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Theory of Spontaneous Vesicle Formation in Surfactant Mixtures

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The curvature elastic energy of bilayer vesicles formed by a mixture of two surfactants, which individually form either micelles or lamellar bilayer phases is described theoretically. In the limit of large bending elastic modulus *K* being much greater than the temperature *T*, the free energy is minimized by vesicles with different concentrations of the two surfactants in each monolayer of the bilayer. Vesicles are more stable than lamellar structures only when interactions or complexing of the two surfactants is taken into account.

RECENT EXPERIMENTS (*1*) PROVIDE strong evidence for the spontaneous formation of an equilibrium phase of large single bilayer vesicles from mixtures of two ionic surfactants with oppositely charged head groups. Indeed, most previous observations of thermodynamically stable vesicles also involved surfactant mixtures (*2–4*) or systems with mixed counterions (*5*). We present a continuum, theoretical model based on curvature energy concepts (*6*) for the equilibrium formation of vesicles with a preferred radius in mixed surfactant systems. Within this model, we find that vesicle formation requires nonideal mixing of the surfactants within a membrane and that it only occurs in the presence of complexing interactions.

Previous theoretical models that predict equilibrium vesicles (as opposed to lamellar or micellar phases) even for single surfactants have focused on the case of either small vescicles (7), or on the case of surfactant bilayers with small (compared to temperature *T*) curvature elastic modulus (*6*) *K*; the vesicles are then stabilized by entropic effects (*7–9*). In our model, which focuses on the limit of large *K* (>0), the most probable state for the pure surfactant solutions is either small micelles or lamellar bilayer phases. However, when the two surfactants are mixed, a vesicular phase can be stabilized by the curvature energy; the vesicles are stable (with respect to the lamellar phase) even in the limit of large curvature elastic modulus. The vesicular phase has lower free energy than the lamellar phase (*10*) because the mixing—when complexing or nonideality is included—allows the formation of surfactant bilayers where the two monolayers have different surfactant concentrations, which may result in equal and opposite monolayer spontaneous curvatures (*6*).

We consider the free energy of a phase composed of a dilute solution of monodisperse, spherical vesicles where surfactants labeled "1" and "2" are mixed in a single solvent. Since our goal is to demonstrate the stabilization of the vesicles by their "internal" free energy, we consider the case where the translational entropy as well as interactions between the layers can be neglected (*10*). The vesicle is composed of inner and outer monolayers with curvatures (*11*) $\pm c$ to lowest order in the ratio of the surfactant thickness δ to the vesicle radius $R = 1/c$. In general, each monolayer has a different composition, and we denote the spontaneous curvatures (*6*) of the inner and outer monolayers by c_i and c_o, respectively. The spontaneous curvature of films formed by pure surfactants "1" and "2" are c_1 and c_2, respectively. The curvature elastic energy (*12*) of the vesicle per unit area f_c is given (*6*) by

$$f_c = 2K[(c + c_o)^2 + (c - c_i)^2] \qquad (1)$$

For simplicity, we have assumed that the bending modulus K of the mixed system is independent of the composition. In the case of the pure surfactants, the curvature energy is minimized by the formation of micelles [when c_1 and c_2 are consistent with the packing constraints (*13*)] or lamellar bilayers [when c_1 and $c_2 << \delta^{-1}$, with δ a molecular size].

The physical origin of the spontaneous curvature of a monolayer is the asymmetry in the packing densities of the polar and nonpolar regions of the amphiphile (*13, 14*). If we assume, for simplicity, that the hydrophobic regions of the two surfactants are identical but that their polar groups are different, the composition dependence of c_o and c_i arises from the composition dependence of the bond distances in the polar parts of each monolayer. Let ψ_i and ψ_o be the molar fraction of surfactant "2" in the inner and outer monolayers, respectively; the molar fraction of surfactant "1" is then $1 - \psi_i$ or $1 - \psi_o$, respectively. A random-mixing (*15*) approximation for the average distance a_i between polar heads in the inner layers yields:

$$a_i = a_1(1 - \psi_i)^2 + a_2\psi_i^2 + (1 - \gamma)(a_1 + a_2)\psi_i(1 - \psi_i) \qquad (2)$$

