# Electrostatics of membrane adhesion

S. Marčelja
Department of Applied Mathematics, Research School of Physical Sciences and Engineering, The Australian National University,
Canberra ACT 2601, Australia

ABSTRACT We consider electrical double layer interaction under the conditions typically encountered during membrane fusion. Within the physiological concentration range of monovalent electrolytes the interaction is repulsive and the Poisson-Boltzmann calculation may be used to evaluate the force. When divalent counterions are added, strong ion–ion correlations make the Poisson-Boltzmann approximation inapplicable. We use the anisotropic hypernetted chain method to show that in the presence of small amounts of divalent counterions in adsorption equilibrium with the surfaces, the double layer interaction turns into attraction. This attractive electrostatic force may be the balancing contribution controlling membrane adhesion.

## INTRODUCTION

Controlled fusion of membranes is an indispensable part of many biological functions. Different experimental lines of evidence show that the fusion is a two-step process (e.g., 1, 2). In the first step, normally initiated by an increase in the concentration of calcium ions, membranes come into close adhesive contact. Divalent ions other than calcium are also effective in inducing membrane adhesion. In the second step, specific functional mechanisms induce fusion of closely apposed membranes.

The control of the adhesion step by divalent counterions is not well understood. This is not surprising, because the electrostatic interaction of weakly charged surfaces in the presence of adsorbing divalent counterions has never been accurately evaluated. It is shown below that due to strong correlations between the positions of divalent counterions, such a calculation is outside of the range of validity of the Poisson-Boltzmann (PB) approximation.

Two accurate methods suitable for the calculation of electrostatic interaction between surfaces in ionic solutions have been developed over the last six years: Monte Carlo simulations (3–5) and anisotropic hypernetted chain (HNC) approximation (6). Results obtained with these methods are in a very good agreement with each other (5, 7, 8). The calculations indicate that in the presence of divalent counterions, the interaction between the surfaces is very sensitive to the correlations which are neglected in the PB approximation. The full electrical double layer contribution to the interaction between the surfaces or interaction between particles is often attractive and in our earlier work (9, 10) we have shown how this attractive double layer interaction affects the swelling of calcium clays. The method and the results up until mid 1989 are reviewed in (11) and (12).

In this report, we present HNC calculations of the electrostatic double layer interaction under the conditions relevant to the fusion of model membranes. Electrostatic interaction between weakly charged surfaces typical of biological membranes is normally repulsive. We show that in the presence of surface adsorption the addition of a low concentration of divalent counterions turns the electrostatic interaction into attraction. This effect significantly changes the adhesion of such membranes.

## METHOD

### Theoretical model

We use the anisotropic HNC approximation applied to a primitive model electrolyte between planar surfaces, as described in our earlier work (6, 11). To recapitulate very briefly, in the primitive model of electrolytes ions are approximated as charged hard spheres and the solvent as a dielectric continuum. In the anisotropic HNC approximation we use the standard HNC expressions adopted for the situation of a nonuniform ionic fluid between the surfaces. The accuracy of the calculation is the same as in the more common bulk electrolyte studies. Because the ionic fluid is never very dense, in absolute terms the accuracy is excellent. Nevertheless, it should be remembered that the primitive model of electrolytes has deficiencies, the worst of which is the neglect of the molecular structure of the aqueous solvent.

The double layer interaction calculated in the HNC approximation contains two contributions neglected in the PB theory: the ion correlation attraction and the hard core repulsion. The overall interaction can be either repulsive or attractive, with the attractive regime found at small surface separations when counterions have a valency of 2 or higher.

For this study, several new elements have been added to the model. The adsorption of counterions to the surfaces is described by the inclusion of a constant negative adsorption energy for any counterion

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in contact with a surface. This requires equilibration between the 3-dimensional distribution of ions in the bulk and the two-dimensional distribution of ions adsorbed to the surface. The equilibration is particularly easy within our calculational method, where space between surfaces is divided into a series of planes with constant ion density. The ions adsorbed to the surface are still free to move in the plane of the surface and they do correlate their positions with all other ions in the double layer.

