Comparison of the van der Waals and Undulation Interactions between Uncharged Lipid Bilayers

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A calculation of the long-range interaction energies between two undulating neutral lipid bilayers was performed. At bilayer spacings above several nanometers, the bilayer interaction can be considered to be composed solely of an attractive van der Waals component plus a repulsive undulation component arising from the entropic confinement of thermally excited out-of-plane motion of the bilayers by neighboring bilayers. The objective of this work was to determine if the long-range swelling behavior that has been observed in neutral multilamellar lecithin systems (e.g., bilayer spacings as large as 150 Å), can in fact be explained by the undulation repulsion, which is the currently accepted explanation. It was found that the relative magnitudes of the long-range van der Waals and undulation energies are essentially determined by the magnitude of the dimensionless ratio $A_H K_b/(kT)^2$, where A_H is the Hamaker constant, K_b is the elastic bending modulus of the bilayer, and kT is the thermal energy. When this value is much greater than 1, the van der Waals attraction dominates at large bilayer spacings, while the opposite is true when this ratio is much smaller than 1. Using the measured physical parameters for lecithin systems, the value of the dimensionless ratio is over 50 and the long-range retarded van der Waals interaction remains well over 1 order of magnitude greater than the undulation repulsion. This difference is far too large to be explained by uncertainties in the values of the key system parameters and suggests that another mechanism is responsible for the swelling of the lecithin bilayers.

Introduction

The observation that multilamellar stacks of uncharged lipid bilayers can spontaneously swell in aqueous solutions has been reported by various authors. Perhaps the most comprehensive study of this phenomenon was performed by Helfrich and co-workers using neutral lecithin bilayers. For example, Harbich et al.[1] found that lecithins above the crystalline−fluid phase transition temperature always swelled and usually disintegrated into giant vesicles. Harbich and Helfrich[2] measured increases in membrane spacing for the well-studied egg lecithin−water system up to 150 Å with no sign of an equilibrium spacing or phase separation. Similar findings were reported by Servuss and Helfrich[3] using egg and dimyristoyl lecithin bilayers and Servuss et al.[4] again using egg lecithin.

At the same time, the exact mechanism responsible for this swelling has been a subject of much interest. Since the membranes are electrically neutral, the swelling can obviously not be due to a long-range screened Coulombic repulsion. Likewise, because of the large spacings involved, the swelling cannot be explained by the traditional hydration forces arising from orientation of water molecules, since the decay length of these forces (2−3 Å) is much too small.[5−10] Instead, the currently accepted opinion is that the repulsion arises from the entropic confinement of thermally excited undulations of the bilayers by neighboring bilayers.

The idea that out-of-plane undulations could lead to repulsive interactions between lipid bilayers was first proposed by Helfrich in the late 1970s.[11] Specifically, because of their inherent flexibility, the bilayers are in a constant state of thermal motion, yielding undulations which can actually be seen under a light microscope.[3] When two bilayers approach, this motion becomes constrained, which is entropically unfavorable, and a repulsive force arises. Helfrich demonstrated that unlike the hydration forces, this undulation repulsion can act over relatively large bilayer spacings and thus could possibly account for the observed swelling.

Recently, Israelachvili and Wennerström[12] proposed three additional types of entropic restrictions that can exist between bilayers: a headgroup overlap, or steric, repulsion; a peristaltic repulsion due to the confinement of squeezing motion; and a protrusion interaction due to the confinement of out-of-plane motion of the individual surfactant molecules comprising the bilayers. However, because these interactions are important only at separations less than 2 nm, they do not contribute significantly to the long-range repulsion needed to explain the swelling behavior.

Although the expression for the undulation repulsion can be derived in different ways, the equation is always of the form

$$P_{\mathrm{und}}(D) \propto (kT)^2/K_b D^3 \qquad (1)$$

where $P_{\mathrm{und}}(D)$ is the repulsive pressure, D is the mean spacing between two bilayers, kT is the thermal energy, and K_b is the elastic bending modulus, which is a unique physical property of each bilayer. The importance of this equation lies in the inverse third dependence on bilayer separation. Specifically, at

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gap widths much larger than the bilayer thickness, the van der Waals attractive force between two thin parallel planar plates decays with distance to the negative fifth power (retardation effects would produce an even faster decay). This means that the undulation repulsion should always dominate at sufficiently large separations, regardless of the physical parameters of the system. For example, Harbich and Helfrich[2] state that "... at all spacings van der Waals attraction is overwhelmed by hydration and undulation forces."

It is clear, however, that this comparison between the interactions is somewhat inconsistent, since the presence of the undulations could have a major effect on the van der Waals interaction. Specifically, because sections of the two bilayers will actually be closer than the mean spacing, the true van der Waals interaction could be significantly larger than that between two parallel planar plates, meaning that it is possible that the undulation repulsion may *never* exceed the van der Waals attraction. Alternatively, the separation distance at which the undulation repulsion actually overcomes the van der Waals attraction may be increased to the point that the undulation interaction cannot, in fact, explain the observed swelling. Our objective in this paper was thus to perform a more consistent comparison of the long-range behavior of the two interactions.

Before proceeding, we should mention that an analysis of the effect of undulations on the interaction between *charged* bilayers was performed by Podgornik and Parsegian.[13] The authors used a Feynman-type variational procedure to obtain the partition function for an undulating bilayer whose out-of-plane displacements were calculated using the solution to the one-dimensional diffusion equation in a confined geometry. This approach allowed examining the coupling between the undulatory motion of the bilayers and the "direct" confinement forces (i.e., hydration, electrostatic and van der Waals forces). The authors showed that when the electrostatic contribution to the confinement force is sufficiently large, the net bilayer interaction can be repulsive at all separation distances.

