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Adhesion of vesicles

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A simple model for the adhesion of vesicles to interfaces and membranes is introduced and theoretically studied. It is shown that adhering (or bound) vesicles can exhibit a large variety of different shapes. The notion of a contact angle governed by tension is found to be applicable only for a restricted subset of these shapes. Furthermore, the vesicle undergoes a nontrivial adhesion transition from a free to a bound state. This transition is governed by the balance between the overall bending and adhesion energies, and occurs even in the absence of shape fluctuations.

Light and electron microscopy have revealed an astonishing complexity of the spatial organization of biological systems.[1] There are two basic problems if one tries to understand the physical mechanisms behind this complexity: (i) these systems contain a large number of different chemical species, and (ii) they represent dissipative structures which involve complex patterns of dynamical processes. It turns out, however, that one can gain some insight into their spatial organization if one considers very simple model systems.

Lipid bilayers or membranes probably represent the simplest models of this kind: (i) they already form in simple binary mixtures consisting only of lipid and water, and (ii) they represent thermally equilibrated states while the number of molecules within the bilayer is (almost) constant on experimentally accessible time scales. In aqueous solution, lipid bilayers form vesicles, i.e., closed surfaces in order to prevent any contact between the hydrocarbon chains of the lipid and the water. It is generally believed that this hydrophobic effect is also the main mechanism behind the formation of vesicles in biological systems. Indeed, these systems usually contain a large number of such structures. The most obvious examples are the big vesicles which represent the boundaries of biological cells. In addition, smaller vesicles are frequently used for various transport processes within cells and across cell boundaries.

Lipid vesicles can be easily isolated and then studied by a variety of experimental techniques.[2] These studies tend to confirm the theoretical idea[3-6] that the shape of a vesicle is mainly controlled by the bending elasticity and thus by the curvature of the bilayer or membrane.

In this paper, we theoretically study a simple model for the adhesion of vesicles. In a biological context, vesicle adhesion represents an essential step for many processes such as, e.g., endocytosis and exocytosis,[1] that is, the transport of small vesicles through large membrane surfaces. Likewise, the adhesion of vesicles is used in biotechnological processes such as, e.g., drug delivery by liposomes.[7]

In our theoretical model, we ignore the details of the molecular structure within the membrane, which is then viewed as a thin flexible sheet. We will assume, however, that this membrane is fluid and thus governed by its bending rigidity.

For a bound vesicle, the membrane segment adjacent to the wall can experience a variety of intramolecular forces, such as van der Waals, electrostatic, and structural forces. In order to have a bound state, the effective interaction potential must exhibit a minimum at a finite distance z_0. This potential range is typically of the order of a few nm. On the other hand, the radius of a vesicle typically varies from 0.1 to 10 μm. Since we are primarily interested in the overall shape of the vesicle, we will ignore spatial variations on the scale of the potential range z_0. Therefore we replace the microscopic interaction potential for adhesion by an effective contact potential.[8]

Starting from a model for fluid membranes which includes both the bending energy and such a contact potential, we study the equation for the shape of a bound vesicle which involves a novel boundary condition. Numerical solutions of these equations reveal a large variety of different shapes. In addition, we find a nontrivial adhesion (or unbinding) transition: as temperature or pressure (or any other macroscopic parameter) is changed, the vesicle undergoes a transition from a bound to a free state in the presence of a nonzero contact potential. This transition is governed by the competition between bending and adhesion energies.

So far, thermally excited shape fluctuations have been ignored. As explained towards the end of our paper, these fluctuations introduce a crossover length R_c, which separates two different regimes. For vesicle size $R < R_c$, the unbinding is driven by the energetic mechanism just described; for $R > R_c$, it is driven by the shape fluctuations and thus by entropy. This latter regime corresponds to the unbinding transition of two roughly parallel membranes as studied previously.[9-11] For phospholipid bilayers, we estimate $R_c \simeq n \times (0.2\ \mu\text{m})$, where n is the number of bilayers within the vesicle surface.

The distinction between these two different unbinding regimes should be important both for experiments and for computer simulations of such adhesion phenomena. Consider, e.g., a surface or wall with a potential strength W and vesicles characterized by a bending rigidity κ. If the length scale $R_a \equiv (2\kappa/W)^{1/2}$ satisfies $R_a < R_c$, the vesicle should unbind for energetic rather than entropic reasons. In this case, the size distribution of bound vesicles is predicted to exhibit a lower cutoff $\sim R_a$, which should be accessible to various experimental techniques. A single vesicle, on the other hand, which adheres to the wall, could now unbind as the temperature is *decreased* since its linear size shrinks as the result of the relatively large thermal area expansivity of the bilayer.

