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PHASE TRANSITIONS IN LIPID BILAYERS

A THEORETICAL MODEL FOR PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDIC ACID BILAYERS

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We present an application of an earlier model to the description of phase transitions in phosphatidylethanolamine and phosphatidic acid bilayers. For the former system we modify the model to allow for very slightly closer chain packing. This small modification alone is sufficient to shift the main phase transition temperature to higher values, and to eliminate the lower transition. For the charged bilayers we incorporate electrostatic contributions into the free energy using double layer theory and we consider the excluded volume of counterions at the bilayer/water interface. Phase transition temperatures and the presence or absence of a lower transition are determined for various pH values, and the results are compared with experiment.

Introduction

While most studies of the physical states of lipid bilayers have involved phospholipids with zwitterionic head groups, such as the phosphatidylcholines, workers have in recent years turned increasingly to bilayers of lipids with different head groups. Among the more commonly studied systems are the ethanolamines [1–4], and various types of charged lipids [5–14]. The latter systems are of special interest because of the possibility of stimulating lipid phase transitions isothermally by changes in pH [11]. The phase behavior of charged lipid bilayers is complex. The neutral phosphatidic acids have their main phase transition temperatures near that of the corresponding phosphatidylethanolamines, and removal of a single proton shifts this temperature only slightly. However, removal of the second proton results in a large drop in transition temperature [7]. In addition, doubly charged phosphatidic acid bilayers exhibit a lower transition with a ripple phase while singly charged and neutral systems apparently do not [9,12]. On the other hand, lipids with larger head groups capable of only a single proton dissociation such as phosphatidylserine and phosphatidylglycerol show a large drop in T_m when the single proton is dissociated [8,13]. Phosphatidylglycerol bilayers also exhibit a lower transition to a ripple phase when charged, but not when neutral [8].

Although a large number of theoretical models now exist for the lipid bilayer phase transition, these models generally are applied to the widely studied phosphatidylcholine systems [14]. Only a few workers have considered the effects of the head group region on the phase properties. Nagle [15] first studied the effect of various head group interaction potentials on his earlier published models for the phosphatidylcholines. Scott and Cheng [16] also modeled the ethanolamine phase transition by adding an empirically constructed potential energy to an earlier computation. The effects of varying pH and/or of charged lipid head groups have been examined theoretically by Forsyth and co-workers [10], and somewhat more crudely by Trauble and Eibl [6] and Jahnig [17]. The latter theories all make use of double layer theory to calculate the electrostatic con-

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tribution to the free energy, but only Forsyth et al. [10] include the calculation of the hydrocarbon region free energy in the model. Very recently, Copeland and Andersen [18] have developed the classical double layer theory, proceeding from a basic statistical mechanical beginning. They derive formulas for the shift in phase transition temperatures as a function of pH and divalent cation concentration. In general, a proper calculation of the lipid bilayer free energy should minimize the entire function (hydrocarbon chains plus head groups plus any other terms) with respect to all unconstrained internal variables. Performing this minimization, Forsyth et al. [10] concluded that the phase transition in charged lipids is not first order. The accuracy of this conclusion is somewhat clouded, however, by assumptions made in the computations.

Recently, we devised a new model for lipid bilayer phase transitions [19] based upon the two-dimensional packing of hard rods of varying length. The hard rods represent shadows, or projections of the lipid hydrocarbon chains plus head groups into the bilayer plane. In this scheme the rod length was determined by the isomeric state of the hydrocarbon chains. Incorporating statistical weighting for the chain states, a lateral pressure of 50 dyne/cm, an approximate van der Waals' energy [20], and Scaled Particle Theory for hard rod packing [22,23], we found that the model was highly successful in its predictions for the temperatures and enthalpy changes for the main lipid phase transition. In addition, the models exhibited a smaller transition at temperatures which correspond well to the lower transition observed in the phosphatidylcholines. The lower transition in the model was due to liberation of long-axis rotational degrees of freedom without changing the isomeric states of the molecules. While changes in chain tilt angle and ripples are not within the capabilities of the model, our results suggest a mechanism for the onset of rotational disorder which may lead to these more complex phenomena.