In contrast to the work of Podgornik and Parsegian, our interest in the present paper is the relative magnitudes of the repulsive undulation and attractive van der Waals energies in *uncharged* systems at bilayer spacings comparable to those observed in the swelling experiments of Helfrich and coworkers. Surprisingly, we find that even when retardation effects are included, the van der Waals interaction between two undulating lecithin bilayers remains at least 1 order of magnitude greater than the undulation repulsion out to bilayers spacing as large as 50 nm. This finding clearly contradicts the assertion of Helfrich that the van der Waals interaction is overwhelmed by the undulation repulsion. A detailed description of this work is presented in what follows.

Theory

Description of Undulating Bilayers. A description of the physical system and definition of relevant variables is presented in Figure 1. An undulating bilayer of thickness t is constrained between two solid plates, also of thickness t. The composition and physical properties of each of the restricting plates are assumed to be the same as that of the undulating bilayer. The distance between the mean surface of the bilayer and the opposing plate is D, the local out-of-plane displacement of the bilayer at any position along its surface is u, and h is the local gap width between the bilayer surface and opposing plate. The minimum approach distance between the bilayer and plate (minimum h), is δ. It is easy to show that h ranges between δ and $2D - \delta$ while u ranges between $-(D - \delta)$ and $D - \delta$.

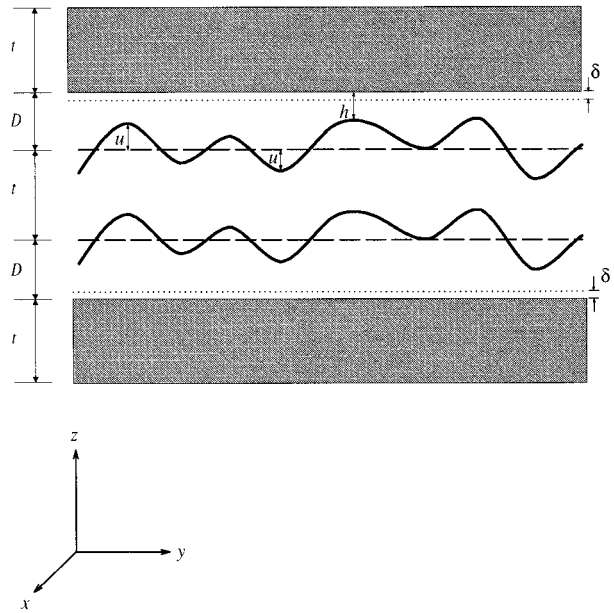

Figure 1. Schematic showing the physical system under consideration and the important variables used. A lipid bilayer of thickness t is confined between two solid plates, each of thickness t, across an average gap width D. The local out-of-plane displacement of the bilayer is u and the distance between the bilayer and top plate at any position is h. The minimum approach distance between the bilayer and plate, which is set by the range of the hydration repulsion, is δ.

Because of thermal undulations, the actual distance between the bilayer and the solid wall, h, will vary over the xy plane. The probability density function describing the displacements of the bilayer out of the xy plane, $\rho(u)$, has been discussed by various authors. If one assumes that the bilayer fluctuations are purely random and uncorrelated, then the distribution function will satisfy the equation

$$2\frac{\partial\rho(u)}{\partial\sigma^2} = \frac{\partial^2\rho(u)}{\partial u^2} \tag{2}$$

which is analogous to a diffusion equation.[13] Here σ^2 is the variance of the out-of-plane displacements of the bilayer. The boundary conditions for eq 2 are that the probability density function must equal zero when the displacement equals $\pm(D - \delta)$, leading to the solution

$$\rho(u) = \frac{\sum_{n=0}^{\infty} \exp[-(1/2)(2n+2)^2(\pi/2)^2(\sigma/\Delta)^2]\cos[(2n+1)(\pi/2)(u/\Delta)]}{2\sum_{n=0}^{\infty}\frac{(-1)^n}{(2n+1)(\pi/2\Delta)}\exp[-(1/2)(2n+2)^2(\pi/2)^2(\sigma/\Delta)^2]} \tag{3}$$

where $\Delta = D - \delta$. (Note that a typographical error appears in the denominator of this equation in the paper of Podgornik and Parsegian.[13]) The value of the variance can be found from

$$\sigma^2 = \int_{-\Delta}^{\Delta} u^2\rho(u)\,\mathrm{d}u \tag{4}$$

Using the approach of Helfrich, the variance of the distribution can be written as a function of the effective gap width

$$\sigma^2 = \mu(D - \delta)^2 \tag{5}$$

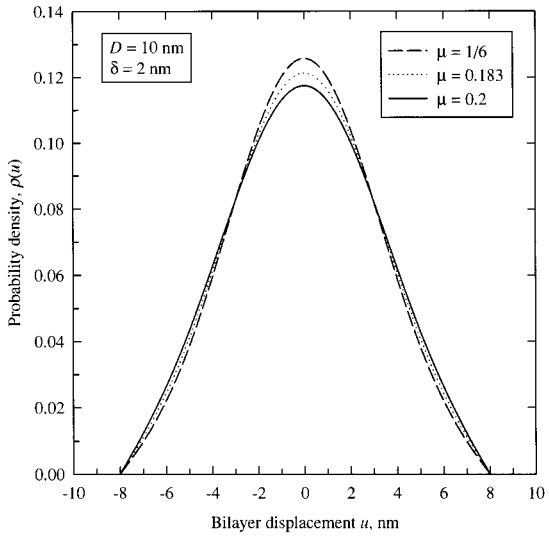

Figure 2. Dentiy distribution of displacements, u, at various values of the dimensionless parameter μ, defined as $\mu = \sigma^2/(D - \delta)^2$, where σ is the standard deviation of the displacements. The values of μ shown correspond to the values recommended by Helfrich (1/6),[11] Podgornik and Parsegian (0.183),[13] and Janke and Kleinert (0.2).[14]