The first and the last layer are assigned to the ions in contact with the surfaces. The ions in those layers are considered to have the energy lowered by a constant adsorption energy. We have chosen to describe the adsorbed layer as a true two-dimensional fluid in equilibrium with the three-dimensional diffuse ion distribution. From the relationship between the respective chemical potentials (see e.g., 6) it then follows that the thickness of the adsorbed layers should be taken as the thermal wavelength $\Lambda = h/(2\pi mkT)^{1/2}$ of the adsorbing ion. In this work we have used the value of $\Lambda = 0.1597$Å, corresponding to calcium ions. There are no specific bonding sites, and bound ions are free to move within the plane of the surface. However, the chemical potential of the adsorbed ions increases with the increasing density and the adsorption density cannot exceed the limit imposed by the finite size of the ions. Except for the constant adsorption energy, the adsorbed layer is treated like any other layer.

Our choice of describing the adsorbed ion layer as a two-dimensional fluid of counterions in contact with the surface is not an important element of the model. Any attractive short-ranged potential acting on the counterions in the immediate vicinity of the surface would have led to the same general results for the behavior of the double layer with adsorption. However, the numerical value of the adsorption energy parameter would have been different, depending on the assumed form of the short-ranged adsorption potential.

The numerical program has also been modified to include one more species of ions, so that it can be run with two species of counterions and one coion. This allows us to model mixed electrolyte systems, for example, a physiological concentration of 1:1 electrolyte with an addition of calcium. The equilibrium concentration of ions in each layer is obtained by maintaining the chemical potential for each species of ions throughout the whole system equal to that of the bulk reservoir. The procedure is the same as in the earlier work, and the equation imposed by the condition of electroneutrality is only slightly more complicated.

As bulk chemical potentials for mixed electrolytes in the HNC approximation were not available, they were calculated using the same program under the condition where surfaces are sufficiently far apart. Trial values for chemical potentials were adjusted until the calculated concentrations in the middle of the layer were equal to the desired bulk concentrations.

## Adsorption energy parameter for typical experimental models of adhesion

In the studies of membrane adhesion and fusion, commonly used model systems are vesicles formed from specific mixtures of neutral and charged phospholipids. To find a realistic value for the adsorption energy parameter needed in the calculation we can, for example, use the electrophoresis data by McLaughlin et al. (13) obtained with large multilamellar vesicles formed from phosphatidylcholine (PC) and phosphatidylserine (PS).

Fig. 4 of ref. (13) shows the zeta potential of vesicles formed from mixtures of PC and PS in the electrolyte containing different concentrations of Ca ions in the 0.1 M NaCl background. For a specified lipid composition, the measurements of the zeta potential indicate the divalent electrolyte concentration where the surface charge is fully compensated. Depending on the PS content, the zeta potential decreases to zero within the range of Ca concentrations between ~8 and 20 mM.

To find the value of the adsorption energy $E_0$, the anisotropic HNC computer program is equilibrated for a given value of the bare surface charge and divalent electrolyte concentration using different values for $E_0$. The resulting net surface charge is then plotted as a function of $E_0$, and an example of such a plot is shown in Fig. 1. The selected surface charge (250 Å$^2$ per unit charge) corresponds to ~3:1 ratio of PC to PS. The zeta potential data indicate that a vesicle membrane with the same PC/PS ratio should neutralize for Ca content between 10 and 20 mM. Referring back to Fig. 1, this indicates that the adsorption energy parameter $E_0$ for the divalent counterion is almost 6 kT. As mentioned above, this value is a model-dependent parameter which is useful only in the context of further calculations within the same model.

## RESULTS

To approximate physiological conditions, in all runs we maintained a background concentration of 0.1 M of 1:1 electrolyte. The surface charge was modeled as continuous and the electrostatic image potential was not included. Our earlier work (11, 14) has shown that the discreteness of surface charge and the electrostatic images have only a minor effect on the interaction between surfaces. The ion radius was set at 2.125 Å. Again, from the earlier work we know that if ionic radii are smaller than some 3 Å, the surface interaction depends only very weakly on the ion size. Under those conditions, the double layer interaction is repulsive (Fig. 2), with a value only slightly different from the corresponding result of the PB theory. For lower surface

