The adsorption of phosphatidylcholine (PC) vesicles (30, 50, and 100 nm nominal diameters) and of dye-labeled PC vesicles (labeled with 6% Texas Red fluorophore (TR) and encapsulated carboxy fluorescein (CF)) to glass surfaces was studied by contact mode atomic force microscopy in aqueous buffer. These studies were performed in part to unravel details of the previously observed isolated rupture of dye-labeled PC vesicles on glass (Johnson, J. M.; Ha, T.; Chu, S.; Boxer, S. G. Biophys. J. 2002, 83, 3371–3379), specifically to differentiate partial rupture, that is, pore formation and leakage of entrapped dye, from full rupture to form bilayer disks. In addition, the adhesion potential of PC vesicles on glass was calculated based upon the adhesion-driven flattening of adsorbed vesicles and a newly developed theoretical model. The vesicles were found to flatten considerably upon adsorption to glass (width-to-height ratio of approximately 5), which leads to an estimate for the adhesion potential and for the critical rupture radius of $1.5 \times 10^{-4}$ J/m² and 250 nm, respectively. Independent of vesicle size and loading with dye molecules, the adsorption of intact vesicles was observed at all concentrations below a threshold concentration, above which the formation of smooth lipid bilayers occurred. In conjunction with previous work (Johnson, J. M.; Ha, T.; Chu, S.; Boxer, S. G. Biophys. J. 2002, 83, 3371–3379), these data show that 6% TR 20 mM CF vesicles adsorb to the surface intact but undergo partial rupture in which they exchange content with the external buffer.

Introduction

Supported lipid bilayers are useful as model systems for biological membranes. Supported bilayers allow study of membrane constituents in a controlled environment and may serve as a platform for biosensors based on naturally existing biomolecules in a milieu that approximates a cell membrane.

Vesicle fusion to a substrate is one of the most convenient ways of preparing supported bilayers, as it does not require sophisticated equipment and reliably produces high quality supported bilayers. Following the pioneering work of McConnell and co-workers, vesicle fusion has been used to form supported bilayers from various lipids on micropatterned supports.


substrates, such as glass,\textsuperscript{19} mica,\textsuperscript{20} self-assembled monolayers,\textsuperscript{21} polymers,\textsuperscript{22} and quartz.\textsuperscript{23}

Much effort has been invested to elucidate the underlying mechanisms of bilayer formation.\textsuperscript{1,24–27} A theoretical model has been developed by Seifert and Lipowsky that predicts the dependence of bilayer formation on properties of the vesicles, such as the bending modulus and curvature, along with the adhesive interaction of the vesicles with the solid substrate.\textsuperscript{28,29} One central result of this theory is the prediction that only vesicles above a critical size adsorbed on the surface will rupture. The existence of a critical vesicle size of 75 nm has been observed for phosphatidylcholine (PC) vesicles adsorbed to mica by atomic force microscopy (AFM) measurements.\textsuperscript{24}

![Figure 1](image)

**Figure 1.** Four step scenario of supported bilayer formation via vesicle fusion comprising (1) vesicle adsorption, (2) fusion of vesicles at the surface to form larger vesicles, (3) rupture of the fused vesicles resulting in bilayer disks, and finally (4) merging of the disks. Here red lipids represent a leaflet fluorophore and green represents an entrapped aqueous dye (ref 1).

In previous work, the steps of bilayer formation depicted in Figure 1 were investigated using a single vesicle assay based on two color fluorescence microscopy.\textsuperscript{1} Fusion was observed by including a self-quenching concentration of Texas Red labeled lipids (TR), and rupture was observed by entrapping solvable carboxy fluorescein dyes (CF) within the vesicles. Four distinct pathways of bilayer formation were observed: (1) primary fusion, as depicted in Figure 1; (2) simultaneous fusion and rupture, in which a labeled vesicle on the surface ruptures simultaneously upon fusion with an unlabeled vesicle; (3) no dequenching, in which loss of fluorescence signal from both dyes occur simultaneously; and (4) isolated rupture (preruptured vesicles), in which a labeled vesicle on the surface spontaneously undergoes content loss, a process that depends on the presence of TR-labeled lipids. The fluorescence signatures alone did not allow differentiation between partial rupture (i.e., pore formation and leakage of entrapped dye) versus full rupture to form a bilayer disk.