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with a similar equation for the average distance between polar heads in the outer layer. In Eq. 2, a_1 and a_2 are the distances between polar heads in monolayers composed of only surfactant "1" or "2", respectively. The term proportional to γ represents the effects of interactions of the two surfactants (*16*), which can either increase ($\gamma < 0$) or decrease ($\gamma > 0$) the distance between a molecule of type "1" and one of type "2," compared with the average bond distance, $(a_1 + a_2)/2$; the case $\gamma = 0$ represents an ideal mixing where the bond distances follow the average [$a_i = a_1(1 - \psi_i) + a_2\psi_i$, for example].

We define $\phi = 1/2(\psi_o - \psi_i)$, that is, the composition difference between the inner and outer layers and note that the average mole fraction of surfactant "2" is $\psi = 1/2(\psi_o + \psi_i)$. The spontaneous curvatures, which are linear functions of the bond distances, can then be written:

$$c_i = \overline{c}(\psi) + \alpha(\psi)\phi - \beta\phi^2 \qquad (3)$$

$$c_o = \overline{c}(\psi) - \alpha(\psi)\phi - \beta\phi^2 \qquad (4)$$

where β is proportional to γ, $\overline{c} = c_1(1 - \psi) + c_2\psi + \beta\psi(1 - \psi)$, and $\alpha = (c_1 - c_2) - \beta(1 - 2\psi)$. We use these expressions in Eq. 2, and then minimize f_c with respect to the curvature c to determine the minimum energy curvature c^* as $c^* = \phi\alpha$. Thus phase separation of the two surfactants in the inner and outer layers (that is, nonzero values of ϕ) implies a finite curvature as the minimum energy state. To determine ϕ, we add to the elastic energy the contribution from the entropy of mixing of the surfactants in the two monolayers. Defining f_m as the ϕ dependent part of this contribution, in a random mixing approximation to order ϕ^4,

$$f_m = K\tau\left\{\frac{\phi^2}{\psi(1 - \psi)} + \frac{1}{6}\phi^4\left[\frac{1}{\psi^3} + \frac{1}{(1 - \psi)^3}\right]\right\} \qquad (5)$$

where $\overline{a}$ is a molecular size and $\tau = T/(\pi K\overline{a}^2)$. When $c = c^*$, the free energy per unit area relative to the lamellar phase, $f = f_c + f_m$, is

$$f = 2K\left(-\frac{1}{2}\epsilon\phi^2 + \frac{1}{4}A\phi^4\right) \qquad (6)$$

where $\epsilon = 8\beta\overline{c} - \tau/[\psi(1 - \psi)]$ and $A = 8\beta^2 + \tau/3[1/\psi^3 + 1/(1 - \psi)^3]$.

For $\epsilon < 0$, the minimum free energy state is composed of flat bilayers where the two monolayers have identical compositions ($\phi = c = 0$). When $\epsilon > 0$, the free energy is minimized by a nonzero value of ϕ and hence a nonzero curvature. However, for this to happen, the product $\beta\overline{c} > 0$. This condition, together with the requirement that $\phi^2 < \psi^2$, constrains the ranges of c_1, c_2, β, and ψ where the vesicles are stable (*17*). For example, if $c_1 \approx c_2 < 0$ (*11*) (that is, the surfactants tend to form micelles in water), then β must be positive, implying attractive interactions are necessary to stabilize vesicles. For large values of K/T, the value of ϕ that minimizes f is $\phi^* = \pm(\overline{c}/\beta)^{1/2}$, where the correct sign is obtained by the requirement that $c^* = \phi^*\alpha$ be positive by convention. In this case, the vesicle free energy is lower than the lamellar free energy by an amount $f = -4K\overline{c}^2$. These values of ϕ^* and c^* are precisely what is needed so that $c^* = c_i = -c_o$; the compositions of the two surfactants in the two monolayers are such that the spontaneous curvatures of the inner and outer layers are equal and of opposite sign. If the complexing is absent (that is, $\gamma = \beta = 0$), this can only occur for the special case of $\overline{c} = 0$; the lowest energy state would be flat bilayers ($\epsilon < 0$) due to entropy of mixing, as indicated by Eq. 6.