The purpose of the present paper is to apply the same model to bilayers composed of different lipids, namely ethanolamines, and phosphatidic acids. In the next section we describe our model for dipalmitoylphosphatidylethanolamine (DPPE) bilayers. We then discuss a model for dipalmitoylphosphatidic acid (DPPA) bilayers in which we consider the effects of varying pH on the transition temperature and the presence or absence of a lower transition. The final section contains our conclusions. Although we only consider a single species of charged lipid we shall show how our model can also describe the observed phase behavior of other charged lipids with larger head groups.

Model for DPPE phase transitions

In general, members of the homologous series of phosphatidylethanolamines have higher phase transition temperatures and enthalpy changes than their phosphatidylcholine counterparts. For example, has $T_m \approx 336$ K and $\Delta H \approx 8.8$ kcal/mol [2], while dipalmitoylphosphatidylcholine (DPPC) has $T_m \approx 314$ K and $\Delta H \approx 8.9$ kcal/mol [1], although the latter value is higher than that reported recently by Chen et al. [24] of 6.8 kcal/mol. In either case, ΔH for DPPE seems to be somewhat higher than that of DPPC [2]. Earlier, theory models [15,20,21] which briefly address these differences have introduced additional potential energy terms to the free energy. There are several possible sources of these interactions, including dipolar interactions or hydrogen bonding, each of which can be modeled only very qualitatively. The early models represent feasibility studies for several of these types of interactions.

A crucial difference between the phosphatidylcholines and the phosphatidylethanolamines is the chain tilt exhibited by the former below the lower transition. Recent work by McIntosh [4] seems to verify a suggestion made earlier by Nagle [15] that the chain tilting in the phosphatidylcholines allows the chains to pack closely in spite of the large head group region (Nagle attributed this packing difference to the glycerol region). In the phosphatidylethanolamines this region does not sterically force the chains apart, so tilting is not necessary. If this enhanced packing is the reason for the chain tilt, then one need not directly include it in a statistical mechanical calculation of the type proposed here. The interactions between the closely packed chains may be viewed along an angle parallel to the chains and the magnitude of the semiempirical van der Waals' energy reflects the principal consequence of tilting.

X-ray studies of phosphatidylcholines and phosphatidylethanolamines support the model of Nagle

and McIntosh, indicating that chain packing in the two systems is very similar [4]. This would suggest similar transition temperatures (given similar chain lengths and van der Waals' energies) for the two systems. Since the T_m values are not similar, and since phosphatidylethanolamine has no lower transition, one must examine the systems more closely. In this paper we choose not to introduce a phenomological interaction of the sort described above. Rather, we consider the fact that, in monolayers, phosphatidylethanolamine films can be compressed to higher densities than their phosphatidylcholine counterparts [25]. Thus, it seems possible that in bilayers closer packing may be possible also for phosphatidylethanolamines. The X-ray data indicate that differences in packing between phosphatidylethanolamines and phosphatidylcholines must be slight, but van der Waals' forces vary strongly at close packing distances. Our approach is the following.

We consider the model for DPPC described in Ref. 19, and in the present calculations all of the properties of this earlier model are retained except, to allow for closer packing, the lengths of the rods are all reduced by a common, small, factor. The resulting phase properties of the models were studied for several values for this size-scaling factor. The results are presented in Table I. Computational details have all been described in earlier publications [19,23] and so will be omitted here. The most significant results contained in Table I are that, with only a 2% reduction in hard rod length in our basic model, the main transition temperature is driven upwards from 41 to 66°C, while the lower transition disappears. It should be recalled at this point that, as described in Ref. 19,

TABLE I

PROPERTIES OF THE C-16 CHAIN MODEL WITH HARD-ROD LENGTHS REDUCED BY THE SCALE FACTOR

Data for DPPC and DPPE are included for comparison.