Isolated rupture events, which may be due to partial rupture or full rupture as discussed above, occurred for approximately 50% of vesicles containing 6 mol % TR. Previously, isolated rupture to form bilayer disks has been reported for vesicles of the same size under similar conditions on mica.\textsuperscript{24} To unequivocally differentiate between partial and full rupture for the adsorption of PC vesicles and dye-loaded PC vesicles on glass surfaces, we have studied the adsorption behavior of PC vesicles to a glass surface directly by in situ AFM under the same conditions that were used in the single vesicle fluorescence assay. In addition, a new theoretical model was developed, which allowed us to calculate the adhesion potential and the critical rupture radius of the vesicles on the basis of the vesicle dimensions as determined by AFM.
Experimental Section

Materials. Egg phosphatidylcholine (egg PC) was purchased from Avanti Polar Lipids (Alabaster, AL). Texas Red 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (TR) and 5-and-6-carboxylfluorescein mixed isomers (CF) were purchased from Molecular Probes (Eugene, OR). Glass coverslips (no. 1.5) were purchased from VWR (Westchester, PA).

Vesicle Preparation. Extruded unilamellar vesicles (referred to as simply as vesicles) were prepared according to the protocol described in Johnson et al.1 Briefly, chloroform was evaporated from the egg PC under a vacuum, and the lipids were then allowed to hydrate in standard buffer (10 mM Tris [pH 8], 100 mM NaCl). The resulting multilamellar vesicles were subjected to 5 freeze/thaw cycles and then extruded through polycarbonate membranes of 30, 50, or 100 nm pore diameter (Avanti Mini-Extruder). The vesicle sizes quoted in this paper refer to the pore size used for extrusion. The vesicle size distribution was not independently measured; however, the conclusions drawn do not depend on knowing vesicle sizes in solution. For labeled vesicles, TR was mixed with egg PC in chloroform; the mixture was dried and resuspended in a solution of 20 mM CF dissolved in 10 mM Tris [pH 8] buffer with the appropriate amount of NaCl to make the solution iso-osmolar (intravesicular solution: 20 mM CF, 10 mM Tris [pH 8], 50 mM NaCl), or Milli-Q water using a DI liquid cell. The temperature inside the cell was 31 °C.

Substrate Preparation. Glass coverslips were prepared by soaking the substrates in heated detergent solution (Linbro 7X Lab Glass Cleaner, ICN Pharmaceuticals) for at least 20 min, followed by exhaustive rinsing. The coverslips were then baked at 400 °C for 4 h. The root-mean-square (rms) roughness of the substrates prepared following this procedure was 0.3 ± 0.1 nm as determined by contact mode AFM in water (5/μm2 scan size).

Atomic Force Microscopy. The AFM experiments were carried out on a NanoScope III (Veeco/Digital Instruments (DI), Santa Barbara, CA) in contact mode (CM). V-Shaped Si3N4 cantilevers were used (Nanoprobes (DI)). The cantilevers had a force constant of k = 0.056 N/m as calibrated by the reference lever method36 and tip radii of 20–40 nm estimated from images of a calibration standard (Silicon-MDT, Moscow, Russia). Measurements were performed on baked glass coverslips in buffer (10 mM Tris [pH 8], 100 mM NaCl) or Milli-Q water using a DI liquid cell. The temperature inside the cell was 31–32 °C. The measurement procedure described in ref 15b was followed, with minimized forces (imaging force < 60 pN). After liquid exchange, data were acquired after the photodiode signal showed a constant reading. If necessary the forces were adjusted during each scan by manually minimizing the set point. Typical scan rates of 3–5 Hz at a resolution of 256–512 pixels/line were used. All images shown were subjected to a first order plane-fitting procedure to compensate for sample tilt and, if necessary, to a zeroth order flattening. The cross-sectional analysis was carried out on images subjected only to a first order plane-fitting procedure. Vesicles with a streaked appearance (see below) were not included in the quantitative analysis. The widths were estimated as mean value of the widths obtained from cross-sectional plots through the vesicles for different relative orientations (see Supporting Information, Figure S1).

Results

Vesicles were adsorbed to glass at low surface coverage to allow imaging of individual vesicles by contact mode AFM (Figure 2). Elevated features were observed with sizes that correlated with the nominal diameter of the vesicles in solution. Due to the upper limit of noninvasive (destructive) imaging forces (<60 pN), tip lift-off frequently made vesicles appear to be streaked in the scanning direction, as opposed to circular. The streaking was observed predominantly for larger vesicles but could be avoided in most cases by choosing appropriate imaging forces and scan rates. For the purpose of investigating the nature of the previously observed partial vesicle rupture upon adsorption to glass, we utilized the lowest possible forces to avoid AFM tip-induced rupture, while for the quantitative determination of the heights and widths of the adsorbed vesicles slightly higher forces and in particular lower scan rates were used to avoid the streaking.