where μ is a constant. Podgornik and Parsegian obtain $\mu \approx 0.183$, which is within 10% of the value of $^1/_6$ estimated by Helfrich.[11] Janke and Kleinert[14] performed a detailed Monte Carlo simulation to obtain the distribution of displacements and determined $\mu = 0.2$ at 20 °C.

A graph of the probability density for these three values of μ is given in Figure 2. The calculations were performed using a bilayer spacing, D, equal to 10 nm and a minimum approach distance, δ, of 2 nm. As seen, the distributions are quite similar, especially at the larger values of u that contribute most to the van der Waals interaction because of the corresponding smaller distance from the opposing plate, h (see Figure 1).

As mentioned above, although the exact expression for the undulation repulsion has been independently derived by various researchers, the result is always of the form given in eq 1. The only differences lie in the value of the numerical prefactor. We can thus write the undulation repulsion as

$$P_{\text{und}}(D) = C\frac{(kT)^2}{K_b D^3} \tag{6}$$

where C is a constant. Israelachvili and Wennerström[12] showed that the form of this equation (everything except the value of the prefactor) could be derived via a simple application of the contact value theorem. Podgornik and Parsegian[13] used a Feynman-type variational principle to express the partition function for a stack of multilayer membranes containing both hard (steric) and soft (electrostatic and van der Waals) confinement potentials. In their system, a single elastic membrane is bounded by two rigid surfaces, much like the situation depicted in Figure 1. The total energy of each membrane was assumed to be composed of an intrinsic component arising from elastic deformation plus a second component accounting for the interaction of the membrane with its neighbors. Their final expression was $C = 1/(64\mu)$, where μ was defined above by the expression $\sigma^2 = \mu D^2$. Janke and Kleinert[14] followed the same procedure as Helfrich by assuming that the undulating bilayer is constrained by its neighbors in a harmonic potential. As the bilayer area increased to infinity, the authors found that the numerical prefactor approached an asymptotic value of $C = 0.060 \pm 0.003$, which is within 30% of the value estimated by Podgornik and Parsegian.

On the other hand, the prefactor suggested by Helfrich was considerably larger.[11] Although he presents a number of possible values of the prefactor, his final recommended value is 0.84, an order of magnitude larger than either of the other two values. He himself-states, however, that this value is not very reliable and suggests that the "... correct value could be between twice and half as large." We have thus chosen to use the value of $C = 0.060$ obtained from the Monte Carlo simulations of Janke and Kleinert for the results presented below.

It is appropriate at this point to discuss the value of the minimum approach distance, δ, and its effect on the above equations. As two bilayers approach, strong, short-ranged repulsive hydration forces dominate the interaction. As mentioned above, the characteristic decay length of this force has been predicted theoretically and observed experimentally by various authors to lie in the range of 2−3 Å. One can account for this force in these calculations by shifting the plane of origin of the undulation force outward from each plate by an amount δ, as shown in Figure 1. Our final expression for the repulsive undulation pressure is thus

$$P_{\text{und}}(D) = 0.06\frac{(kT)^2}{K_b(D-\delta)^3} \tag{7}$$

Regarding the value of δ, Servuss and Helfrich[3] suggest that the hydration force manifests itself as "... an effective thickening of the bilayers by 1 nm on each side." This corresponds to a distance of closest approach, δ, equal to 2 nm. The sensitivity of both the undulation and van der Waals interactions to the value of δ is discussed in greater detail below.

According to eq 7, the undulation repulsion is inversely proportional to the elastic bending modulus, K_b, which is specific to each type of bilayer. For the well-studied lecithin bilayers, experimental values range from 9×10^{-20} to 2×10^{-19} J.[15] For example, Servuss et al.[4] measured the average curvature of various lecithin bilayers under a phase contrast microscope and obtained a value for egg lecithin of $(2.3 \pm 0.3) \times 10^{-19}$ J. For very flexible systems, such as the SDS bilayers used by Safinya et al.,[15] the value can be as low as one kT (4×10^{-21} J). Brochard and Lennon[16] estimate the value of K_b for human red blood cell membranes to lie in the range of $(1.3-3) \times 10^{-20}$ J.

Although direct measurements are difficult, there have been some experimental results supporting the general form of eq 7. Safinya et al.[15] used X-ray scattering to study the structure of lamellar liquid crystals composed of SDS bilayers in a mixture of pentanol, water, and dodecane. For membrane gap distances between approximately 4 and 16 nm, the power-law behavior of the first harmonic of the scattering structure factor was accurately described using eq 7. Abillon and Perez[17] used the surface force apparatus to measure the repulsive pressure between two mica surfaces confining swollen CTAB/hexanol/brine bilayers. The range of gap widths studied was too small to allow an accurate determination of the distance scaling exponent; however, the authors used the Helfrich expression for the undulation repulsion to estimate an elastic bending modulus of $5.5 \pm 3\ kT$ for the bilayer. This value was somewhat higher than those measured in other similar systems but of the same order of magnitude.