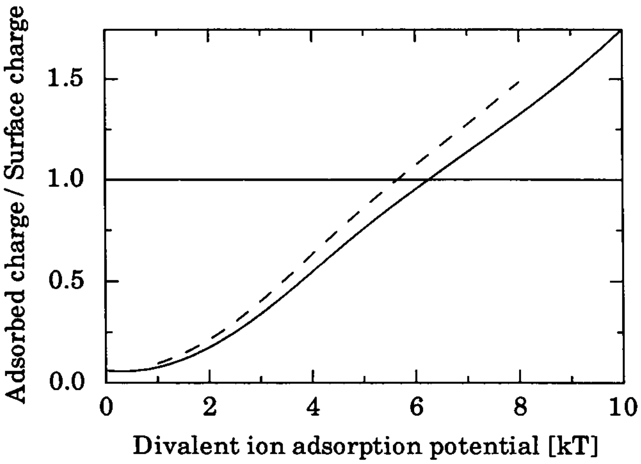

FIGURE 1 HNC results for the adsorption of divalent counterions to a surface with a bare charge of one elementary charge per 250 Å$^2$ in a background of 0.1 M 1:1 electrolyte. The ratio of the adsorbed charge to the bare charge is plotted as a function of the adsorption energy parameter $E_0$ for the 2:1 electrolyte concentrations of either 10 mM (*full line*) or 20 mM (*dashed line*).

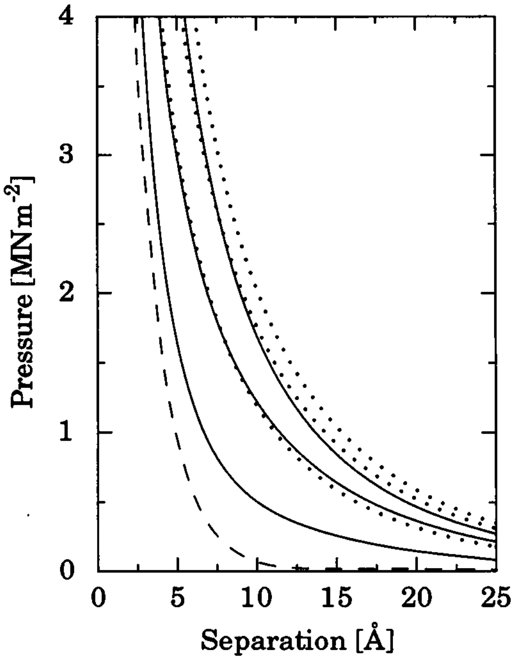

FIGURE 2 Electrostatic respulsion between the surfaces with a charge density of one elementary charge per 250 Å$^2$. Full lines, from top to bottom: HNC results in a background of 0.1 M 1:1 electrolyte with the addition of 0, 1 mM and 10 mM of 2:1 electrolyte respectively. (*Dotted lines*) The corresponding PB results. (*Dashed lines*) Only a 10 mM 2:1 electrolyte, without the monovalent background. It is assumed that the counterions do not adsorb to the surfaces.

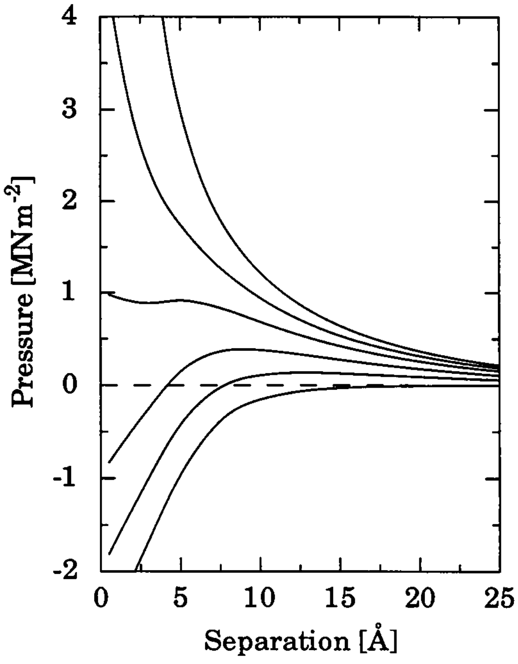

FIGURE 3 HNC results for the electrostatic repulsion between the surfaces bearing one elementary charge per 250 Å$^2$ in a solution of 0.1 M of 1:1 electrolyte and 1 mM of 2:1 electrolyte. The adsorption energy parameter values, from top to bottom: 0, 3 kT, 4 kT, 5 kT, 6 kT, and 8 kT.

charges, the HNC results are even closer to the PB theory.

It should be noted that in all figures, the zero separation is defined as the closest possible approach of the surfaces. For the HNC theory this is the separation where one layer of counterions remains confined between the surfaces, while for the PB theory (where ions have no size) the zero separation is the actual contact of the surfaces.