The appearance of the features observed in Figure 2 changed rapidly if imaged under invasive (destructive) imaging conditions. For imaging forces exceeding ~60 pN, insufficient gain, or too rapid scanning, the elevated features changed appearance from one scan line to the subsequent scan line as illustrated in Figure 3. The resulting feature (in the slow scanning direction) was a much flatter elevated patch, with a typical height of 5.0 nm. This height agrees well with the thickness of a complete bilayer, as previously measured by AFM.31,32 The observed features in Figures 2 and 3 are therefore attributed to adsorbed vesicles, which undergo rupture to form bilayer disks under invasive imaging conditions. By using carefully adjusted set points, the tip-induced rupture

Figure 2. Contact mode AFM height images of vesicles adsorbed on glass and corresponding section analyses (mean vesicle diameter: (a) 100 nm; (b) 50 nm; (c) 30 nm) (vesicle concentration: (a) 5 × 10⁻⁴ mg/mL; (b) 5 × 10⁻⁴ mg/mL; (c) 5 × 10⁻³ mg/mL). The gray scale in the images covers a range of 50 nm from dark to bright. Images were obtained in buffer at minimized forces. The section analyses show the profiles along the corresponding lines indicated in the AFM images.
could be reliably avoided. AFM tip-induced rupture of adsorbed vesicles has been previously reported.\footnote{33}

From these observations,\footnote{34} we can infer that vesicles with mean diameters between 30 and 100 nm adsorb intact on glass at low vesicle concentrations. We have not observed the adsorption of vesicles from solution or desorption of intact vesicles in real time. Similar observations were also made in the case of 50 nm diameter vesicles with 6\% TR and entrapped CF (Supporting Information, Figure S2), which previously showed isolated rupture events for approximately 50\% of vesicles.\footnote{37} At higher solution concentrations, the surface coverage increases, until at some critical solution concentration ($>$6 $\times$ 10$^{-3}$ mg/mL) a bilayer is formed.\footnote{28,29}

The lipid bilayer has an almost featureless appearance with an rms roughness of <0.3 nm (glass, <0.4 nm) measured on 1 $\mu$m$^2$. If scratched with the AFM tip, the height of the bilayer is measured to be 4.7 nm. This also allows an estimation of the maximum size of any defects present in the bilayer (see Supporting Information, Figure S3).

A more quantitative analysis of the vesicles shows a linear correlation between measured diameter and height with a nonunit slope (Figure 4). The widths and heights were determined from sectional plots as described in the Experimental Section (see also Supporting Information, Figure S1). For this analysis, we have corrected the measured width by subtraction of the measured tip diameter.\footnote{36} We minimize possible flattening of the vesicles as a result of normal forces induced by the AFM tip\footnote{36} by using the lowest possible imaging forces ($\sim$30 to $\sim$50 pN).

An estimate of the indentation length indicates that the flattening can be neglected for the conditions used in our experiments.\footnote{37}

The solid line in Figure 4 is a linear least-squares fit of width versus height with a slope of 5.2. The dashed line shows a slope of unity for comparison, which would be the correlation of width versus height for vesicles that adsorbed without flattening. This result is consistent with the view that vesicles adsorb to glass surfaces in a flattened configuration.\footnote{28,29}

Labeled vesicles were also observed to rupture in response to osmotic stress. When the buffer solution was replaced with Milli-Q water, many vesicles were found to be ruptured, resulting in isolated bilayer disks. However, we also observed a significant number of vesicles which were stable for hours in salt-free water and did not rupture under these conditions. Intact vesicles were imaged in Milli-Q water, as well as in buffer after exchanging the imaging liquid. The data shown in Figure 5 were acquired in buffer to avoid the effect of double layer repulsion, which may result in height artifacts.\footnote{38} The right panel of Figure 5 is a plot of width versus height for adsorbed vesicles and bilayer disks determined in buffer. The bilayer disks show widths from 75 to 425 nm and a height of 4 nm (circles). Adsorbed vesicles before osmotic rupturing (squares) show the same width versus height distribution as adsorbed vesicles that remained intact after osmotic rupturing and exchange of the imaging liquid (triangles).