Comparison of the Interactions When the van der Waals Interaction Is Calculated as for Planar Films. A comparison between the undulation repulsion and van der Waals attraction is shown in Figure 3. The physical values used ($K_b = 2 \times 10^{-19}$

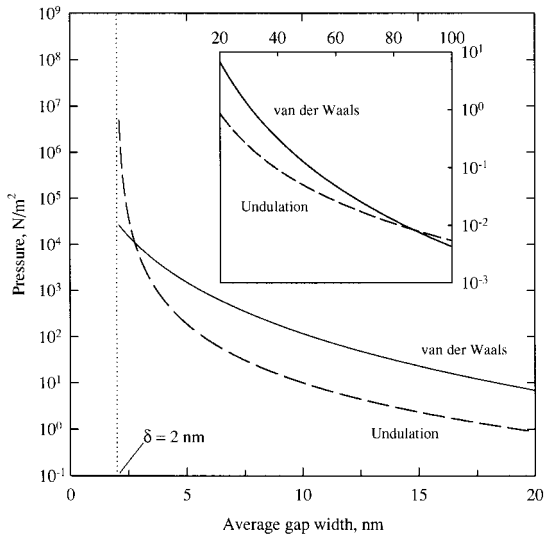

Figure 3. Comparison of the magnitude of the undulation repulsive pressure (eq 7) between two lipid bilayers to the van der Waals attraction calculated by treating the bilayers as two smooth planar plates (eq 8). The thickness of each bilayer, t, was taken to be 4 nm, the Hamaker constant was 5×10^{-21} J, and the elastic bending modulus, K_b, was 2×10^{-19} J. The insert shows the two forces at very large gap widths. Note that the van der Waals force shown here does not include retardation effects.

J, $A_H = 5 \times 10^{-21}$ J, and $t = 4$ nm) are typical for egg lecithin bilayers.[4,12,18] The van der Waals force was calculated assuming two planar films (no undulations) and ignoring retardation effects. Under these conditions, the van der Waals pressure can be calculated analytically using the expression

$$P_{vdw}(D) = -\frac{A_H}{6\pi}\left[\frac{1}{D^3} + \frac{1}{(D+2t)^3} - \frac{2}{(D+t)^3}\right] \qquad (8)$$

where t is the film thickness and A_H is the Hamaker constant. (The negative of this pressure and/or energy is presented in this and all subsequent graphs.)

For these calculations, δ was set to 2 nm; thus the undulation repulsion diverges as this separation is approached. The results of interest here, however, are the long-range behavior. As seen, for gap widths between 10 and 20 nm, the van der Waals interaction remains roughly an order of magnitude larger than the undulation repulsion. At larger separations, however (seen in the insert), the faster decay of the van der Waals interaction becomes apparent such that eventually the undulation repulsion again dominates. It is interesting, however, that this does not occur until the bilayer spacing reaches some 90 nm.

van der Waals Interaction between Undulating Surfaces. Calculation of the van der Waals interaction between the undulating bilayer shown in Figure 1 and one of the opposing plates was performed using a summation approach. The exact procedure involved two steps. First, the retarded interaction profile (energy vs separation distance) between two smooth plates of equal thickness t was found as described below. The interaction energy at any gap width D for the undulating case was then calculated using the expression

$$E_{vdw}(D) = \int_{-(D-\delta)}^{D-\delta} \rho(u) E_{pp}(D-u)\, du \qquad (9)$$

where $\rho(u)$ is the probability distribution of displacements given by eq 3 and $E_{pp}(D-u)$ is the retarded van der Waals attraction between two parallel plates separated by distance h (related to u as $h = D - u$), calculated as described below. The integration is performed over all displacements u. The justification behind the use of this equation is as follows. Consider a bilayer whose lateral dimensions are much larger than both the average gap width and the characteristic lateral dimensions of the undulations. Since an additivity approach is used, the order in which the summation is performed is irrelevant. In addition, the interaction of any "box" of the undulating bilayer of lateral dimensions Δx and Δy and thickness t depends only on the separation distance of that box, $h(x,y)$, from the opposing smooth plate. This means that the undulating bilayer can be sliced into a set of boxes, each of dimension Δx and Δy, and then rearranged so that all boxes having equal $h(x,y)$ are placed together, forming a set of parallel plates. The relative lateral area of each plate is described by the probability distribution function of eq 3.

As mentioned above, the first step of this procedure was to calculate the retarded interaction profile between two smooth plates of thickness t. This was done using the Hamaker additivity approach, in which the interaction between two differential volume elements, dV_1 and dV_2, in each plate separated by distance r is calculated as

$$E = -\frac{\beta}{r^6}\rho_1\rho_2\, dV_1\, dV_2 \qquad (10)$$

where β is the London interaction coefficient and ρ_1 and ρ_2 are the number density of molecules in dV_1 and dV_2, respectively. The Hamaker constant, A_H, is related to these quantities via $A_H = \pi^2\beta\rho_2\rho_2$. To account for possible retardation at larger separation distances, the empirical Overbeek correction factor,[19] $f(p)$, which is based on the Casimir-Polder[20] equations, was used. Thus

$$E = -\frac{\beta}{r^6}\rho_1\rho_2 f(p)\, dV_1\, dV_2 \qquad (11)$$

where p is a dimensionless separation distance defined as $p = 2\pi r/\lambda$ and λ is the characteristic retardation wavelength of the interaction. Overbeek gives the following expression for $f(p)$

$$f(p) = 1.01 - 0.14p \qquad \text{for } 0 < p < 3$$
$$= \frac{2.45}{p} - \frac{2.04}{p^2} \qquad \text{for } p > 3 \qquad (12)$$

The complete van der Waals interaction is obtained by inserting eq 12 into eq 11 and numerically integrating the resulting expression over the volumes of both plates (a sixth order integral). A 15-point Gauss−Legendre routine was used to compute the integrals. For each separation distance, the lateral dimensions of each plate (x and y dimensions) were taken to be at least 2 orders of magnitude larger than the gap width to minimize edge effects.

