

Phase separation and curvature of bilayer membranes

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Phase separation of two-component mixtures in fluid bilayers is shown to result in a stable one-phase vesicle region near the critical composition for the mixture. The resulting phase diagram exhibits tricritical behavior: The critical point for phase separation lies on the line of continuous transitions between lamellar and vesicle phases. Near the continuous transitions, the polydispersity of the resulting vesicles is large. Possible implications for experiments on two-component surfactant systems are also discussed.

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Two-dimensional fluid membranes composed of amphiphilic molecules such as phospholipids or surfactants form a variety of structures of scientific as well as technological importance. In particular, fluid bilayer membranes can form open, flat lamellae or closed, curved vesicles. Recent experimental studies [1,2] have concerned membranes composed of mixtures of oppositely charged ionic surfactants. These studies have shown that a wide variety of such mixtures can result in stable (equilibrium) vesicles, in contrast with single surfactant systems. Motivated by these experiments, previous theoretical work [3,4] has focused on the nature of interactions between the two species which can lead to complex formation and to spontaneous vesicle formation. Complex formation, however, leads to stable vesicles only in special cases. In this work, we show quite generally that for amphiphilic mixtures in bilayers, phase separation within the membrane can lead to curved bilayers, and in fact, to closed vesicles of well-defined size. In addition, we compute the polydispersity of vesicles and show that near lamellar-vesicle phase boundaries, the polydispersity can grow without bound. Finally, we discuss the relevance of this work to experiments as well as previous theoretical work.

In simple binary liquid mixtures, attractive interactions between like species result in phase separation in an appropriate temperature and composition range. The coexisting phases are of different compositions and the sample can contain metastable domains of these phases. Due to the surface tension or excess free energy associated with the interfaces between these domains, the equilibrium configuration corresponds to two macroscopic regions separated by an interface with minimal surface area, such as occurs when water and oil are mixed at room temperature. In this case there is no characteristic length scale for the domains in the thermodynamic limit. In contrast, we find that for binary mixtures of amphiphiles in fluid bilayers, phase separation chooses an intermediate length scale for the formation of stable vesicles. This is due to the coupling of composition and curvature degrees of freedom [3–5]. Another feature of phase separating fluid

bilayers is that the two phases can coexist on opposite sides of a bilayer; the phase separation can occur without interfaces between the two phases. We predict a stable, one-phase vesicle region near the critical composition, with a simple dependence of the vesicle size on composition. We also determine the regions of coexistence of vesicles with lamellae.

In order to illustrate this behavior, we introduce a model free energy, which we then treat within mean-field theory. First, we assume that the bilayer membrane is composed of a mixture of two amphiphiles, for which the relevant interactions (e.g., those which promote phase separation) are primarily between molecules in the same monolayer. This should apply, for example, to a mixture for lipid molecules in water, which molecules differ only in their polar head groups [6]. In this case, the only interfaces which we need consider are those between regions of different (polar) composition within each monolayer.

For simplicity, we consider only isotropic curvatures of the membrane. We thus focus on the transition from lamellae to spherical vesicles. Furthermore, we assume that the local mean curvature of the membrane is free to adjust to minimize the curvature energy. In other words, only equilibrium membrane shapes are considered. Since the spontaneous curvature comes from a delicate balance of the head and chain packing densities, we assume that the composition (e.g., relative fraction of one of the amphiphiles) primarily affects the spontaneous curvature and not the bending modulus. Again, this should apply to mixtures of amphiphiles which differ only in their polar segments, since bending moduli are determined principally by chain properties [7].