Scale factor	T_m (°C)	ΔH_m (kcal mol)	T_L (°C)
DPPC (expt.) [5,24]	41.4	6.6 [24], 7.7 [5]	35.1
1.0 [19]	41.0	9.0	34.0
0.99	54.0	10.9	none
0.98	66.0	12.7	none
DPPE (expt.) [2]	63	8.8	none

the lower transition in our model is interpreted as the spontaneous onset of long-axis molecular rotation. In earlier studies, we found that models with closer packing do not always exhibit a lower transition. The present results suggest that the major differences between phosphatidylethanolamine and phosphatidylcholine bilayers can be explained in terms of chain packing, without resorting to new mechanisms such as hydrogen bond schemes. Our theory is consistent with the conclusions drawn by McIntosh [4] which indicate similar, but not necessarily identical, packing for DPPC and DPPE. If our models are correct, then long-axis rotation plays an important role in bilayers below T_m. Experimental evidence in support of the long-axis rotation in the phosphatidylcholines is discussed in Ref. 19. For DPPE, very recent results [26] suggest the presence of slow, long-axis rotation below T_m. Full details of these experiments have not yet been published, to our knowledge. In the present models, the equilibrium state below T_m is orientationally biaxial, although domains of different orientation or slow orientational motion are not ruled out. Near T_m the fraction of orientationally disordered molecules in the model is about 10^{-2}. Theoretical predictions of the enthalpy change at the main transition are too high in general, although the trend to a higher ΔH for DPPE than for DPPC is consistent with our close chain packing hypothesis. We now make use of the above results to define a model describing phase transitions in DPPA at various pH values.

Model for DPPA phase transitions

Monolayers and bilayers of the various phosphatidic acid homologs have been studied by different groups as models for charged biological lipids in membranes [5–7,27–28]. The phase properties of phosphatidic acid have been determined by bilayer [5] and monolayer [27] experiments at various pH values. These data suggest that like DPPE, $DPPA^-$ packs more closely than DPPC, has its main phase transition at 67°C [5] (T_m = 63°C for DPPE), and does not exhibit a lower transition. The enthalpy change for $DPPA^-$ is, however, much lower (5.2 kcal/mol [1]). At higher pH, DPPA becomes doubly charged, its phase transition temperature drops to 58°C, and the enthalpy change is reduced to about

2.9 kcal/mol [5]. Doubly charged diether phosphatidic acid bilayers exhibit a lower transition as shown by freeze-fracture and scanning calorimetry at high pH [12]. X-ray studies [9] indicate that chain tilt is also induced by increased surface charge.

With these data in mind, we have extended our basic lipid bilayer model described above and in detail in Ref. 19 to the charged lipid systems, in particular to $DPPA^-$ and $DPPA^{2-}$. The procedure is somewhat similar to that of Forsyth et al. [10]. We use Guoy-Chapman double layer theory to calculate the electrostatic contribution to the Gibbs free energy at fixed molecular area. The details of this part of the calculation are given in the Appendix. The total free energy is then minimized with respect to molecular area as well as the distribution of molecular conformations (rod sizes). For neutral DPPA and $DPPA^-$ we assume the set of allowed rod sizes is the same as that for DPPE, since the monolayer packing and bilayer transition temperatures are very similar. As the pH is increased, one gets more $DPPA^{2-}$ in the system. In our calculations we found that if we assumed $DPPA^{2-}$ molecules packed in the same manner as $DPPA^-$ and DPPE, then the phase transition temperature varies little even at high pH, contrary to experiment. However, monolayer studies show doubly charged phosphatidic acid films cannot be compressed to the extent that slightly charged films can [27]. There may be several possible reasons for this difference, but it seems to us that the simplest hypothesis is that the extra counterions packed among the doubly charged head groups force the system to larger areas by the excluded volume effect. Fig. 1 shows the relative sizes of the rod we use to describe the ground state and a 1 Å radius counterion. The excluded volume effect need not be as important in $DPPA^-$ bilayers, because the small size of the head group could allow for a sufficient number of counterions to be present without affecting the molecular packing arrangements. We, therefore, assigned an enlarged hard rod length to all $DPPA^{2-}$ molecules in their ground or all-*trans* state. The amount by which the length was enlarged varied only between 1 and 2%. All other parameters in the model are unchanged. Additional experimental input for these calculations consists of the pK values for the two ionization reactions. The first proton dissociates from DPPA at pH 2–3, while the second dissociates at pH 8–9 [5]. As

