# The Energetics of Forming Equilibrated Bilayer Vesicles

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# The Energetics of Forming Equilibrated Bilayer Vesicles

Magnus Bergström and Jan Christer Eriksson*

*Department of Chemistry/Physical Chemistry, Royal Institute of Technology,
S-100 44 Stockholm, Sweden*

On the basis of detailed model calculations we demonstrate that the free energy required to form an equilibrated spherical bilayer vesicle is made up of two main contributions: the (size-independent) work of bending the constituent monolayers and the work of stretching the bilayer that is determined by the planar bilayer tension. A major part of the bending free energy arises due to a reduction of the overall hydrocarbon chain volume upon forming a spherically shaped bilayer shell out of a planar monolayer, while keeping the total hydrocarbon/water contact area fixed and the chemical potentials constant. This contribution is only partially counterbalanced by a sum of contributions due to curvature-dependent molecular free energies of various origins. The overall free energy of bending a pure (charged) SDS bilayer into a vesicle is found to be quite large ($\sim$90$kT$). However, admixing with a long-chain alcohol brings it down substantially and is thus crucial for spontaneous vesicle formation to occur.

## Introduction

The potential use of vesicles and liposomes as vehicles for on-site delivery of drugs, genetic material, and cosmetics has generated a growing interest as to the basic physicochemical aspects of this particular kind of amphiphile aggregate, and during the last decade, a number of fundamentally oriented investigations have been presented.[1–7] Mostly owing to electrostatic reasons, at certain solution states a planar geometry is the favored geometry for a charged aggregate surface, and then bilayer disks may form.[8] However, to eliminate the disadvantageous rim of a large bilayer disk, closure into a spherical vesicle may occur.

The bending properties of bilayers and their influence on the formation (and shape) of vesicles have previously been treated in a number of papers.[3–5] According to Safran *et al.*,[4,5] specific interactions between the surfactants in a mixed bilayer can result in a nonvanishing spontaneous curvature and may, in addition, significantly promote spontaneous vesicle formation.

We have shown, however, that apart from the energetic effects of bending, the residual tension of the *planar* bilayer and a statistical factor related to the fluctuations in chain packing density, composition, and shape play major roles for the genesis and size distribution of large bilayer vesicles.[9,10] In the present work we concentrate on obtaining curvature and stretching free energies from model calculations carried out for equilibrated sodium dodecyl sulfate (SDS)/dodecanol (DOH) bilayer vesicles. No specific intermolecular interactions between the aggregated monomers are invoked in our model, but we take into account electrostatics, hydrocarbon chain conforma-
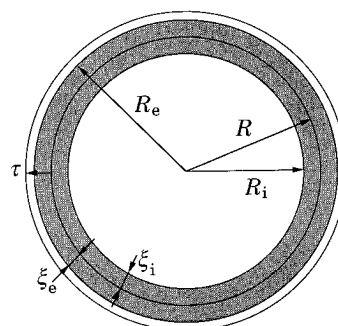


**Figure 1.** Model of a spherical unilamellar bilayer vesicle. The thicknesses of the outer and inner monolayers are $\xi_e$ and $\xi_i$, respectively, and the two monolayers adhere to each other at the radial distance $R$.

tional free energies, as well as hydrocarbon/water contact free energies. Furthermore, for the most part we consider (pure and mixed) bilayers which have zero spontaneous curvature.

The bending of a bilayer is first of all associated with a free energy change related to the *intrinsic* bilayer properties. In addition, however, the enclosure of appreciable amounts of water implies that the vesicle surfaces are brought close to each other even at rather low volume fractions of the bilayer constituents, resulting in *intervesicular* interactions of non-negligible magnitude. Large vesicles occupy a large volume of the solution even at very low bilayer concentrations. Hence, the free energy of forming vesicles out of an infinite, planar bilayer can be written as a sum of the work of bending and an intervesicular interaction potential: $F = F_{bend} + F_{int}$. In this paper we do not consider the second contribution but, still, we underline that to minimize the overall free energy, one would in general have to also invoke the intervesicular interaction.

A spherical vesicle made up solely of SDS monomers can be considered geometrically as being composed of three separate parts (Figure 1): an inner (i) and an outer (e) monolayer with a total number of $N_s = N_s^i + N_s^e$ aggregated surfactant molecules and a water core containing $N_w^i$ water molecules. In addition, $N_w^e$ water molecules are mixed into the outer monolayer which strictly is defined by an external shell located at the radial distance $\tau$ from the bilayer midplane at $R$. We may typically imagine $\tau$ to correspond to the length of the extended hydrocarbon chain, for a $C_{12}$ chain $\approx$ 16.7 Å.[11] Beyond this outer shell,

(1) Israelachvili, N.; Mitchell, D. J.; Ninham, B. W. *Biochim. Biophys. Acta* **1977,** *470,* 185.

(2) Helfrich, W. *J. Phys. (Paris)* **1986,** *47,* 321.