We shall consider a model for phase separation of surfactant heads in a bilayer. For simplicity, we shall consider the Landau free energy

$$f_{\phi} = \frac{1}{2}t\phi^2 + \frac{1}{4}\phi^4, \quad (1)$$

where ϕ is the composition relative to the critical composition ϕ_c , and $t \sim (T - T_c)$ is the reduced temperature. In

the bulk, this would lead to coexisting phases of compositions $\phi = \pm\sqrt{-t}$, for $t < 0$. A gradient term in f_ϕ results in an excess free energy or surface tension associated with interfaces between coexisting phases [8]. As will be shown below, such a term is not important, provided that the bending rigidity is large. The curvature energy [9] of a mixed amphiphilic monolayer can be expressed as [10]

$$f_c = \frac{1}{2}\kappa[c - c_0(\phi)]^2, \quad (2)$$

where $c_0(\phi) = c_0 - \gamma\phi$ is the spontaneous curvature as a function of composition ϕ , and c_0 is the spontaneous curvature of the monolayer at the critical composition ϕ_c . We have assumed a simple linear dependence of spontaneous curvature on ϕ for illustrative purposes. This linear assumption is valid for systems where the spontaneous radius of curvature is much larger than the correlation length associated with phase separation and a simple averaging over the local spontaneous curvatures is appropriate. For a bilayer, the free energy is

$$f = \frac{1}{2}t(\phi_1^2 + \phi_2^2) + \frac{1}{4}(\phi_1^4 + \phi_2^4) + \frac{1}{2}\kappa[(c + \gamma\phi_1)^2 + (c - \gamma\phi_2)^2] - \mu(\phi_1 + \phi_2), \quad (3)$$

where ϕ_1 and ϕ_2 refer to the compositions of the inner and outer layers, which may or may not be homogeneous, and μ is the chemical potential. For a closed system, the Lagrange multiplier, μ is determined by the constraint of fixed composition. We have also eliminated terms proportional to c_0 , which merely shift the chemical potential.

Minimizing the free energy with respect to ϕ_1 and ϕ_2 gives

$$t\phi_1 + \phi_1^3 + \kappa\gamma^2\phi_1 = \mu - \gamma\kappa c, \quad (4)$$

$$t\phi_2 + \phi_2^3 + \kappa\gamma^2\phi_2 = \mu + \gamma\kappa c. \quad (5)$$

We shall denote the mean composition of a bilayer by $\psi = (\phi_2 + \phi_1)/2$ and the composition asymmetry by $\phi = (\phi_2 - \phi_1)/2$. For $t + \kappa\gamma^2 \geq 0$, the compositions ϕ_1 and ϕ_2 of each layer are uniform by Eqs. (4) and (5). As a result, we need not consider a gradient term in Eq. (1). Hence, ϕ and ψ are constants, and the free energy can be written as

$$f = (t + 3\psi^2)\phi^2 + \frac{1}{2}\phi^4 + (t + \kappa\gamma^2)\psi^2 + \frac{1}{2}\psi^4 - 2\mu\psi, \quad (6)$$

after minimization with respect to c . The mean curvature of the bilayer is related to the composition difference ϕ by $c = \gamma\phi$. The orientation of the bilayer (i.e., choice of “inside” and “outside”) is arbitrary, as is the sign of the bilayer curvature: one monolayer has curvature $\gamma\phi$, while the other has curvature $-\gamma\phi$. Thus the system is degenerate with respect to the sign of ϕ .

For $\mu = 0$, which corresponds to a closed system at the critical composition, the system is macroscopically uniform, and $\psi = 0$. Below $t = 0$, the bilayer spontaneously breaks the symmetry in composition between the inside and outside, such that $\phi = \pm\sqrt{-t}$. By our assumption that the curvature of the membrane is isotropic, the equilibrium is characterized by vesicles of radius $R = (\gamma|\phi|)^{-1}$. This has a lower free energy than a lamel-

lar phase.

A more detailed analysis of the free energy shows that vesicles are indeed more stable than lamellae in a region around the critical composition. This is due to the ability of the vesicles with different compositions in the inner and outer layers to relieve the frustration of the curvature energy. The possibility of vesicle formation precludes the usual coexistence of lamellar systems with two compositions for temperatures near $t = 0$ where we find a transition from lamellae with uniform compositions to vesicles with composition asymmetries. At lower temperatures, vesicles are stable for compositions near $\psi = 0$ and a two-phase coexistence of symmetric lamellae and vesicles is predicted for larger values of ψ .