It is interesting to compare the shape of a bound vesicle with the shape of a liquid droplet adhering to an interface. Such a droplet has a well-defined contact angle as given by the Young-Dupré relation in terms of the various interfacial tensions. The work presented below implies that such a relation does not exist, in general, for adhering vesicles.[12] In fact, in the presence of a nonzero bending rigidity, the contact potential no longer determines the contact angle (which is always equal to π) but the contact curvature. However, we also show that the concept of an effective contact angle is useful provided (i) the pressure inside the vesicle exceeds the outside pressure, and (ii) the bending rigidity is sufficiently small.

Our results are obtained by minimizing the free-energy functional F,

$$\begin{aligned} F &= F_\kappa + F_W + F_P + F_\Sigma \\ &= (\kappa/2)\oint dA\,(C_1+C_2-C_0)^2 - WA^* + P\int dV \\ &\quad + \Sigma \oint dA\ . \end{aligned} \tag{1}$$

The first term F_κ is the widely accepted expression of Helfrich[3] for the curvature energy, which depends on the two principal curvatures C_1 and C_2, on the spontaneous curvature C_0, and on the bending rigidity κ. (We assume a fixed spherical topology of the vesicle and thus do not include the Gaussian curvature.) In the second term, $W \geq 0$ denotes the contact potential for adhesion and A^* is the contact area. The last two terms in (1) refer to the constraints for the volume and the area of the vesicle. These constraints depend on the physical situation and define different statistical ensembles. On short time scales, the enclosed volume is essentially constant. The Lagrange multiplier P must then be adjusted in order to ensure this prescribed volume. If one allows for changes in the volume, e.g., by a different osmotic pressure inside and outside the vesicle, P denotes this difference $P = P_{\text{ext}} - P_{\text{int}}$. Likewise the parameter Σ is either a Lagrange multiplier to ensure a fixed total area A or represents a lateral tension thus allowing for nonconstant total area.

In the absence of the contact term F_W, minimization of the free energy as in (1) leads to the shape equation for free vesicles. We consider axisymmetric shapes for which the shape equation is given by a system of nonlinear ordinary differential equations.[4-6] These equations remain valid for bound vesicles since the adhesion term F_W enters only as a boundary condition at the contact point. First, the contact angle ψ is always given by $\psi = \pi$, since any other contact angle implies an infinite curvature energy. Second, variation of the contact point yields the novel boundary condition[13]

$$C_1^* = (2W/\kappa)^{1/2}\ , \tag{2}$$

where C_1^* denotes the principal curvature along the meridian of the vesicle at this point. This equation determines the contact curvature in terms of the contact potential W; it holds for any value of C_0 and Σ irrespective of the chosen ensemble.[14] Numerical solutions of the shape equation with the boundary condition (2) lead to a large variety of shapes, some of which are shown in Fig. 1.

For $\kappa = 0$, minimization of the free energy as given by (1) leads to the Laplace equation with the Young-Dupré equation as a boundary condition. The latter equation determines the contact angle ψ via

$$W = \Sigma(1+\cos\psi)\ . \tag{3}$$

Strictly speaking, this relation loses its meaning for any $\kappa \neq 0$ since $\psi = \pi$ as discussed above. However, for small

