

THE ATTRACTIVE FORCES BETWEEN POLAR LIPID BILAYERS

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ABSTRACT Long-range attractive forces between lipid bilayers are not well described by the Lifshitz theory of Van der Waals forces between macroscopic media. It is shown that when correlations between polar headgroups are taken into account, the predicted forces take a qualitatively different form consistent with the measured data.

INTRODUCTION

The attractive forces operating between lipid bilayers inferred from osmotic pressure experiments (1, 2) or measured directly (3–6) show some puzzling anomalies. For the first class of experiments (1, 2), the Hamaker constants are deduced by balancing an extrapolated repulsive hydration force with an assumed Van der Waals force at the equilibrium bilayer separation. The magnitude of the forces deduced varies by a factor of about five above or below that expected from Lifshitz theory for hydrocarbon-water systems, depending on the lipid involved (2). For the second set of experiments, the attractive forces have been measured accurately over a water thickness range from 20 to 60 Å. Again there are anomalies: sometimes the forces are about an order of magnitude larger than expected, and sometimes lower (4–6). More importantly, the decay with distance does not follow the power law dependence predicted by theory.

We are not concerned here with the strongly repulsive short range hydration force operating between lipids. The (larger) separation regimes where attractive forces dominate can be clearly demarked. In this distance regime we show that the apparent anomalies disappear if a previously neglected component of the forces is taken into account. These additional contributions arise from correlations between polar headgroups and can be treated in a dielectric continuum model explicitly (7). The forces within the model are due to the direct interaction between correlated dipoles on opposite surfaces, as well as to interactions with image charges which arise from the dielectric disparity between the intervening water phase the hydrocarbon bilayer interior. The previous perturbation analysis (7) was shown to describe the predictions of the model over experimental distance regimes of interest. Those theoretical results are used here. The effect of the headgroup correlations is to give a force contribution that is at first attractive

but which turns over to a repulsion at larger separations. The Lifshitz interaction can be substantially changed when the polar headgroup contribution is added, although in the cases we have examined the total force is always attractive.

We have reexamined the measured attractive forces between bilayers deposited on mica surfaces (4–6). We analyze data for plant digalactosyl-diglyceride (DGDG), L- α -dipalmitoyl-phosphatidyl-choline (DPPC), and L- α -dipalmitoyl-phosphatidyl-ethanolamine (DPPE). When the new force contributions are included, the experimental data is well described.

THEORY

The attractive pressures between bilayers deposited on mica substrates were originally analysed (4–6) using simple Hamaker theory for half spaces,

$$P = \frac{-A}{6\pi d^3} \quad (1)$$

where d is the thickness of the aqueous phase and the Hamaker constant for hydrocarbon, water is $A \sim 6 \cdot 10^{-21}$ J. A glance at the figure shows that the theory with this value does not fit the data. More suggestive is the difference in the qualitative behavior, since it is evident that the measured pressures decay faster than the cubic law predicted by the Hamaker theory. Since this theory is a very crude approximation to the Van der Waals forces acting between the bilayers, before seeking an alternative explanation for the data it is first necessary to compute the predictions of the complete Lifshitz theory. We use the Lifshitz theory for a mica-hydrocarbon-water triple film (8, 9). The required dielectric data are taken from the literature, for mica (10) and for water (11) and no ultraviolet interpolation is used (11). For the hydrocarbon, we used the measured thickness, ~ 50 Å (6), and dielectric

data for bulk hexane ($\epsilon_{hc} = 1.89$, $\omega_{uv} = 1.54 \cdot 10^{16} \text{ s}^{-1}$). There is a slightly larger attraction if values for bulk dodecane ($\epsilon_{hc} = 2.04$, $\omega_{uv} = 1.40 \cdot 10^{16} \text{ s}^{-1}$) are used instead. Since Lifshitz theory is formulated to describe interactions between uniform macroscopic dielectric media, it is more appropriate to use these bulk values than for example, the value ascribed to Lecithin bilayers, $\epsilon_{hc} = 2.143$ (12). This latter contains contributions from the polar headgroups, and we intend to treat these separately. Also, this value taken together with a hydrocarbon ionisation potential, ω_{uv} , predicts too large an attraction for the phospholipid data at large separations.