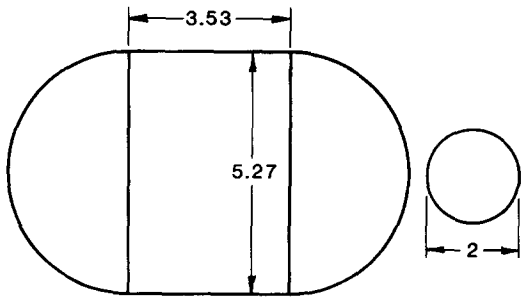

Fig. 1. Relative sizes of ground-state rod used in calculations for DPPE and $DPPA^-$, and a 1 Å radius counterion.

shown in the Appendix our calculation requires only the difference $pK_2 - pK_1$ which we take to be 6.

We have calculated phase transition temperatures and enthalpy changes, and searched for a lower transition for a variety of values of the pH, and for different excluded volume assigned to the $DPPA^{2-}$ ground state. The results are summarized in Table II and Fig. 2. Table II shows that we observe no lower transition in these models when DPPA is singly or only partially doubly ionized. When the ground-state rod length of $DPPA^{2-}$ is increased from 3.528 to 3.544 Å, we still observe no lower transition even at very large pH. However, when this length is set at 3.600 Å (the value we use for DPPC ground state in Ref. 19) then $DPPA^{2-}$ exhibits a lower transition. The enthalpy changes for our models are all larger than the experimental values, but the trends that that model data show are consistent with expeiment, that is ΔH-

TABLE II

PROPERTIES OF THE MODEL FOR DPPA AT VARIOUS $pH-pK_2$ AND $pH-pK_1$ USING A SCALE FACTOR OF 0.98, AND SETTING THE ROD LENGTH FOR THE GROUND STATE OF DOUBLY CHARGED MOLECULES EQUAL TO 3.600

$pH-pK_1$ ($pH-pK_2$)	% singly ionized	% doubly ionized	T_m (K)	ΔH_m (kal/mol)	T_L (K)
−3 (+2)	100	0	337	12.9	none
−4 (+2)	100	0	337	12.9	none
−6 (+0)	100	29	321	12.1	none
−8 (−2)	100	95	309	9.6	none
−9 (−3)	100	100	308	9.4	307