For a given temperature t and chemical potential μ the minimum of f with respect to ψ is determined. From the value ψ_* corresponding to this minimum, both ϕ and $c = \gamma\phi$ are determined. Vesicle and lamellar phases are distinguished by the order parameter ϕ which breaks the composition symmetry between inside and outside: if $\phi = 0$, the bilayer is flat, which we identify with the lamellar phase; if $\phi \neq 0$, the bilayer is curved, which we identify with the vesicle phase. For $0 > t > -\kappa\gamma^2/6$, the transition from vesicles to lamellae is second order. At a critical value μ_c the asymmetry, ϕ and the curvature c go continuously to zero. Below $t_c = -\kappa\gamma^2/6$, the transition becomes first order. This is illustrated by the phase diagram in Fig. 1. For a closed system, the first-order transition corresponds to the coexistence of uniform, flat lamellae with vesicles of a finite radius and composition difference between inside and outside. This two-phase re-

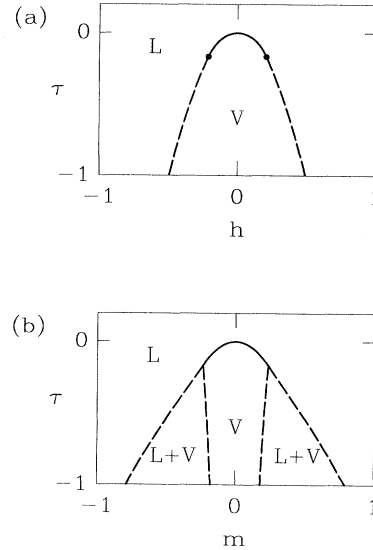


FIG. 1. (a) The phase diagram as a function of rescaled chemical potential $h = \mu(\kappa\gamma^2)^{-3/2}$, and temperature $\tau = t/(\kappa\gamma^2)$. Vesicle (V) and lamellar (L) phases are labeled. Below the tricritical point (●), the transition becomes first order, as indicated by the dashed line. (b) The phase diagram as a function of reduced temperature τ and composition $m = \psi\sqrt{\kappa\gamma^2}$. The region of coexistence of vesicles with lamellae is denoted by V+L. In this example, we have set $\kappa\gamma^2 = 1$.

gion is indicated by “ $L + V$ ” in Fig. 1(b).

The symmetry of the phase diagrams about $\psi=0$ and $\mu=0$ is a consequence of the $\psi^2\phi^2$ coupling in Eq. (6). A $\psi\phi^2$ is also allowed by symmetry. Such a term, however, results in phase diagrams which, although asymmetric, have the same form.

The calculated phase diagrams in Fig. 1 are reminiscent of diagrams for metamagnets and for ^3He - ^4He mixtures at low temperatures [11]. In particular, these systems exhibit a critical point for phase separation which lies on a line of continuous transitions (e.g., the λ line). In fact, it can be shown that the present model is formally equivalent to the mean-field theory of metamagnets in Ref. [11]. At the critical point, a uniform lamellar phase and two degenerate (ordered) phases, with positive and negative ϕ , coexist [12,13]. In the limit of large $\kappa\gamma^2$, coexistence of vesicles with lamellae is not expected, since $t_c = -\kappa\gamma^2/6$ becomes unphysical. We expect, however, that the intermediate case ($\kappa\gamma^2 \lesssim 1$) is most relevant for experiments, since the bending modulus κ may be significantly renormalized by composition fluctuations, and since the (dimensionless) curvature c and coupling γ are expressed relative to a molecular dimension.

Similar phase behavior has been obtained for models of the so-called sponge phase [14]. In this phase there are two relevant order parameters, one of which is an *inside-outside* order parameter. Although the model of Ref. [14] differs from the present one in certain essential respects, the resulting phase diagrams also exhibit tricritical behavior. Provided that the bending modulus and the coupling γ are sufficiently large, the present work predicts vesicle and lamellar phases. For small bending modulus and low surfactant concentration, on the other hand, a dilute vesicle phase may exist (as predicted in Ref. [14]). However, such vesicles are stabilized by their entropy of dispersion. As a result there is no single characteristic size for these vesicles, in contrast with the present model.