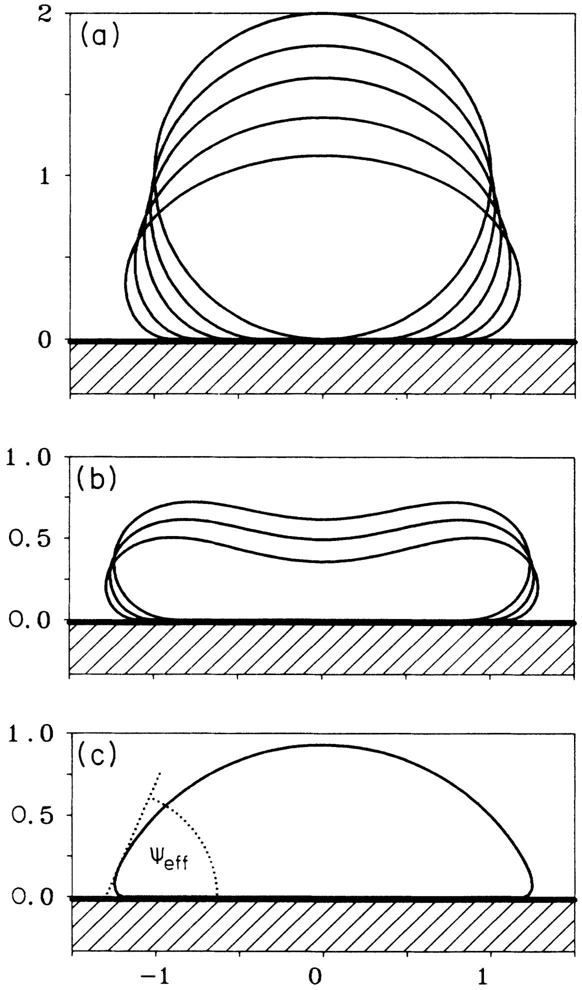

FIG. 1. Shapes of bound vesicles which all have the same area $A = 4\pi$: (a) for reduced pressure $p = 0$, and reduced contact potential $w = 2.0, 2.9, 4.1, 6.4, 10.2$ with increasing contact area A^*. Shapes for $p < 0$ look similar; (b) $(p,w) = (14.6, 2.0), (14.0, 6.1), (14.1, 12.9)$ with increasing A^*; and (c) $(p,w) = (-386.5, 375.2)$. This shape has a reduced tension $\sigma = 265.3$ and an effective contact angle $\psi = 65.5°$ in agreement with (3).

κ, the bound vesicle becomes a spherical cap for $P<0$, which exhibits a rounded contact region on the length scale $R_a=(2\kappa/W)^{1/2}$. The effective contact angle ψ_{eff} of the spherical part with the wall as indicated in Fig. 1(c) indeed obeys the Young-Dupré equation (3) with $\psi=\psi_{\mathrm{eff}}$. Note that this effective contact angle is only defined in the limit where the length scale R_a is small compared to the linear size $R\equiv\sqrt{A/4\pi}$ of the vesicle.

Although the shape equation and hence the shapes do not depend on the choice of constraints for A and V, the phase diagram does, because the relevant free energies for the free and adhering solution depend on this choice. For a fixed total surface area A and a given pressure difference P, this free energy is the enthalpy $F_\kappa+F_W+F_P$. The free solution has no contribution to F_W but typically a lower bending energy F_κ. The competition of F_W and F_κ (and F_P) leads to nontrivial phase transitions. A typical phase diagram is shown in Fig. 2 for $C_0=0$ and fixed area. The phase diagrams for $C_0\neq0$ and other choices of constraints exhibit similar features as will be discussed elsewhere.

The phase diagram in Fig. 2 is basically divided into two parts which may be characterized by means of the reduced variables

$$p\equiv PR^3/\kappa,\quad w\equiv WR^2/\kappa,\quad \sigma\equiv\Sigma R^2/\kappa\ , \tag{4}$$

with $A\equiv4\pi R^2$. For reduced contact potential $w>w_a(p)$ the vesicle is bound to the wall, while for $w<w_a(p)$ it is free even in the presence of an attractive wall. The character of the adhesion (or unbinding) transition between both states depends crucially on the value of p. Several cases must be distinguished: (i) For $p<p_1$, with $p_1=4$ ($p_1=4-2C_0R$ for general C_0), and especially whenever the pressure inside is higher than outside, the phase boundary (C_a) represents a continuous transition at $w_a=2$ or, equivalently, at $R_a=(2\kappa/W)^{1/2}$. Thus, for fixed κ and W, small vesicles with radii $R<R_a$ are free, while the larger ones are bound within the above model. For these bound vesicles, the contact area A^* vanishes as $A^*\sim R^2\Delta w/|\ln\Delta w|$ with $\Delta w=w-2$ at (C_a). The energy difference ΔF between the bound and the free states scales as