Since the curvature is proportional to ϕ, Eq. 6 can also be viewed as the curvature free energy of the vesicle. Although f is minimized by a finite value of the curvature, the vesicle does not have a spontaneous curvature in the sense of a microemulsion droplet (*18*); there is no term in the effective energy that is linear in curvature. Instead, the finite value of c^* arises from the balance of a negative term quadratic in the curvature with a positive quartic term. Thus the effective bending modulus is negative in these systems in which the degree of freedom of composition variations stabilizes curved layers (*19*).

We propose a model wherein vesicles of a preferred size are the most probable state in systems composed of mixed surfactants that interact or form complexes. This model may explain the enhanced stabilization of large vesicles by the mixing of oppositely charged surfactants (*1*); the resulting complexes would tend to decrease the average polar bond length so that $\gamma > 0$. Although we have used a particular microscopic interaction for the composition dependence of the polar bond distances, Eqs. 3 and 4 can apply quite generally to mixed, interacting surfactant films; the interactions can arise from either the polar heads or the chains. Since these vesicles are stabilized by the curvature energies, these effects should be largest in stiff systems with $K >> T$. Surfactant-like long-chain polymers (or block copolymers), where K increases with molecular weight, may be another class of materials where these vesicles can be formed (*20*).

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11. We use the convention that the inner monolayer has positive curvature.
12. We have omitted the saddle-splay [see (*6*)] term in the elastic energy since we consider only spherical vesicles and define the monolayer spontaneous curvatures as the curvatures at which the elastic energy is minimized. We thus write the elastic energy as $f_c = 1/2K'(c_1 + c_2 - 2c_0')^2 + \overline{K}'(c_1 - c_2)^2$, where K', $\overline{K}'$, and c_0' can be simply related to the standard (*6*) K, $\overline{K}$, and c_o. In this rewriting of f_c, the term proportional to the saddle-splay $\overline{K}'$ vanishes for spherical structures and c_0' is the curvature of the minimum energy sphere. This is the motivation for Eq. 1, where the primes have been dropped for notational convenience.
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Developmental Arrest During Larval Life and Life-Span Extension in a Marine Mollusc

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The length of larval life in the nudibranch *Phestilla sibogae* is determined by a chance encounter with a specific metamorphic stimulus associated with the post-larval benthic habitat. A developmental hiatus begins at the onset of larval metamorphic competence and ends at metamorphosis; aging is suspended during this hiatus. Because the duration of post-larval life is unaffected by the duration of larval life, total life-span varies with the length of the larval period. Developmental control of the timing of expression of life-history stages is an important factor regulating aging and senescence in animals with complex life cycles.

ORGANISMS WITH COMPLEX LIFE cycles undergo a metamorphic transition that is prerequisite for post-larval growth and development (*1*). For many marine invertebrate species with planktonic larvae, metamorphosis is induced by environmental cues associated with the benthic habitat, and this helps place juveniles in appropriate post-larval settings (*2*). The reliance of metamorphic induction on chance encounters with environmental cues imparts variation to the duration of the larval period [that is, the time between hatching and metamorphosis (*3*)] and, consequently, to the temporal age at which post-larval life begins. The effects of larval duration on post-larval life reveals developmental relations among life-history stages that influence aging and senescence and the evolution of complex life cycles in benthic marine invertebrates.

Evolutionary theories of life-span determination (*4*) propose that deleterious genetic effects expressed late in life result in aging and senescence. These effects may arise pleiotropically from genes that are favored early in life or from the accumulation of mutations in genes that are expressed during post-reproductive life. From these theories it follows that an extension of the larval period should extend life-span by delaying subsequent life-history stages in which these deleterious genetic effects are expressed.