As already remarked, we use the perturbation theory (7) to describe the interactions between two, two-dimensional, rigid planar surfaces of mobile orientable point dipoles which represent the headgroups. Results calculated for zwitterions are of little difference at the separations we study here. The surfaces containing the dipoles are embedded in the central dielectric, $\epsilon_1 \approx 80$, and separated by a distance h . A distance w behind each surface is a different medium with dielectric constant ϵ_2 (see inset in the figure). Hence the width of the central dielectric is $d = h + 2w$. Since the dielectric constants of hydrocarbon and of the backing mica sheets are both low compared with water, we take the outer region to be a semi-infinite half-space with $\epsilon_2 \approx 2$. In perturbation theory one systematically expands the exponential of the interaction Hamiltonian. Here we carry out the expansion through second order; this gives the leading terms in ΔF , the interaction-free energy per unit area (7):

$$\Delta F_{10} = \frac{1}{4\pi^2} \left[\hat{G}_{xx}^{\circ}(0) \int f_+(k) dk + \hat{G}_{zz}^{\circ}(0) \int f_-(k) dk \right] \quad (2a)$$

$$\Delta F_{11} = \frac{-\beta}{2\pi\epsilon_1} \left\{ [\hat{G}_{xx}^{\circ}(0)]^2 \int kf_+(k) dk - [\hat{G}_{zz}^{\circ}(0)]^2 \int kf_-(k) dk \right\} \quad (2b)$$

$$\begin{aligned} \Delta F_{20} = & \frac{-\beta}{8\pi^2} \left[(\hat{G}_{xx}^{\circ}(0))^2 \int \{ [f_+(k)]^2 + [g_1(k) + g_+(k)]^2 \} dk \right. \\ & + 2\hat{G}_{xx}^{\circ}(0)\hat{G}_{zz}^{\circ}(0) \int \{ [f_1(k)e^{kd} \sinh(kh)]^2 \\ & + [g_1(k) - \epsilon_D f_1(k) \sinh(kh)]^2 \} dk \\ & + [\hat{G}_{zz}^{\circ}(0)]^2 \int \{ [f_-(k)]^2 \\ & \left. + [g_-(k) - g_1(k)]^2 \} dk \right], \quad (2c) \end{aligned}$$

where $\Delta F = \Delta F_{10} + \Delta F_{11} + \Delta F_{20}$ and we have defined

$$f_{\pm}(k) = \frac{2\pi}{\epsilon_1} \frac{2\epsilon_D k}{e^{2kd} - \epsilon_D^2}, \quad g_1(k) = \frac{2\pi}{\epsilon_1} k e^{-kh}$$

$$f_{\pm}(k) = f_1(k) [e^{kd} \cosh(hk) \pm \epsilon_D],$$

$$g_{\pm}(k) = f_1(k) [e^{kd} \pm \epsilon_D \cosh(hk)]$$

$\epsilon_D = (\epsilon_1 - \epsilon_2)/(\epsilon_1 + \epsilon_2)$ is a measure of the strength of the image interaction and $\beta = 1/k_B T$ is the inverse temperature. These functions arise from the two-dimensional Fourier representation of the direct and image electrostatic interaction potentials. The latter represent the dielectric response of the media to the surface dipoles. $\mathbf{G}^0(\mathbf{r})$ is the polarisation-polarisation correlation tensor of the isolated surface, which is long ranged (cf., reference 13).

ΔF_{10} is a first order perturbation contribution from the image interaction between dipoles on the same surface, using only the static susceptibility, $\hat{G}^0(0)$ —the Fourier transform of the polarisation correlation tensor $\hat{G}^0(\mathbf{k})$ evaluated at $k=0$. The resultant contribution to the pressure is repulsive, going like h^{-4} at large separations. ΔF_{20} is a second order term which again employs only the static susceptibility. It includes the direct correlational attraction between dipoles on opposite surfaces. While the corresponding contribution to the pressure decays as h^{-5} , for large enough susceptibilities its magnitude is such that it dominates for most of the separations of experimental interest, and is responsible for an increased attraction. The fact that the polarisation correlation tensor is long ranged leads to a subtlety in the perturbation theory and an additional term. Inclusion of the term linear in k (from the long range part of the correlation tensor) in the first order perturbation yields ΔF_{11} . This contribution to the pressure also decays asymptotically as h^{-5} . Since it arises from the long-wavelength behavior of the susceptibility, it represents an extension beyond the Lifshitz theory which uses only macroscopic k -independent dielectric constants. The pressure follows by differentiating these three leading terms in the expansion for the free energy with respect to separation.