$$\Delta F\sim\kappa(\Delta w)^2/|\ln\Delta w|\quad\text{at } C_a\ . \tag{5}$$

The limit $\Delta w\to0$ is singular since $C_1^*=2/R+0(\Delta w/R)$, while $C_1=1/R$ for the free spherical vesicle. (ii) For $p_1<p<p_2$, with $p_2\simeq11.4$, the free spherical vesicle coexists with a bound state of finite A^*, corresponding to a discontinuous transition (D_a^{sp}). (iii) The phase boundaries C_a and D_a^{sp} meet at the special tricritical point (S_1). At S_1, we find $A^*\sim R^2\Delta w$ and $\Delta F\sim\kappa(\Delta w)^2$. The phase boundary ($D_a^{\mathrm{sp}}$) is given by $\Delta w\sim-\exp(-\mathrm{const}/\Delta p)$ where $\Delta p=p-p_1$ and $A^*\sim R^2\exp(-\mathrm{const}/\Delta p)$ along D_a^{sp}. (iv) At $p=p_2$, the free spherical vesicle undergoes a first-order transition (D_f^{pr}) to a prolate ellipsoid. This leads to a change in slope of the phase boundary at the triple point (S_2). The curve D_a^{pr} denotes the coexistence curve between the bound state and the free prolate shape. (v) Within the region of the phase diagram where the free prolate ellipsoid represents the stable state, the vesicle undergoes transitions between different metastable states. In Fig. 2, these transitions are displayed by dashed lines. The phase boundary (D_a^{sp}) is continued into this metastable region. At the critical end point (S_3) with $p_3=12$ ($p_3=12-2C_0R$ for $C_0R>-1.2$), the free sphere loses its metastability and undergoes a continuous transition (C_f) to an oblate ellipsoid. For $p>p_3$, the curve (D_a^{ob}) denotes the coexistence between this oblate ellipsoid and a metastable bound state. At the azeotropic point (S_4), with $p_4\simeq13.7$ for $C_0=0$, the curvature of the bound vesicle vanishes at the top and bottom while the contact area A^* vanishes as $A^*\sim R^2w^{1/2}$. For $p>p_4$ the phase boundary increases again, separating free and adhering metastable shapes which are concave at the top; see Fig. 1(b). (vi) These transitions between metastable states become transitions between stable states for $C_0R<-1.2$. In this case, the free spherical vesicle undergoes a first-order transition (D_f^{ob}) to an oblate ellipsoid at $p=p_2$. Consequently, the adhesion transition (D_a^{ob}) separates a stable bound state from this stable free oblate shape.

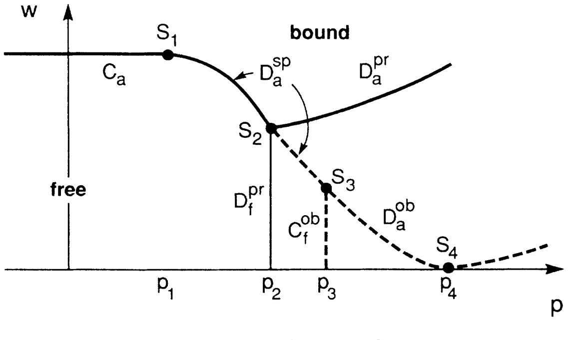

FIG. 2. Schematic phase diagram for $C_0=0$. Above the phase boundary ($C_a,S_1,D_a^{\mathrm{sp}},S_2,D_a^{\mathrm{pr}}$) the vesicle is bound, below it is free.

The adhesion transitions just described are driven by the overall curvature of the vesicle. If the gain in adhesion energy does not overcome the cost in bending energy, the vesicle unbinds from the wall. In real systems, these transitions are preempted by thermal activation since the vesicles have a finite size and thus a finite contact area. Indeed, in the presence of a large amount of water, any bound vesicle will unbind on sufficiently large time scales of order $\sim\exp(|\Delta F|/T)$ where ΔF is the difference in free energies of the bound and the free state. Close to an adhesion transition, this free-energy difference becomes small. At the transition C_a, for example, the behavior of ΔF as given by (5) leads to the estimate that such an activated unbinding process dominates for $\Delta w\simeq\Delta w_{\mathrm{act}}\equiv(T/\kappa)^{1/2}$ with $\Delta w=w-2$. For fluid bilayers at room temperature, one has $\kappa\simeq10^{-19}$ J, $T=T_{\mathrm{room}}\simeq4\times10^{-21}$ J, and thus $\Delta w_{\mathrm{act}}\simeq0.2$.

The theory presented here does not yet include the effect of thermally excited fluctuations about the extremal vesicle shape. Such fluctuations can, in fact, be observed by optical methods both for free[15,16] and for bound[17] vesicles. They will lead to an entropic contribution to the free energy[18] and should increase the tendency of the vesicle to unbind. For an investigation of this effect one has to use a more microscopic model in which the adjacent segment of the vesicle experiences an interaction potential $V(z)$ where z denotes its distance from the wall. The attractive part of $V(z)$ is given by $V_{\mathrm{at}}(z)=-U(z_0/z)^r$, where $U>0$ may be identified with the contact potential W of the coarse-grained description in (1). As long as $r\geq 2$, which includes the important case of van der Waals forces, an infinite membrane unbinds at a finite value[9] U_a^∞ of the amplitude U. Above this transition, the free-energy density f scales as $f\sim U_a^\propto(U/U_a^\propto-1)^2$. Consequently, a finite membrane segment of linear size R will unbind via thermal activation at $U_a(R)\approx U_a^\infty+a_1(TU_a^\infty)^{1/2}/R$, where a_1 is a numerical coefficient of order unity. Equating $U_a(R)$ with $W_a(R)$ yields the crossover radius $R_c\approx(2\kappa/U_a^\infty)^{1/2}$ for the phase boundary C_a, which is then given by $W_a(R)\approx 2\kappa/R^2$ for $R\ll R_c$ as in the absence of fluctuations and by $W_a(R)\approx U_a(R)$ for $R\gg R_c$. A numerical estimate of R_c may be obtained from Monte Carlo data,[10] which yield for a square-well potential with range z_0 the critical depth $U_a^\infty\simeq 0.2T^2/(\kappa z_0^2)$. For $z_0=3$ nm, $T=4\times 10^{-21}$ J, and a single bilayer with $\kappa=10^{-19}$ J, we find $R_c\simeq 0.2\ \mu\mathrm{m}$.