Various estimates of the value of λ have appeared in the literature, ranging from 100[19] to 200 nm.[21,22] Recently, Suresh and Walz[23] obtained values of 40−50 nm by fitting the interaction between two planar half-spaces calculated using the Hamaker approach to that calculated using Lifshitz theory. One explanation given for the lower values was the additional screening of the interaction produced at long range by electrolytes in solution.

To check the accuracy of the numerical integration, a comparison was made with the analytical equation for the interaction energy between two infinite, thin planar films

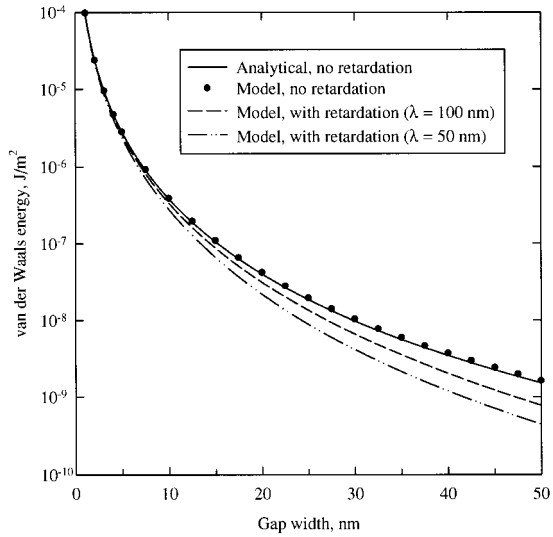

Figure 4. Magnitude of the van der Waals interaction energy between two thin planar plates each of thickness t equal to 4 nm. The solid line was calculated using the analytical expression of eq 13 while the symbols were calculated using the model described in the text, ignoring retardation. The two lower lines (dash and dash-dot-dot) were also calculated using the model; however, retardation effects were included via eq 12. For the dashed line, the retardation wavelength, λ, was set to 100 nm while for the dash-dot-dot line, λ was set to 50 nm.

ignoring retardation. For two plates of thickness t, this equation is

$$\frac{E_{\mathrm{vdw}}(D)}{\mathrm{area}} = -\frac{A_{\mathrm{H}}}{12\pi}\left[\frac{1}{D^2} + \frac{1}{(D+2t)^2} - \frac{2}{(D+t)^2}\right] \quad (13)$$

which can be derived by integrating eq 8 from infinity to any bilayer spacing D. The results are shown in Figure 4, using $t = 4$ nm and $A_{\mathrm{H}} = 5 \times 10^{-21}$ J. (Again, the negative of the interactions is shown here.) At all gap widths, the difference between the model results (symbols) and the analytical results (solid line) is less than 10%. Also shown is the interaction when retardation is included via eq 12. Results obtained with values of the characteristic wavelength, λ, equal to 50 and 100 nm are given for comparison. For both cases, retardation effects clearly become important at gap widths above 10 nm. The faster decay associated with the smaller retardation wavelength is also clearly visible. For example, the interaction energy at a separation distance of 20 nm calculated with λ equal to 50 nm is approximately 33% lower than the corresponding value calculated with λ equal to 100 nm.

Results

The effect of undulations on the retarded van der Waals interaction is shown in Figure 5. For these and all subsequent results, the retardation wavelength, λ, has been set to 100 nm. (The effect of this value is reviewed in the Discussion section.) The top graph (Figure 5a) shows the dependence on the distribution of displacements (characterized by μ) at a constant minimum approach value of $\delta = 2$ nm, while the bottom graph (Figure 5b) shows the dependence on δ at a constant distribution of displacements ($\mu = 2$). In each graph, the solid line gives the van der Waals interaction with no displacements (i.e., two thin planar plates), while the dashed line is the interaction calculated using the recommended values of μ and δ for lecithin bilayers, 0.2 and 2 nm, respectively, as discussed above.

Several trends in these results should be emphasized. First, the presence of the undulations significantly increases the

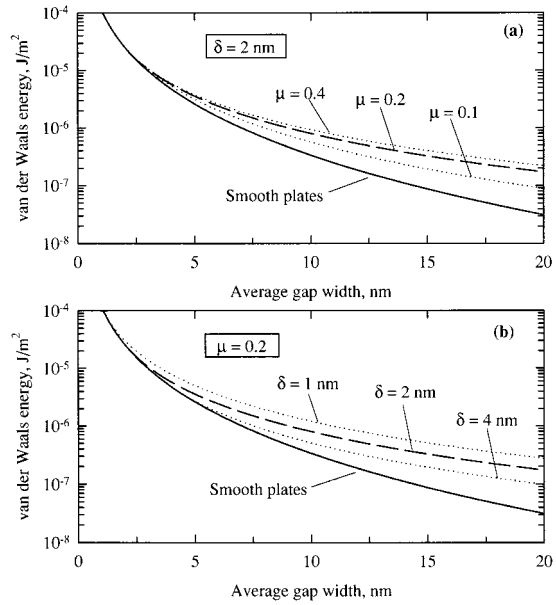

Figure 5. Effect of undulations on the van der Waals interaction energy between the undulating and smooth bilayers depicted in Figure 1. (a) Effect of changes in the displacement parameter μ at constant minimum approach distance, δ; (b) Effect of changes in δ keeping a constant μ. Also shown in each graph is the result between two smooth planar plates (i.e., no undulations present).