In principle one could calculate the pressure numerically for this primitive model using one of the sophisticated numerical techniques of statistical mechanics, eg., the hypernetted chain (HNC) closure of the Ornstein-Zernike intergral equation. This has previously been done for mobile dipoles constrained to the perpendicular orientation (14, 7). A comparison with the results of the present perturbation analysis for the same system has shown that the latter does indeed predict accurate pressures, even at separations of the order of Angstroms (7). Accordingly, since our approximate treatment remains transparent and tractable without sacrificing accuracy, we use it here. Note that primitive model analyses do not include those particular bulk permanent dipole fluctuations that give rise to the zero frequency Van der Waals—Lifshitz interaction between the dielectric half-spaces. We emphasise that this term must be added to any calculated pressures (13). When one adds the two contributions, the repulsions due to images calculated before (7) lower the total attractive forces, but never dominate the Lifshitz term. For the present problem, we added the dipolar headgroup correlation contribution (Eq. 2) to the complete Lifshitz triple film calculation. The net force is then enhanced or reduced

with respect to the Lifshitz result, depending on both the headgroup susceptibility and on the particular separation.

COMPARISON OF THEORY AND EXPERIMENT

The experimental data presented in Fig. 1 is derived from Marra (6). The measurements were made via the jump method; i.e., by measuring the separation at which the gradient of the attractive force equalled the spring constant K_s . Then using the Derjaguin approximation, the pressure between two flat surfaces at that separation is $P = -K_s/2\pi R$, (R is the mean radius of the crossed mica cylinders). Plotting the data in the present form no longer implies a particular theory of the attractive force. However, experimental scatter and departure from theory are more apparent here than in the original presentation (4–6). It can be seen that the results for the two phospholipids lie on approximately the same curve and this is substantially below the data for the galactolipid. Upon the addition of 0.2 M NaCl, the attraction for DGDG is reduced. The separation is the experimental distance from $D = 0$, the anhydrous bilayer contact. Ambiguities in the interpretation of this distance (in the context of our point dipole model for the headgroups) have minimal effect at these large separations. These ambiguities do become more severe in interpreting the osmotic pressure experiments (1, 2). Note that

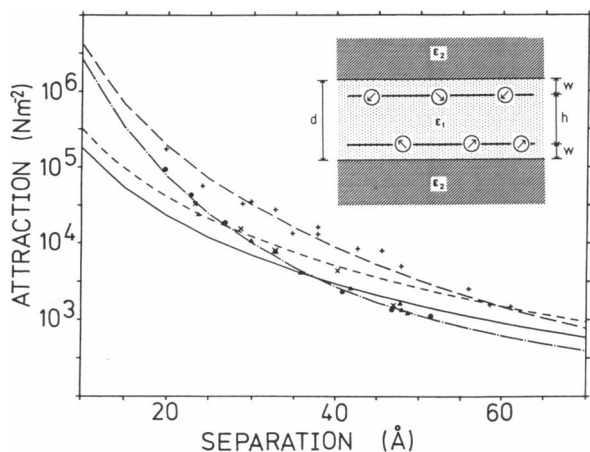


FIGURE 1 Direct force measurements (6) of the attractive pressure between digalactosyl-diglyceride (DGDG, +), phosphatidyl-choline (DPPC, O) and phosphatidyl-ethanolamine (DPPE, Δ) bilayers compared with various theories. It is clear that simple Hamaker theory, $A = 6.0 \cdot 10^{-21}$ J (----) has the wrong decay to ever fit the data. The same is true for the full retarded, triple-film Lifshitz theory (—) which uses dielectric data for bulk water, mica and hexane. However, the data can be described by adding to the Lifshitz result the pressure due to zero frequency correlations between dipoles of the polar headgroups, Eq. 2. Thus for DGDG (----) the susceptibility fitted with $\chi = 0.3$, and the distance of the dielectric discontinuity behind the dipoles was $w = 4$ Å. For the phospholipids (-·-·-·-) the parameters fitted were $\chi = 0.11$ and $w = 0.1$ Å. Note that the data for DGDG in 0.2 M NaCl (X), which we have not attempted to fit, indicates some additional headgroup contribution to the attraction at nonzero frequencies. *Inset.* Geometry of the primitive model of the polar headgroup interactions.

the large separation experimental attractions were measured by a quite different method to that used to measure the small separation adhesion energies. We do not analyze the latter here.