Addition of small amounts of divalent counterions decreased but did not eliminate the double layer repulsion. However, upon addition of divalent ions the differences between the HNC and PB results increased, with the accurate HNC values being significantly less repulsive. This is illustrated in Fig. 2 for the case where 1 or 10 mM of 2:1 electrolyte is added to the background of a monovalent electrolyte.

The effect of divalent counterion adsorption on the double layer repulsion is shown in Fig. 3. The rapid decrease in the repulsion is brought on by two effects: as the surface charge is partially compensated, the osmotic contribution to the repulsion proportional to the ion density in the middle of the double layer $(\rho_{mid} - \rho_{bulk})kT$ is diminished. As surfaces are brought closer together, more and more counterions adsorb to the surfaces (see Fig. 4). The osmotic contribution hence does not diverge as is the case for systems without adsorption.

The second effect is due to the adsorbed charge, which is still present as a discrete charged species on the surface. Adsorbed ions contribute to the overall interaction by correlating positions with the ions in the diffuse layers and on the other surface. This ion–ion correlation contribution is attractive and acts to further diminish the double-layer repulsion. A situation where at small surface separations the double layer interaction turns into an attraction is already reached for the adsorption energy parameter values lower than $E_0 = 6$ kT estimated for the PC/PS membranes in the methods section.

The adsorption of divalent counterions for the realistic value of $E_0 = 6$ kT and 1 mM of divalent salt is shown in Fig. 4. At large surface separations the surface charge on the membranes is decreased by about one half. The approach of the surfaces leads to increased adsorption of divalent counterions.

The strength of the double layer attraction under those conditions is compared to the corresponding PB result and to the Van der Waals interaction in Fig. 5. In the PB theory, we only see the double layer repulsion reduced by partial compensation of the surface charge

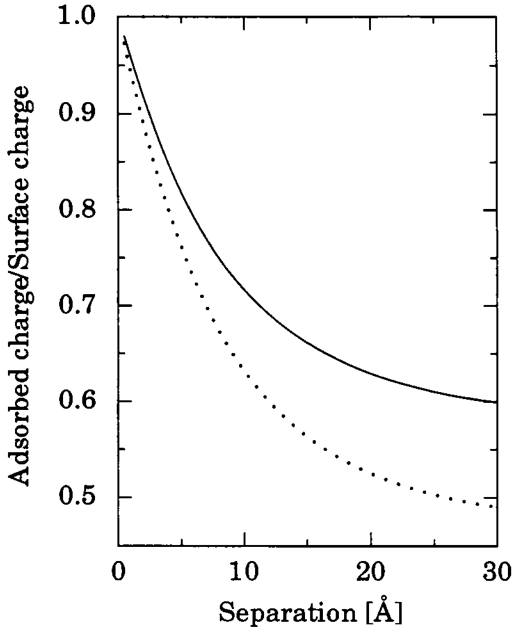

FIGURE 4 Partial compensation of the surface charge by divalent counterion adsorption shown as a function of the separation between the surfaces. The ionic solution contains 0.1 M of 1:1 electrolyte and 1 mM of 2:1 electrolyte, and the bare charge on the surfaces is assumed to be one elementary charge per 250 Å$^2$. The adsorption energy parameter is 6 kT. The HNC result is shown as a full line and the PB result as a dotted line.

by adsorbed divalent counterions. In the HNC calculation both free and adsorbed divalent counterions significantly contribute to the attractive correlation force between the surfaces.

As the Van der Waals force is not accurately known for very small separations, it can only be included to illustrate possible forms of the combined interaction. The main points of uncertainty are the exact position of the plane of the origin of the Van der Waals interaction and the contributions of the headgroup dipoles and quadrupoles (15). For the last 5 or 10 Å, even the continuum description of the Van der Waals interaction becomes progressively less accurate. In this example, we have taken the separation of the surfaces where Van der Waals force originates as 5 Å larger than the separation at the contact, accounting for the combined headgroup layers of the two membranes. The simplest nonretarded formula has been used with the Hamaker constant of $6 \cdot 10^{-21} J$.

## DISCUSSION

Could electrostatic interaction be a controlling factor in membrane adhesion? Earlier investigators have considered the reduction of the double-layer repulsion brought by the partial compensation of the surface charge upon adsorption of divalent counterions. In contrast, we have shown here that in the presence of divalent counterions the double layer interaction actually turns attractive and favors contact between weakly charged adsorbing surfaces. With the accurate evaluation of the attractive contribution due to ion–ion correlations, the effect of counterion adsorption on the double layer interaction is about twice as large as that predicted by the approximate PB theory.