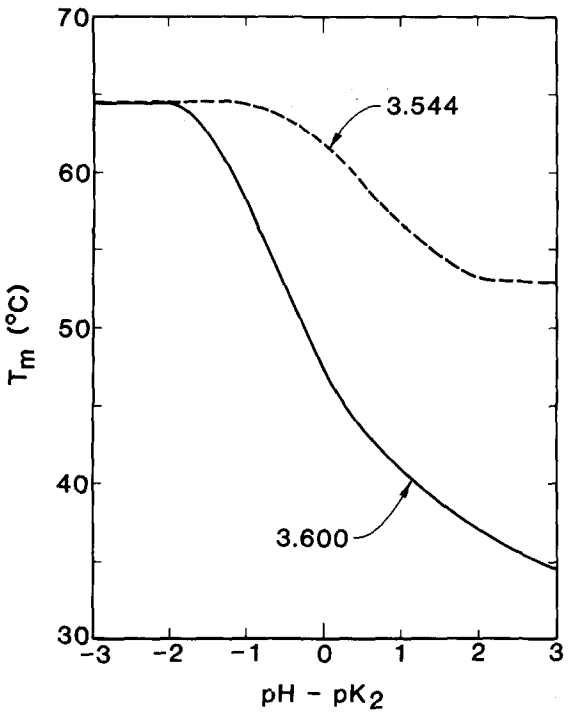

Fig. 2. A plot of phase transition temperature vs. pH. (———) Rod length for the ground state of $DPPA^{2-}$ = 3.600 Å. (- - - - -) Rod length for the ground state of $DPPA^{2-}$ = 3.544 Å.

$(DPPA^{2-}) < \Delta H(DPPE)$. In Fig. 2 we plot the transition temperature against the relative pH. The top curve describes the system in which the ground-state rod length for doubly charged molecules is increased from 3.528 to 3.544 Å, while for the lower curve this length is increased to 3.600 Å. Both curves have the same qualitative shape as the experimental curves [6]. However, the lower curve shows a drop in T_m of over 30°C, while the upper curve shows a drop in T_m of about 10°C over the pH range studied, which is closer to the experimental result of 9°C [5]. Moreover, the system described by the top curve does not show a lower transition while the bottom curve system has a lower transition at high pH (see Table II).

Conclusions

The theoretical models described in this paper are basically two-dimensional circularly capped hard rod systems. The hard rods represent the projections of the lipids onto the bilayer plane, so that molecules in which the hydrocarbon chains are disordered cast longer shadows, and we represent them by longer rods. The hard rods also may exist in several orientational states in which rods may make angles of $\kappa\pi/3$ (κ = 0,1,2) with each other. Continuous rotational freedom is very difficult to treat numerically so we have not considered this case. The excluded volume problem is approximately accounted for using Scaled Particle Theory, which has proven to be an excellent technique for other systems [23]. The van der Waals' energy of the system is represented by a function:

$$E_{vd\omega} = -C/(A - 38)^{1.5} \quad (1)$$

as was also used in Refs. 19 and 20. This functional form led to values for the excess van der Waals' energy at the main phase transition which agreed very well with experiment [19,20]. A third feature of our models is the lateral pressure exerted on the film of 50 dyne/cm, an effective contribution to the free energy which keeps the lipids packed as in a monolayer. Values significantly lower than 50 dyne/cm for this pressure led to very large molecular areas in the fluid phase, while values much higher than 50 dyne/cm also seem unlikely. The main phase transition in these models is, as usual, a hydrocarbon chain melting transition, in which, in the theory, a macroscopic fraction of the molecules have elongated projections on the bilayer plane, while in the ordered phase all molecules have the smallest possible projection. When the length-to-width ratio of the rods is greater than some minimal value, a lower transition occurs in which the rods change from an all-parallel alignment to one in which all possible intermolecular angles are allowed but with no accompanying chain disordering. We have interpreted this first-order phase change as a mechanism for the onset of the observed lower transition in the phosphatidylcholines (see Ref. 19 for details of the argument). We concluded that this onset of long-axis rotation may be correlated with mechanisms for the onset of other features associated with the $P_{\beta'}$ phase, such as ripples [29,30].

In the present work we have extended this model to different systems. The extensions were made in such a way that the only changes made were those which seem well supported by experimental data and/or well known differences between the new systems