From the area of each observed bilayer disk following osmotic rupturing, the size of the corresponding original vesicle can be estimated.\footnote{39} To within the experimental error, we observe a reasonable correspondence of the mean vesicle diameter estimated from the (deconvoluted) size

\begin{itemize}
\item[(34)] After thermal equilibration, the data for a given set of conditions were acquired within $<$2 h.
\item[(37)] The indentation length $L$ of a vesicle (with radius $R = 100$ nm) probed by an AFM tip ($\text{radius } r = 30$ nm) using a force ($F$) of 30 pN can be calculated as 1 nm using $L = (RF/r)^2$, where $k$ denotes the stretching modulus of the vesicle.
\end{itemize}
of the observed bilayer patches (estimated diameter = 70 ± 36 nm) and the nominal vesicle size (nominal diameter = 50 nm).

Discussion

In previous work, isolated rupture events were observed for 6% TR vesicles. These results resulted in loss of internal dye signal (CF) from approximately 50% of vesicles adsorbed to glass. These isolated rupture events were not observed for vesicles labeled with 0.5% TR and entrapped CF. In this current work, we have observed that vesicles labeled with 6% TR and containing CF adsorbed intact to glass surfaces, and no isolated bilayer disks resulting from individual vesicles were observed. Thus, the rupture events may be interpreted as exchange of the internal content of vesicles due to an instability, such as pore formation. Transient pore formation has been previously observed in giant vesicles under tension or in contact with black lipid membranes.40,41 We believe that high TR content and adsorption of the vesicle to the surface destabilize the membrane enough to allow rupture pores to form, but not enough to induce complete rupture of adsorbed vesicles to form bilayer disks.42

The measured ratio of width to height shown in Figures 4 and 5 can be used to calculate the strength of the adhesion potential of the glass surface. The experimental values of the width-to-height ratio are compared with those predicted by shape dependent energy functionals as introduced by Lipowsky and Seifert.28 Generally, the shape of an adhering vesicle is determined by the competition between the free energy cost associated with the curvature elasticity of the membrane and the gain in adhesion energy. The equilibrium shape of adhering vesicles corresponds to the minimum of the free energy functional

\[
F = \frac{1}{2} \int dA(2H)^2 - WA_{adh}
\]  

(1)

where \( W \) is the adhesion potential (with units of energy per area), \( x \) the bending rigidity (with units of energy), \( H \) the mean curvature (with units of inverse length), and \( A_{adh} \), the contact area of the vesicle.28,29 In determining the minima of eq 1, the volume and area of the vesicle have to be kept fixed.

General solutions of eq 1 are not known. However, in the limit of infinitely strong adhesion \( W \rightarrow \infty \) (where curvature energy can be neglected with respect to the adhesion energy) the vesicle shapes can be calculated analytically. In these limits, adhesion of vesicles becomes equivalent to adhesion of conventional liquids on substrates and correspondingly the shape of the vesicles is given by spherical caps, as illustrated in Figure 6.

In this case, the free energy can be written as

\[
F_0 = -WA_{adh}^0 + \sigma_0 A + p_0 V
\]  

(2)

where the area and volume constraint have been explicitly taken into account. Here, \( \sigma_0 \) is the membrane surface tension and \( p_0 \) the pressure difference between outside and inside of the vesicle, which act as Lagrange multipliers for the area and volume constraints, respectively. The analogy to conventional wetting can be made more explicit by writing eq 2 as

\[
F_0 = (\sigma_0 - W)A_{adh}^0 + \sigma_0(A - A_{adh}) + p_0 V
\]  

(3)

which is the free energy of a liquid droplet of phase \( \beta \) wetting a flat substrate (\( \gamma \)) in the presence of a vapor phase (\( \alpha \)). In this analogy, \( \sigma_0 - W \) corresponds to \( \Sigma_{\alpha} - \Sigma_{\gamma} \), \( \sigma_0 \) corresponds to \( \Sigma_{\beta} \), where \( \Sigma_{\gamma} \) is the interfacial tension between phases \( i \) and \( j \), and \( p_0 = P_\alpha - P_\beta \) is the pressure difference between outside and inside of the droplet. Thus, the shapes of infinitely strongly adhering vesicles are given by spherical caps with contact angle \( \theta_0 \) and radius \( R_0 \).

In eq 1, the curvature contribution is scale invariant whereas the adhesion energy depends on the size of the vesicles. Therefore, the description of vesicles as spherical caps becomes more appropriate for larger vesicles, as the adhesion energy becomes large compared with curvature cost.

