads

$$\mathrm{d}F^{\mathrm{el}} = -S^{\mathrm{el}}\mathrm{d}T - \Pi^{\mathrm{el}}\mathrm{d}f + \mu^{\mathrm{el}}\mathrm{d}n_{\mathrm{H}} . \tag{70}$$

(In principle also the concentrations of other ions may be included as external variables.) The above definition of Π^{el}, eq. (67), is a direct consequence of this relation, the quantity μ^{el} being defined analogously.

The physical basis of Π^{el} is the same as discussed in the preceding section. But we are now able to vary Π^{el} externally. Changing the proton concentration n_{H} the charge q can be varied between 0 and the maximal value $-e$ allowing the surface pressure and the charge-induced shift of T_t to be switched on and off. To some extent q can also be varied by changing the con-

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* Usually the constant K depends on temperature as $K = K_0 \exp(-\phi/kT)$, its constancy then being included in the constant temperature.

centration of non-binding ions, e.g., monovalent metal ions (these contribute to the total electrolyte concentration n like the protons). This effect of salt concentration occurs in addition to the small salt effect discussed in section 4.

A quantitative comparison between the theoretical result as described by eqs. (69) and (66) and experimental data has been carried out by TTWE and excellent agreement was found.

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