under study and the previously studied phosphatidylcholines. In the case of DPPE, we found that a 2% reduction in the lengths of the rods associated with all the possible molecular conformations which are the same as those for DPPC [11] allowed closer packing and increased the main phase transition temperature by the amount observed experimentally. The enthalpy change calculated was more than the experimental value, probably because the area per molecule in the fluid state for DPPE turned out to be about 70 $Å^2$/mol, which seems too high. Thus, a stronger lateral pressure might be necessary for DPPE in the fluid phase, since this phase occurs at a higher temperature. Equally significant is the fact that the lower rotational phase transition does not occur in the DPPE model, a result consistent with observations. In the theory, the rotational transition occurs at a temperature which depends strongly upon the length-to-width ratios of the rods in the system. This means that for a given ratio, there is a unique critical density at which this transition occurs. By allowing a very slight increase in packing density for phosphatidylethanolamine over phosphatidylcholine (within the limits of error of the X-ray data, we believe) all the main properties of the phosphatidylethanolamines, which differ from the phosphatidylcholines, emerge. This result gives further support to our suggestion [19] that long-axis rotation plays an important role at temperatures below the chain melting phase transition. As we also stressed [19], while the lower transition in this theory is a direct result of hard rod packing, other observed phenomena such as ripples are not ruled out, and, in fact, may occur as consequences of stresses or other changes brought about by the very slight area increase at the lower phase transition.

In the charged lipid systems we modeled, we basically followed a procedure used by Forsyth et al. [10] with two important additional features. Firstly, we considered two possible ionization states using the Poisson-Boltzmann double layer theory outlined in the Appendix. Secondly, we explicitly considered the counterion excluded volume for doubly charged DPPA. Our rationale was that counterions in a double layer with singly charged DPPA would not disrupt the lipid packing due to the relatively small size of the phosphate group, but the additional counterions and H_2O molecules introduced when the second proton is dissociated might have an important effect. Similar results could have been achieved by changing the rod lengths slightly, as was done for DPPE, but we feel the counterion presence provideds a more physically appealing mechanism. There are important similarities between the different types of bilayers which this model suggests. In all cases the lower transition, if present, signals the onset of long-axis rotation. This lower transition only occurs in systems with larger head groups or neighboring counterions, either of which can force chains to larger molecular areas (and which thereby leads to chain tilting as a mechanism to decrease the attractive energy). The reason for the large values of ΔH in the charged systems is essentially the same as that discussed above for DPPE. However, even though the trend in ΔH predicted by the theory is the same as the experimental trend, the differences are rather large, and so other mechanisms or features of the charged systems may not be included in the theory. It is interesting to note that the lower transition appears at high pH for systems in which the excluded volume attributed to the counterions is sufficiently large.

The theory described above can be easily modified to describe phosphatidylserine or phosphatidylglycerol bilayers. In these cases, one expects pH vs. T_m curves like those of Fig. 2 with only a single proton dissociation. The model presented here will produce such a curve if we suppose that the proton dissociation leads to a slightly enlarged head group (as we did for the second proton loss for DPPA). This seems reasonable for phosphatidylserine and phosphatidylglycerol, since these lipids have much larger head groups than phosphatidic acid to begin with. We note also that the magnitude of the change in T_m, as well as the presence (or lack) of a lower transition both depend very sensitively upon the lengt-to-width ratio of the rods used.

Very recently, Copeland and Andersen [18] have applied their earlier theory to singly and doubly charged lipid bilayers.

They are able to reproduce the experimental pH vs. T_m curves for both types of systems by adjusting several parameters, including the solid and fluid phase molecular areas used in the theory. While this approach is different from the theory presented here, the two seem consistent in that larger molecular areas are required in both cases to facilitate the large drop in T_m.

Appendix

Calculation of the double layer free energy

If we solve the Poisson-Boltzmann equation for an infinite flat sheet in the presence of monovalent electrolyte, we obtain for the surface charge density at the sheet [31,32]:

$$\sigma = \frac{2n\epsilon kT}{\pi} \sinh\left(\frac{e\psi_0}{2kT}\right) \tag{A1}$$

where σ is the charge density, ψ_0 the surface potential, n the electrolyte concentration far from the interface, ϵ the solvent dielectric constant, kT Boltzmann's constant times the absolute temperature, and e the electronic charge.