effective van der Waals attraction between the bilayers. For example, using the recommended values of μ and δ, the interaction is 2.4 times larger than that between two parallel plates at an average gap width of 10 nm, and 5.1 times larger at a 20 nm gap. Second, the changes in the effective interactions with changes in μ and δ are as expected. For example, as δ is increased at a constant μ (i.e., increasing minimum approach distance), the van der Waals interaction is reduced. On the other hand, when μ is increased at a constant minimum approach distance, meaning a greater fraction of the bilayer will be at smaller values of h, the van der Waals interaction increases.

A comparison of the revised van der Waals interaction (including undulations) with the undulation repulsion, calculated with the Janke and Kleinert[14] parameters, is shown in Figure 6. These results, which are really the crucial results of this study, indicate that when the currently accepted values of the physical parameters are used (e.g., Hamaker constant, elastic modulus, distribution of displacements), the van der Waals attraction remains substantially greater than the undulation repulsion at bilayer spacings between 5 and 20 nm. Specifically, at bilayer spacings of 5, 10, 15, and 20 nm, the ratio of the van der Waals energy to the undulation energy is 12.8, 20.3, 22.0, and 22.3, respectively. (Note that if a value of δ equal to 1 nm had been used in these calculations, corresponding to a shorter length scale for the hydration repulsion, these ratios would be increased to 16.2, 27.5, 30.8, and 31.9, respectively.) The meaning of these results is discussed in greater detail below.

It is interesting that at a fixed value of the displacement distribution parameter μ, both the undulation repulsion and the van der Waals attraction profiles can be defined by essentially two physical parameters—the elastic bending modulus and the Hamaker constant. Specifically, the ratio of the two interactions (i.e., $E_{\mathrm{vdw}}/E_{\mathrm{und}}$) will be a function of the dimensionless ratio $A_{\mathrm{H}}K_{\mathrm{b}}/(kT)^2$. A graph showing this dependence is shown in Figure 7. As seen, at separations well above the distance of minimum approach δ, the ratio of the two energies reaches a nearly constant value, independent of the bilayer gap width. This arises because the modified van der Waals interaction decays es-

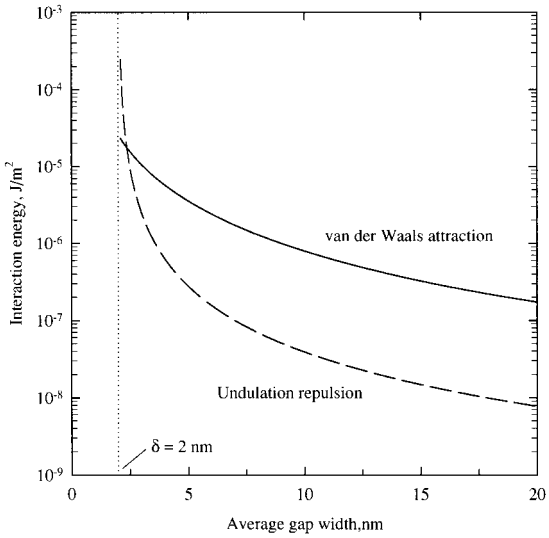

Figure 6. Magnitudes of the van der Waals and undulation interaction energies calculated using the currently accepted values for the key physical parameters (i.e., $A_H = 5 \times 10^{-21}$ J, $K_b = 2 \times 10^{-19}$ J, $t = 4$ nm, $\delta = 2$ nm, and $\mu = 0.2$). At an average gap width of 20 nm, the van der Waals attraction is approximately 23 times larger than the undulation repulsion.

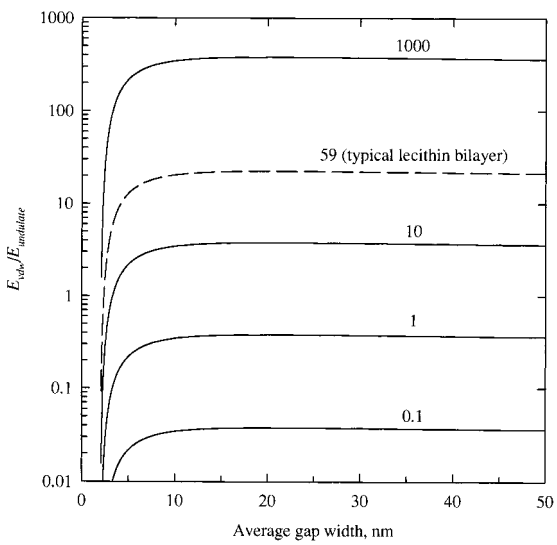

Figure 7. Ratio of the van der Waals attractive and undulation repulsive energies for various values of the dimensionless parameter $A_H K_b/(kT)^2$. The dashed line corresponds to a typical lecithin bilayer, calculated using the values given in the caption to Figure 6. The nearly constant ratio at larger spacings arises because both interactions decay with approximately the same decay rate (distance to the inverse second power) in this range.

sentially with distance to the inverse second power over this range of gap widths, just like the undulation repulsion (the larger undulation repulsion at small gaps results from the use of $D - \delta$ in eq 7). This trend was also predicted in the theoretical work of Podgornik and Parsegian.[13]

We should mention that because of retardation, the decay of the van der Waals interaction with distance is actually slightly faster than the inverse second power. This faster decay, however, would only become important at much larger bilayer spacings. Over the range of spacings observed in the swelling experiments of Helfrich and co-workers (i.e., $\leq$20 nm gap widths), the inverse second power decay is clearly valid.