We have compared the simple Hamaker theory (Eq. 1) with the Lifshitz triple film calculation. It can be seen that a traditional value of the Hamaker constant ($A = 6 \cdot 10^{-21}$ J) overestimates the Van der Waals attraction. Note that if the Lifshitz data is fitted to a Hamaker form, the Hamaker “constant” varies from $3.4 \cdot 10^{-21}$ J at a separation of 10 Å to $4.0 \cdot 10^{-21}$ J at 60 Å. The Hamaker “constant” should decay with separation due to retardation. The weak variation is due to the simultaneous increase in the relative contribution of the mica to the triple film calculation. Previous authors (4–6) claimed a good description of the data was given by simple Hamaker theory but close inspection shows this to be untrue. Even by moving the so called plane of origin of the Van der Waals force (with respect to $D = 0$), or by choosing a different Hamaker constant, the data cannot be fitted. This is because the measured force decays faster than that given by Hamaker (or Lifshitz) theory between half-spaces. Since the contributions from surfaces decay faster than those from semi-infinite half-spaces (9), the measured data indicates that there is a substantial effect from the headgroups. Also the quantitative differences between the various lipids can be understood because of their different polar headgroups.

We have fitted the data by adding the contribution from the headgroup correlations (Eq. 2) to the Lifshitz results. While the model is fully anisotropic, we have considered only isotropic susceptibilities here, and these we present in the nondimensional form, $\chi_{xx} = \chi_{yy} = \chi_{zz} = \chi = \hat{G}^0(0)/q^2$ (q is the charge). For DGDG we have used a susceptibility of $\chi = 0.3$ and have located the effective dielectric discontinuity to be $w = 4$ Å behind the surfaces containing the dipoles. For the phospholipids the fitted parameters are $\chi = 0.11$ and $w = 0.1$ Å. Note that while the data for DGDG is always above the Lifshitz result, the phospholipid data lies below it for larger separations. Thus here with this choice of parameters, and indeed generally for this model, the phospholipid headgroups must experience an image repulsion (ΔF_1 dominates). This lowers the net attractive force for these lipids in this large distance regime. Changing to $w = 1$ Å decreases the fitted attraction by 30%. This is for the phospholipid at small separations; at larger separations the results are quite insensitive to the value of w . Similarly, doubling the susceptibility increases the attraction by about 70%. The fitted values for the susceptibility are of the same order as those found by Monte Carlo simulations of the model system (Bo Jönsson, pers. com.). The qualitative behaviour of the curves, in particular the initially steep decay, is similar for any other reasonable parameters.

These parameters enable one to obtain quite a good fit to the experimental data, but their actual value obviously has to be treated with caution. The primitive model is a gross

simplification of reality. Besides this, it is clear that there must be some further effects not taken into account. To see this consider the forces in the presence of high salt. Here all the zero frequency terms (including the polar headgroup correlations) should be completely screened (9). Since the data for DGDG in 0.2M NaCl remains above the Lifshitz result (at smaller separations at least) and still exhibits a faster decay, it can be presumed that the measured forces include additional contributions from higher frequency correlations associated with the headgroups. Since all of these effects are subsumed in our zero-frequency model dipolar correlation term, the parameters must be regarded as effective rather than literal.

The primitive model perturbation analysis predicts that correlations between the dipolar headgroups can give rise to attractions many times larger than the conventional Van der Waals force between dielectric half-spaces. Here we have analyzed data for some polar lipid bilayers and shown it to be well described when these surface terms are included. The actual values taken by these fitted parameters are not as important as is our main theme: that headgroup correlations must be involved in order to account for both the variation with lipid type and especially the rate of decay of the forces. The data cannot be described by the Lifshitz theory of macroscopic media. The different susceptibilities we fit reflect a specificity of the polarisation correlations due to the different lipid headgroups (size, polarity, mobility, and flexibility), as one might expect. Depending on the particular headgroup, and on the separation, the attractive force can be above or below the usual Hamaker or Lifshitz prediction. The variation in Hamaker constants deduced from osmotic pressure experiments (2) might similarly be ascribed to different headgroups. This is not clear at this stage because of ambiguities in assigning a model distance and in extrapolation of the fitted hydration force. That technique measures repulsive forces only and so one cannot directly analyse such data with this theory. It appears that one cannot ignore the contributions of the polar headgroup correlations to the attractive forces between lipid bilayers.

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