It has been shown by Tordeux et al. that the corrections to the limit of infinitely strong adhesion can be calculated analytically for axisymmetric shapes (shapes that are symmetric under rotation around an axis). By introducing the expansion coefficient

\[
\epsilon = \frac{1}{L} \sqrt{\frac{\kappa}{W}} \ll 1
\]  

(4)

(where \( L \) is the radius of the adhesion area of the vesicle), the vesicle shape, its surface area and volume can be calculated in a systematic expansion in \( \epsilon \). We expect \( \epsilon \approx 0.1 \) for vesicles with \( \kappa \approx 10kT \) and radius 50 nm adhering to a substrate with \( W \approx 10^{-3} \) J/m². Thus, higher-order corrections in \( \epsilon \) should be small, and we can restrict the analysis to first order in \( \epsilon \).

Volume \( V \), surface area \( A \), and width (2L) to height (h) ratio of the adhering vesicles are then in first order of \( \epsilon \) given by

\[
V = \frac{1}{3} \pi R_0^3 \sin(\theta_0) + O(\epsilon^2)
\]  

(5)

\[
A = \pi R_0^2 \sin(\theta_0) + 2\pi R_0 \sqrt{\frac{2\kappa}{W}} + O(\epsilon^2)
\]  

(6)

\[
\frac{2L}{h} = 2 \left( \frac{l_0(\theta_0) + l_1(\theta_0) \sqrt{\frac{\kappa}{WA}}}{h_0(\theta_0) + h_1(\theta_0) \sqrt{\frac{\kappa}{WA}}} + O(\epsilon^2) \right)
\]  

(7)

All coefficients appearing in the last equations are defined in the Appendix.

The main problem in determining \( W \) from the experimental data by using eqs 5–7 is that the surface area and volume of the adhered vesicles are not known. We therefore have to make an additional assumption about the shape of the adhering vesicles. Since we are interested in large values of the flattening ratio 2L/h, the adhering vesicles will mostly belong to the pancake-regime, where their shape can be approximated by a cylinder of height \( h \)

With this value of hered to mica, estimated to be 3.5 reported by Reviakine and Brisson for PC vesicles an order of magnitude smaller than the value previously values of 2 and eq 6 equal to eq 5 equal to remaining unknowns (results of this analysis for vesicles with radii $R$ average flattening ratio of 6.8 width larger than 200 nm. This subset of vesicles has an to an adhesion potential of $W$ it is justified to neglect the higher-order terms in the relation

$$R_t = \frac{2\Lambda - g(2\pi W)^{1/2}}{W}$$  \hspace{1cm} (10)$$

where $\Lambda$ is the line tension of the bilayer disk and $g$ is a numerical constant on the order of 1$^{23,28}$ Taking similar values for $\kappa$ and $\Lambda$ used by Reviakine et al., $5 \times 10^{-29}$ J and $1.3 \times 10^{-10}$ J/m, respectively, our estimated adhesion potential of $1.5 \times 10^{-24}$ J/m$^2$ would yield an estimated $R_t$ of approximately 2 $\mu$m for PC vesicles on glass. However, values typically reported for $\Lambda$ are on the order of $2 \times 10^{-11}$ J/m$^2$,$^{40,44}$ which yields an $R_t$ of 250 nm for $W \approx 1.5 \times 10^{-4}$ J/m$^2$. Both these values are above the radius of the vesicles used to estimate $W (R \approx 50$ nm), which is consistent with the fact that no vesicles were observed to undergo isolated rupture to form bilayer disks. Furthermore, if Reviakine et al. had used the value of $\Lambda = 2 \times 10^{-11}$ J/m, they would have estimated an adhesion potential on mica much closer to our estimate for glass ($W \approx 5 \times 10^{-4}$ J/m$^2$).