In these estimates of the fluctuation effects, we did not include the overall constraint arising from the closure of the membrane. This constraint presumably leads to an effective tension. As shown in Ref. 12, a lateral tension acts to reduce the amplitude of the shape fluctuations and thus to increase the tendency for adhesion. In the present context, the effective tension arising from the closure constraint must vanish for large vesicle size R. For small R, on the other hand, the relevant contact potential $W=W_a(R)=w_a\kappa/R^2$ is large and thus is hardly renormalized by the shape fluctuations. Therefore the possible influence of such an effective tension should be restricted to intermediate R values.

In summary, we have introduced and studied a simple model for the adhesion of vesicles that leads (i) to a variety of different shapes for bound vesicles and (ii) to nontrivial adhesion transitions between bound and free vesicle states. Our estimates indicate that these phenomena should be accessible to experiments using standard optical methods, such as, e.g., phase contrast microcopy.

Our theory can be extended in several ways. Adhesion to a curved wall, mutual adhesion of vesicles, and the influence of long-ranged adhesion potentials can be treated within the same theoretical framework. Likewise, we could include additional elastic terms into the free energy in order to study the adhesion of vesicles composed of crystalline or polymerized membranes. A more elaborate problem is a local stability analysis of bound vesicle states which will presumably show the importance of nonaxisymmetric shapes for certain regions of the phase diagram. Finally, one may study the effect of an attractive wall on the topology of vesicles. Indeed, we have found that adhesion can change the topology since it can induced vesicle fusion.[19]

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[1] See, e.g., B. Alberts, D. Bray, J. Lewis, R. Raff, K. Roberts, and J. D. Watson, *Molecular Biology of the Cell* (Garland, New York, 1983).

[2] See, e.g., *Physics of Amphiphilic Layers,* Vol. 21 of *Springer Proceedings in Physics,* edited by J. Meunier, D. Langevin, and N. Boccara (Springer, Berlin, 1987).

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[12] R. M. Servuss and W. Helfrich, J. Phys. (Paris) **50**, 809 (1989), have previously reached a similar conclusion for the adhesion of a membrane segment subject to an external tension Σ. In this case, one may define an effective contact angle as long as the linear size of the membrane is large compared to the length scale $(\kappa/\Sigma)^{1/2}$.

[13] Variation with a variable contact point leads to a transversality condition which is equivalent to the boundary condition (2). For a general discussion of transversality conditions, see, e.g., R. Courant and D. Hilbert, *Methods of Mathematical Physics* (Interscience, New York, 1953), Vol. 1.

[14] The same boundary condition also applies if one considers a stretchable membrane with an elastic energy term $F_k=(k/2)(\Delta A/A_0)^2$ or if one adds a shear energy term to (1). For adhesion at a curved wall, the boundary condition (2) reads $C_1^*=(2W/\kappa)^{1/2}+C_W^*$, where C_W^* is the curvature along the meridian of the axisymmetric wall at the contact point.

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[17] E. Evans (private communication) has experimentally studied the shape fluctuations of bound vesicles by micropipet techniques.

[18] In general, there are additional contributions to the entropy arising, e.g., from the translational degrees of freedom. The latter contribution will affect the size distribution in a system with a large number of vesicles.

[19] For large w, the energy ΔE gained by the fusion of two bound vesicles of size R scales as $\Delta E\approx 4\pi g(\sqrt{2}-1)\kappa\sqrt{w}$ with $g\simeq 2.8$; see R. Lipowsky and U. Seifert, in Proceedings of the 13th International Liquid Crystal Conference, Vancouver, 1990 [Mol. Cryst. Liq. Cryst. (to be published)].