The free energy of the system may be calculated as a sum of chemical and electrostatic terms [31]. The electrostatic free energy per unit area may be obtained by inverting Eqn. A1 and evaluating the integral:

$$\Delta F_{el} = \int_0^{\sigma} \psi(\sigma')\, d\sigma' \tag{A2}$$

The chemical term is determined by calculating the difference in free energy between an interface consisting of neutral, singly, and doubly charged lipids, and in ideal solution of the same composition. Under idealized conditions this difference is given by an entropy of mixing plus a chemical potential term:

$$\Delta F = \boldsymbol{k}\left[\ln\left(\frac{N_L!}{N_1!N_2!(N_L - N_1 - N_2)!}\right)\right] + \mu_0 \Delta N \tag{A3}$$

where N_L is the total number of lipids and N_1 and N_2 are the number of singly and doubly charged lipids, respectively, and ΔN is the total number of protons removed from the interface. In Stirling's approximation the combinational term becomes:

$$-N_L[\Gamma_1 \ln \Gamma_1 + \Gamma_2 \ln \Gamma_2 + (1 - \Gamma_1 - \Gamma_2)\ln(1 - \Gamma_1 - \Gamma_2)] \tag{A4}$$

where $\Gamma_1 = N_1/N_L$ and $\Gamma_2 = N_2/N_L$. To simplify further we write the equations for the reaction rates at the surface for the two dissociation reactions:

$$\frac{\Gamma_1 n_{H^+}}{1 - \Gamma_1 - \Gamma_2} = K_1 e^{e\psi_0/kT} \tag{A5}$$

$$\frac{\Gamma_2 n_{H^+}}{\Gamma_1} = K_2 e^{e\psi_0/kT} \tag{A6}$$

where K_1 and K_2 are the equilibrium constants for the first and second proton dissociation, respectively, and n_{H^+} is the surface proton concentration.

If we solve Eqns. A5 and A6 for $\ln\Gamma_1$ and $\ln\Gamma_2$ and substitute into Eqn. A4, we obtain:

$$-N_L[e\psi_0/kT(\Gamma_1 + 2\Gamma_2) + \ln(1 - \Gamma_1 - \Gamma_2) - \Gamma_1\Delta_1 - \Gamma_2(\Delta_1 + \Delta_2)] \tag{A7}$$

where $\Delta_1 = \ln K_1 - \ln n_{H^+}$ and $\Delta_2 = \ln K_2 - \ln n_{H^+}$. The first term in Eqn. A7 is simply the charge density σ times surface potential divided by kT. The terms involving Δ_1 and Δ_2 are cancelled by $\mu\Delta N$ terms involving the shift in protons from surface to solution [31] and so we are left with:

$$\Delta F = A\int_0^{\sigma_0} \psi(\sigma')\, d\sigma' - A\sigma\psi_0 + NkT\ln(1 - \Gamma_1 - \Gamma_2) \tag{A8}$$

or, integrating by parts (A, area):

$$\Delta F = -A\int_0^{\psi_0} \sigma(\psi)\, d\psi + NkT\ln(1 - \Gamma_1 - \Gamma_2) \tag{A9}$$

Our numerical procedure is as follows: we solve Eqns. A5 and A6 for Γ_1 and Γ_2, obtaining:

$$\Gamma_{-1} = \frac{e^{y_1}}{1 + e^{y_1} + e^{y_1 + y_2}} \tag{A10}$$

$$\Gamma_{-2} = e^{y_2}\Gamma_{-1} \tag{A11}$$

where $y_1 = e\psi_0/kT + (\mathrm{pH} - \mathrm{p}K_1)$ and $y_2 = e\psi_0/kT + (\mathrm{pH} - \mathrm{p}K_2)$. Substituting Eqns. A10 and A11 into Eqn. A1 with $\sigma = (e\Gamma_{-1} + 2e\Gamma_{-2})/A$ yields a transcendental equation for ψ_0 as a function of T, y_1 and y_2. This equation is solved numerically for ψ_0 for each data point during te minimization scheme described in the text. The results substituted into Eqn. A7 give the additional contribution to the free energy from the double layer at the head group/water interface.

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