In the present model, we have not considered either the entropy of dispersion or possible interactions between vesicles. As mentioned before, for dilute solutions the entropy of dispersion tends to favor vesicles over lamellae. Furthermore, related experiments [2,15] have shown that vesicles remain stable up to relatively high concentrations (i.e., comparable with close packing of vesicles). This suggests that interactions between vesicles will not significantly affect vesicle stability at lower concentrations. Thus, our results are strictly valid in the limit of dilute solutions. At finite concentrations, for example, the second-order transition may become first order.

The bending modulus κ is also the principal factor determining the polydispersity of the vesicles. The effective bending modulus may be significantly reduced by composition fluctuations, especially near the line of continuous transitions in the figure. For example, just above the point $\psi=0$, $t=0$ ($m=0$, $\tau=0$ in the figure),

$$f \simeq \kappa c^2 - 2\kappa\gamma\phi c + (t + \kappa\gamma^2)\phi^2. \quad (7)$$

After minimization with respect to ϕ , the effective bilayer curvature energy is $f_{\text{eff}} \simeq \kappa_{\text{eff}} c^2$, where

$$\kappa_{\text{eff}} = \kappa \frac{t}{t + \kappa\gamma^2} \simeq \frac{t}{\gamma^2} < \kappa. \quad (8)$$

Similarly, below the transition $\kappa_{\text{eff}} \simeq 2|t|/\gamma^2$ and the polydispersity (which is inversely related to the rigidity [4]) diverges at $t \rightarrow 0$. Thus both the width of the distribution as well as the mean vesicle size depend on temperature and composition: the polydispersity diverges near the line of continuous transitions between vesicles and lamellae. This is in contrast with the work of Ref. [4], where complex formation alone results in a small polydispersity of order kT/κ .

The present work demonstrates that mixtures of interacting amphiphiles can stabilize vesicles with different compositions in the inner and outer layers. These vesicles are predicted to form spontaneously and to be energetically favored over flat, lamellar phases for compositions near the critical composition for phase separation. The physical origin of the vesicle stability is the coupling between the tendency for the amphiphiles to phase separate and the composition dependent, spontaneous curvature of each monolayer.

Experiments on mixtures of surfactants with oppositely charged polar head groups have investigated in detail the nature of equilibrium vesicle formation and the resulting structures and phase diagrams [2]. In these systems, one expects *attractive* interactions between the two different surfactant species A and B due to the Coulomb interactions. Previous theoretical studies have shown under what conditions complexation can lead to stable vesicles. When the vesicle curvature is small and the interactions are weak, these conditions are expected to apply only in special cases. For *strong* interactions, on the other hand, the result is an effective mixture of surfactants A (assumed to be the majority) and B' (complexes of the two species A and B), and in the absence of repulsive interactions between these two effective species, spontaneous vesicle formation does not occur: the system is predicted to be lamellar. In the present work, we have shown how phase separation (i.e., *repulsive* interactions between the two different species) can stabilize vesicles for a range of compositions and temperatures. These results may be applicable to the mixed-charge systems if one considers the usual case of strong Coulomb interactions, where complexing occurs. In this limit, the result is an effective mixed-amphiphile system, where the two species are the surfactant complexes and the unpaired majority surfactants. The interaction between these two effective surfactants may indeed be repulsive, leading to phase separation of complexes from the excess majority phase. Such an interaction may arise from variations in the surfactant density in the mixture: each complex corresponds to a distortion of the local head group packing within the membrane, and the resulting elastic energy can be reduced by the aggregation of complexes [16]. A similar sort of *indirect* interaction has been considered in the context of surfactant films with absorbed polymer [17]. In the present case, the low-temperature state would be one where all of the complexes are on one side of the bilayer with the excess species on the other side of the bilayer. Thus one would expect two critical compositions

of $\psi_c = \frac{1}{4}, \frac{3}{4}$ with single-phase vesicle regions near ψ_c . By the same token, one would also expect that the compositions of $\frac{1}{4}$ and $\frac{3}{4}$ would be the most stable in the surfactant concentration-composition plane; this seems to be consistent with the experimental phase diagrams for surfactants with comparable chain lengths and oppositely charged head groups [15,18].

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