The attractive contribution to the double-layer interaction can best be understood as a very low frequency part of the Van der Waals interaction. It is important when counterions are divalent, because the interaction energy between the ions is then larger in comparison with the thermal energy. This leads to significant correlations between the positions of both free and adsorbed ions, the corresponding lowering of the free energy of the system and the attractive force between the membranes.

Both PB and HNC calculations show strong increase

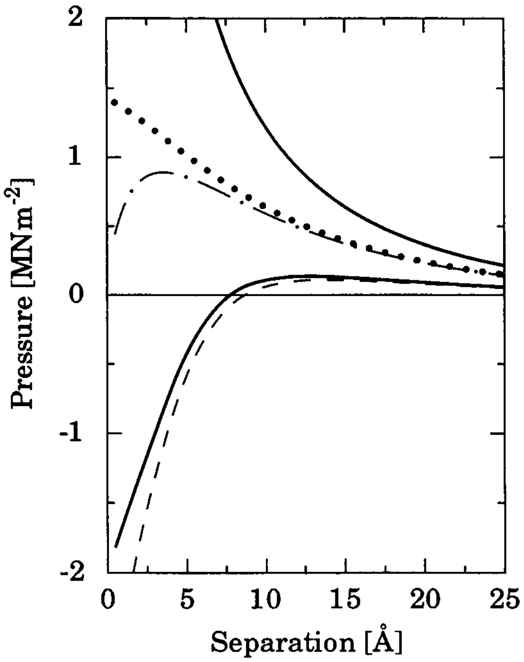

FIGURE 5 Electrostatic repulsion between the surfaces with a charge density of one elementary charge per 250 Å$^2$ in a solution of 0.1 M of 1:1 electrolyte and 1 mM of 2:1 electrolyte. The HNC results are shown without adsorption (*upper full line*) and with the adsorption energy parameter of 6 kT (*lower full line*). The PB result with the adsorption energy parameter of 6 kT is shown as a dotted line. The dashed and the dash-dotted curves are respectively the HNC and the PB electrostatic results in the presence of adsorption with the addition of the Van der Waals interaction, as described in the text.

in Ca adsorption as membrane surfaces are brought closer together. Nevertheless, the binding is not as cooperative as that seen by Feigenson (16) in the experiments on PS and PS/PC multilayers. In the later case, for closely apposed PS/PC multilayers strongly cooperative binding leads to the separate $Ca(PS)_2$ phase (16, 17).

As we have not considered the effects of solvent discreteness, the problem of membrane adhesion is certainly more complicated than our model can admit. Nevertheless, both theoretical (18) and experimental (10) work indicates that solvent or other short-range forces are approximately additive to the double-layer interaction. Other interactions which may affect membrane–membrane adhesion and have not been considered in this work are the contributions due to head group hydration and molecular motion.

In spite of these and other uncertainties of the electrical double layer description within the primitive model, experimental measurements of the adhesion strength for model bilayers by Evans and Needham (19) indicate that in 0.1 M NaCl, the free energy potentials for membrane adhesion accurately follows the predictions calculated from the usual double-layer repulsion and Van der Waals attraction. We have seen that for 1:1 electrolytes the PB calculations are accurate, and an agreement between the experiment and theory is in this case expected. However, in the present context the most interesting finding of Evans and Needham (19) is that the free energy of adhesion vanishes when the charged to neutral lipid ratio reaches the value between 0.05 and 0.10.

The surface charge on biological membranes is within this range, and we might speculate one step further. The membrane–membrane interaction seems to be electrostatically adjusted to a value where it is easily switched between the repulsion and attraction. At this point the adhesion energy is close to zero. Under such conditions, even the relatively weak double-layer attraction which is induced by the addition of divalent counterions can be a deciding factor in promoting membrane adhesion.

Our numerical examples relate only to model membranes, where quantitative adsorption data are available. In situations where biological membranes adsorb to each other, controlling concentrations of Ca are only of the order of $\mu$M. However, specific receptors increase the binding affinity for Ca by orders of magnitude. It therefore appears desirable to further examine the hypothesis where attractive electrostatic double-layer interaction is a mechanism which controls the adhesion of biological membranes.

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