The results in Figure 7 also allow using the value of the $A_H K_b/(kT)^2$ as a criterion for determining the relative magnitude of the van der Waals and undulation interactions. Specifically, when the ratio is much larger than 1, the van der Waals attraction will dominate at large bilayer spacings. Conversely, when the ratio is much smaller than 1, the undulation repulsion will dominate. The dashed line in Figure 7 is the ratio of the two interaction calculated using the values typical of neutral lecithin bilayers, i.e., $K_b = 2 \times 10^{-19}$ J and $A_H = 5 \times 10^{-21}$ J. As seen, these values lead to a value of approximately 59 for $A_H K_b/(kT)^2$, meaning that the long-range van der Waals attraction should dominate at bilayer between 5 and 20 nm. This finding is clearly inconsistent with the swelling behavior observed by Helfrich and co-workers. Possible causes of this inconsistency are reviewed in the Discussion section below.

Before continuing, it is appropriate to apply the above criterion to some of the other experimental findings referenced earlier. For example, Safinya et al.[15] found that the structure factor in their X-ray scattering experiments agreed well with the inverse third dependence on bilayer spacing predicted by eq 7. However, for this system, the value of K_b was previously measured to be of order 1 kT (4×10^{-21} J),[24] which is 1/50 of the value of the lecithin bilayers. If the Hamaker constants of the two systems were the same, the value of $A_H K_b/(kT)^2$ would be approximately 1, meaning that the undulation repulsion may in fact play a role in this system. In addition, because SDS is an anionic surfactant, electrostatic repulsive forces could also be significant. Specifically, Podgornik and Parsegian[13] showed that for undulating bilayers, the undulation, van der Waals, and electrostatic contributions to the total interaction energy will all decay with distance to the inverse second power, meaning that the corresponding force will decay with distance to the inverse third power, consistent with the decay rate observed by Safinya et al. In other words, the inverse third power dependence observed by the authors could have been due to the electrostatic repulsion between the membranes and not specifically to the undulation repulsion. A similar situation exists with the experiments of Abillon and Perez with cationic CTAB (cetyltrimethylammonium bromide) bilayers.[17] These authors used the Helfrich expression for the undulation repulsion to estimate a K_b value of 2.2×10^{-20} J (5.5 kT), which is 1/9 of the value of the lecithin bilayers.

Discussion

The results presented in Figures 6 and 7 indicate clearly that when the accepted governing equations and physical parameters are utilized, the long-range swelling of neutral lecithin bilayers cannot be explained by the repulsive undulation interaction. Before speculating about other possible causes of this swelling, however, it is first appropriate to examine in greater detail some of the assumptions and physical constants used in obtaining these results.

We should again emphasize that our results predict that for neutral lecithin bilayers, the long-range undulation repulsion remains substantially smaller than the van der Waals attraction and thus cannot explain the swelling observations of Helfrich and co-workers.[1−4] Thus if the undulations are in fact the true cause, then we must be either greatly underestimating the magnitude of the undulation repulsion or overestimating the magnitude of the van der Waals attraction. We will thus use these two criteria to guide this sensitivity analysis.

Values of δ and μ. As can be seen from Figure 5, two crucial parameters in these calculations are the minimum approach distance between two parts of the bilayers, δ, and the displacement distribution parameter, μ. Both the undulation and van der Waals interactions depend on these parameters. The issue

is whether the values of δ and μ needed to explain the discrepancy between our results and the experimental observations are within reasonable limits.

According to Figure 5 and eq 7, decreasing the ratio of the van der Waals energy to the undulation energy requires increasing the value of δ. The results in Figures 6 and 7 were calculated using a value of 2 nm, which roughly corresponds to the characteristic length of the hydration repulsion. In order to have the long-range van der Waals and undulation interactions equal at large bilayer spacings (i.e., >10 nm), this distance would need to be increased to over 10 nm. Although there have been various causes and length scales proposed for the hydration repulsion, in no case was the decay length much greater than several angstroms and the range of the interaction greater than 2 nm.

The correct distribution of the displacements is a bit more complicated. As seen from Figure 5, lowering the van der Waals attraction would require reducing μ. The lower limit of μ is of course zero, corresponding to the case of two flat plates. In addition, Helfrich,[11] Podgornik and Parsegian,[13] and Janke and Kleinert[14] showed that the magnitude of the undulation repulsion is inversely proportional to μ; thus decreasing this value further reduces the ratio of the van der Waals and undulation interaction energies.

The value of μ (0.2) used for the results in Figures 6 and 7 is consistent with the values recommended by Podgornik and Parsegian (0.183) and Helfrich (1/6 = 0.167). If the lowest value of 1/6 recommended by Helfrich were used instead, the ratio of the two energies at large separations would still be over 16. If fact, μ must be lowered nearly 1 order of magnitude before the undulation repulsion becomes significantly larger than the van der Waals attraction. Considering the fact that the values obtained independently by three separate researchers differ by less than 20%, it seems unlikely that the true value μ could be this low.