The effects of extended larval life on durations of the juvenile (metamorphosis to first egg laying) and adult (first egg laying to death) periods and on reproductive output were assessed in the aeolid nudibranch *Phestilla sibogae*. Veliger larvae from each of 35 egg masses hatched 5 days after fertilization and were fed phytoplankton freely for the duration of the larval period (*5*). Larvae from each egg mass were sampled 7, 14, 21, and 28 days after hatching. A subset of larvae from each sample was induced to metamorphose by exposure to a fragment of the nudibranch's coral prey (*5*). The remaining sampled larvae and some of the 24-hour post-metamorphic juveniles were used for weight determinations (*6*). Post-larval life was followed in juveniles and adults raised as isolated pairs on their coral prey (*7*). At first reproduction, some adult pairs were sacrificed for weight determinations, and egg masses produced by the remaining pairs were collected daily and weighed (*6*).

Larval weights remained the same throughout all larval periods, and the weights of 24-hour-old juveniles were not significantly affected by the duration of the larval period (Table 1). The maximum experimental larval period, 28 days, represented a more than threefold increase over the minimum developmental time needed to attain the morphology and physiology necessary for successful metamorphosis (that is, 5 days of embryonic development plus 3 days of precompetent larval development). In spite of this great increase in larval period, durations of the juvenile and adult periods were essentially unchanged (Fig. 1). Consequently, both the average life-span and the maximum achievable life-span (oldest individual) increased by the number of days that the larval period was extended. Extending the larval period to 28 days increased average life-span 20 days (26%) over that obtained when an individual metamorphosed soon after it achieved metamorphic competence (that is, 7-day larval period).

The data presented here demonstrate that extension of the planktotrophic larval period beyond the time required to attain metamorphic competence does not significantly affect the duration of post-larval life. Because the timing of expression of post-larval life-history stages is influenced by development (*8*), a hiatus in development must occur during the competence phase of the larval period in order to account for the unchanged post-larval duration. This hiatus begins with the onset of metamorphic competence and ends with the induction of metamorphosis. That a developmental hiatus does occur is further supported by two additional observations: larvae of *P. sibogae* show no signs of morphological differentiation or growth during the competence phase of the larval period (*9*); and the metamorphic transition from larva to juvenile requires induction by a chemical signal from the nudibranch's coral prey. Until this signal is encountered, larvae do not undergo juvenile development (*10*).

Delay or arrest of larval development has been experimentally induced in a variety of organisms by changes in nutrition and temperature regimes (*11*). However, these experimental regimes invariably affect metabolism as well (*12*), complicating the assessment of developmental effects on post-larval life history. Insect diapause also alters developmental and metabolic rates in response to seasonal environmental changes (*13*). In contrast, the experiments on *P. sibogae* were conducted at normal (ambient seawater)

Table 1. Mean (± SE) ash-free dry weights of larvae, 24-hour-old juveniles, and adults at first reproduction for individuals that were held for increasing durations as larvae. Sample sizes (*n*) are the number of weight determinations used to estimate larval or juvenile weights (*10*), and the number of adults used to estimate adult weight. Regressions were not statistically significant for larval, juvenile, or adult weights.

Larval period	Larval weight (μg)	Juvenile weight (μg)	Adult weight (mg)
At hatching	0.76 ± 0.02 (*n* = 6)		
7 days	0.70 ± 0.03 (*n* = 11)	0.62 ± 0.05 (*n* = 5)	32.8 ± 1.4 (*n* = 20)
14 days	0.72 ± 0.04 (*n* = 9)	0.67 ± 0.06 (*n* = 5)	33.4 ± 1.2 (*n* = 20)
21 days	0.77 ± 0.03 (*n* = 4)	0.66 ± 0.04 (*n* = 5)	30.7 ± 1.0 (*n* = 37)
28 days	0.73 ± 0.04 (*n* = 5)	0.63 ± 0.06 (*n* = 4)	31.6 ± 1.2 (*n* = 33)

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