**Conclusion**

Our AFM data show that the adsorption of egg PC vesicles to glass surfaces leads to isolated intact vesicles at low concentrations of vesicles in solution. Vesicles labeled with 6% TR also adsorb intact. Taken in conjunction with previous work,$^3$ these results suggest that 6% TR vesicles form rupture pores but do not fully rupture to form bilayer disks when adsorbed to glass. For increasing vesicle concentrations, there is an increase in surface coverage; however, a coexistence of bilayer disks originating from ruptured vesicles and adsorbed vesicles has not been observed. This observation is in contrast to reports of vesicle adsorption on mica, where vesicles of 100 nm diameter in solution were observed to adsorb both as intact vesicles and as bilayer disks.$^{24}$ However, our data are in agreement with recent quartz crystal microbalance (QCM) measurements of vesicles on glass, which also showed intact vesicles of 100 nm diameter adsorbing.$^{26}$ We have estimated the adhesion energy ($W$) of egg PC vesicles adsorbed to glass to be on the order of $1.5 \times 10^{-4}$ J/m$^2$, which is of the same order of magnitude as the tip-induced tension required for vesicle rupture. We can approximate the critical rupture radius ($R_t$) as being ~250 nm, depending on what value is used for the line tension of a bilayer disk. For high vesicle concentrations, continuous lipid bilayers with a thickness of ~5 nm are formed, which possess no detectable defects.

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Supporting Information Available: Top view and cross-sectional analysis of an AFM height image of an adsorbed vesicle; AFM height images of adsorbed, labeled vesicles at increasing solution concentrations; AFM image and section analysis of a scratched bilayer. This material is available free of charge via the Internet at http://pubs.acs.org.

Appendix: Expansion around the Strong Adhesion Limit

In this Appendix, we briefly define the coefficients appearing in eqs 5-7. Surface area and volume of the adhering vesicle are given by

\[ A = A_{\text{cap}} - \delta A \] (A1)

\[ V = V_{\text{cap}} + O(\epsilon^2) \] (A2)

where

\[ V_{\text{cap}}(\theta, R) = \frac{1}{3}\pi R^3 [2(1 - \cos \theta) - \sin^2 \theta \cos \theta] \equiv \frac{1}{3}\pi R^3 \tilde{V}(\theta) \] (A3)

and

\[ A_{\text{cap}}(\theta, R) = \pi R^2 [2(1 - \cos \theta) + \sin^2 \theta] \equiv \pi R^2 \tilde{A}(\theta) \] (A4)

and

\[ \delta A = 4\pi [\cos \frac{\theta}{2} - \cot \frac{\theta}{2}] \sqrt{\frac{2\kappa}{\kappa}} L_0 + O(\epsilon^2) \equiv 4\pi \delta \tilde{A}(\theta) \sqrt{\frac{2\kappa}{\kappa}} L_0 + O(\epsilon^2) \] (A5)

where \( L_0 = R_0 \sin \theta_0 \).

For large (but finite) \( \kappa \), the shape of the vesicles is also characterized by a contact angle \( \theta \) and radius \( R \) given by \( \theta = \theta_0 + \delta \theta \) and \( R = R_0 + \delta R \). The first order corrections are given by

\[ \delta \theta = \frac{2\left[ \frac{\theta_0}{2} - 1 \right] \left[ 2 + \cos \theta_0 \right]}{R_0 \sin \theta_0} \sqrt{\frac{2\kappa}{\kappa}} + O(\epsilon^2) \equiv \frac{\delta \tilde{\theta}(\theta_0)}{R_0} \sqrt{\frac{2\kappa}{\kappa}} + O(\epsilon^2) \] (A6)

and

\[ \delta R = \frac{2\left( 1 - \sin \frac{\theta_0}{2} \right) \sin^2 \frac{\theta_0}{2}}{(1 - \cos \theta_0)^2} \sqrt{\frac{2\kappa}{\kappa}} + O(\epsilon^2) \equiv \frac{\delta \tilde{R}(\theta_0)}{\sqrt{\frac{2\kappa}{\kappa}}} + O(\epsilon^2) \] (A7)

In first order in \( \epsilon \), the ratio between \( L \) (the radius of the adhesion area of the vesicle) and the height \( h \) of the vesicle is given by

\[ \frac{L}{h} = \frac{l_0 + l_1 \sqrt{\frac{\kappa}{WA}}}{h_0 + h_1 \sqrt{\frac{\kappa}{WA}}} + O(\epsilon^2) \] (A8)

where

\[ l_0 = \sqrt{\frac{1 + \cos \theta_0}{\pi (3 + \cos \theta_0)}} \] (A9)

\[ l_1 = -\sqrt{2} \frac{\cos \theta_0 \sqrt{W}}{1 + \sin \theta_0 / 2} \] (A10)

\[ h_0 = \sqrt{\frac{1 - \cos \theta_0}{\pi (3 + \cos \theta_0)}} \] (A11)

\[ h_1 = -2\sqrt{2} \left( 1 - \sin \frac{\theta_0}{2} \right) \] (A12)

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