We should also mention, however, that in the approach of Podgornik and Parsegian,[13] the distribution of bilayer displacements is a function of the direct interactions between the bilayers (i.e., steric, electrostatic, van der Waals). The value of 0.183 recommended by these authors is for the case when only steric repulsion is present. When van der Waals interactions are taken into account, the authors suggest that the correct value of μ at large bilayer spacings is obtained by solving the equation

$$\frac{1}{\mu^2} = \frac{1}{\mu_s^2} - 2\left[\frac{64K_bA_H}{12\pi(kT)^2}\right]G'(\mu) \tag{14}$$

where μ_s is equal to 0.183 (steric confinement only) and $G'(\mu)$ is the derivative with respect to μ of the following function

$$G(\mu) = \frac{\sum_{n=0}^{\infty}(-1)^n(2n+1)(\pi/2)\mathrm{ci}[(2n+1)\pi]\exp[-(2n+1)^2(\pi/2)^2\mu]}{2\sum_{n=0}^{\infty}\frac{(-1)^n}{(2n+1)(\pi/2)}\exp[-(2n+1)^2(\pi/2)^2\mu]} \tag{15}$$

Here ci is the standard integral cosine function. Equations 14 and 15 reveal that the value of μ increases as the Hamaker constant increases. (In fact, for the values of A_H and K_b typical of neutral lecithin bilayers, these equations predict μ to diverge to infinity. However, the range of validity of these equations is not clear.) This dependence of μ on the Hamaker constant is expected intuitively because the van der Waals attraction between the bilayers stimulates the oscillations, thus increasing their entropic freedom and hence decreasing the undulation repulsion. Consequently, the ratio of the van der Waals and undulation energies is even higher than that presented in Figure 7, calculated using the smallest value of μ ($\mu = \mu_s = 0.183$).

Values of Hamaker Constant and Retardation Wavelength. Various values for the Hamaker constant between lipid bilayers in water have been reported in the literature. Parsegian[25] and Israelachvili[18] used the optical properties of bilayers to predict values ranging from 4.26×10^{-21} J to $(7-9) \times 10^{-21}$ J. Hamaker constants deduced from osmotic pressure measurements range between 4.9×10^{-21} J to as large as 76×10^{-21} J.[9,26] The results in Figures 6 and 7 were obtained using a value of 5×10^{-21} J, which is near the lower limit of this range of values. In the Hamaker pairwise additivity approach, the van der Waals interaction is directly proportional to the Hamaker constant. Thus, for the undulation repulsion in lecithin bilayers to exceed the van der Waals attraction at large bilayer spacings, the Hamaker constant would have to be lowered by a factor of approximately 1/23 (to 2×10^{-22} J), well outside of the range of either the predicted or measured values.

A somewhat related parameter is the value of the retardation wavelength, λ, in eq 12. As mentioned above, estimates of the correct value of λ have ranged between 40 and 200 nm, while a value of 100 nm was used in calculating the results in Figures 6 and 7. Repeating the calculations using 50 nm instead, which is near the lower limit of the reported range of λ, reduces the van der Waals interaction at 20 nm by 33%. However, this still produces a long-range van der Waals interaction that is more than 1 order of magnitude larger than the undulation repulsion.

Layered Stack of Bilayers. As opposed to two single bilayers, membrane systems typically consist of layered stacks of such bilayers, and thus it is worthwhile to consider how such multiple bilayers would affect the van der Waals interaction energy of a single bilayer. For the nonretarded case, the interaction energy for one planar bilayer in an infinite stack of such bilayers can be calculated by simply summing eq 13 over each bilayer. The resulting equation is

$$\frac{E(D)}{\text{area}} = -\frac{A_H}{12\pi D^2}\sum_{j=0}^{\infty}\left\{\frac{1}{1+j[1+t']^2} + \frac{1}{[(j+1)(1+t')+t']^2} - \frac{2}{[(j+1)(1+t')]^2}\right\} \tag{16}$$

where $t' = t/D$. It can be easily shown that this interaction is actually larger than that between two bilayers.

Validity of Hamaker Approach. The errors inherent in the Hamaker approach are well-known. Unfortunately, the more rigorous Lifshitz theory has not been applied to bodies of such complex morphology as the undulating bilayers described here. Ninham and Parsegian, however, used the Lifshitz theory to calculate the interaction energy of a flat bilayer in a multilayer stack of such bilayers.[27] When their results were compared to those predicted using eq 16, they found that the Hamaker approach greatly *underestimated* the energy.

Conclusions

The discussion above makes clear that any errors introduced by the assumptions used in the model cannot explain the discrepancy between results presented in Figures 6 and 7 and the assertion of Harbich and Helfrich[2] that the van der Waals

interaction is "... overwhelmed by the hydration and undulation forces." In addition, a sensitivity analysis on the major physical parameters reveals that it is highly unlikely that uncertainties in any one parameter is the cause of the discrepancy. It is of course possible that two or more parameters, such as the displacement distribution and the retardation wavelength, are in error and their cumulative effect is large enough to explain the disagreement, however this is difficult to evaluate.

On the other hand, if we assume that the governing equations and physical parameters are in fact valid, then the only possible conclusion is that an additional repulsive interaction exists between the bilayers at large spacings. For example, Ruckenstein recently showed that a lamellar liquid crystal can be stable when only van der Waals interactions are present between planar bilayers.[28] In these cases, a force due to the interfacial free energy of the bilayer interfaces is compensated by a force due to the thinness of the lamellae and to the van der Waals interactions among the lamellae. Additional studies in this area are clearly required before a complete understanding of the causes of swelling in these neutral lipid systems can be achieved.

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