(3) Ou-Yang, Z.; Helfrich, W. *Phys. Rev. A* **1989,** *39,* 5280.

(4) Safran, S. A.; Pincus, P.; Andelman, D. *Science* **1990,** *248,* 354.

(5) Safran, S. A.; Pincus, P.; Andelman, D.; Mackintosh, F. C. *Phys. Rev. A* **1991,** *43,* 1071.

(6) Kumaran, V. *J. Chem. Phys.* **1993,** *99,* 5490.

(7) Porte, G.; Ligoure, C. *J. Chem. Phys.* **1995,** *102,* 4290.

(8) Eriksson, J. C.; Ljunggren, S. *Langmuir* **1990,** *6,* 895.

(9) Eriksson, J. C.; Bergström, M.; Ljunggren, S. *Prog. Colloid Polym. Sci.* **1993,** *93,* 225.

(10) Bergström, M.; Eriksson, J. C. *Langmuir,* submitted for publication.

at the distance $\tau$ from the midplane, the water solution is virtually unaffected by the presence of the vesicle. The corresponding thickness of the hydrocarbon part of each monolayer is only about 11 Å.[12]

For a *mixed* vesicle we denote the number of aggregated alcohol molecules in the inner and outer monolayers by $N_{OH}^i$ and $N_{OH}^e$, respectively.

The radii of the spherical hydrocarbon/water interfaces (which we shall use as dividing surfaces) at the inner and outer monolayers, $R_i$ and $R_e$, respectively (Figure 1), are related to the bilayer midplane radius $R$ by the exact geometrical relationships

$$R_i^3 + \frac{3v_s}{a_i}R_i^2 - R^3 = 0 \qquad (1)$$

and

$$R_e^3 - \frac{3v_s}{a_e}R_e^2 - R^3 = 0 \qquad (2)$$

where $a_i = A_i/N^i = 4\pi R_i^2/N^i$ and $a_e = A_e/N^e = 4\pi R_e^2/N^e$ denote the surface areas per chain at the hydrocarbon/water interface, in the inner and outer monolayer, respectively, and $v_s = 351$ Å$^2$ is the volume of a $C_{12}$ hydrocarbon chain, and where $N^i$ stands for $N_s^i + N_{OH}^i$ and $N^e$ for $N_s^e + N_{OH}^e$.

Considering the thicknesses of the two hydrocarbon monolayer films, $\xi_i = R - R_i$ and $\xi_e = R_e - R$, as parameters we can rewrite eqs 1 and 2 so as to explicitly express the chain packing densities at the hydrocarbon/water interfaces, $\Gamma_i = N^i/A_i = 1/a_i$ and $\Gamma_e = N^e/A_e = 1/a_e$, as functions of the radii $R_i$ and $R_e$, respectively,

$$\Gamma_i = \frac{\xi_i}{v_s}\left(1 + \frac{\xi_i}{R_i} + \frac{\xi_i^2}{3R_i^2}\right) \qquad (3)$$

and

$$\Gamma_e = \frac{\xi_e}{v_s}\left(1 - \frac{\xi_e}{R_e} + \frac{\xi_e^2}{3R_e^2}\right) \qquad (4)$$

Moreover, we note that by making use of the equilibrium conditions (*cf.* eq 40 below) we can eventually obtain $R_i$ and $R_e$ for each $R$-value or, in other words, $\xi_i$, $\xi_e$, $a_i$, and $a_e$ become fully determined for every vesicle radius $R$. In addition, for the mixed case, we can compute the monolayer mole fractions $x_{OH}^i(R_i)$ and $x_{OH}^e(R_e)$, respectively.

## Thermodynamics of a Bilayer Vesicle

We have previously evaluated size distributions of equilibrated vesicles by means of a multiple equilibrium approach yielding the total volume fraction of vesicles determined by the expression

$$\phi_{tot} = \int_0^\infty S(R)e^{-\epsilon(R)/kT}\,dR \qquad (5)$$

where the preexponential factor $S(R)$ is related to fluctuations in composition, chain packing density, and shape, and $\epsilon(R)$ is the excess free energy for the full equilibrium case, *i.e.*, when the vesicle is perfectly spherical and the compositional states in the outer and inner monolayers

are optimal. Note that the free energy function $\epsilon(R)$ must be positive for all $R$-values to compensate the negative fluctuation entropy. Below we discuss how to evaluate $\epsilon(R)$ for pure and mixed vesicles.

**Pure Vesicle Case.** The *excess* free energy relative to the external surface at $R + \tau$ of a vesicle composed of both an ionic surfactant and a long-chain alcohol can be expressed in the following way

$$G = F + p_e V - \mu_w n_w - \mu_s^+ n_s^+ - \mu_s^- n_s^- - \mu_{OH} n_{OH} \qquad (6)$$

$F$ is the Helmholtz free energy of the whole aggregate on which a bulk solution pressure $p_e$ acts and its volume $V = 4\pi(R + \tau)^3/3$ is determined as the sum of the volumes of the molecules encompassed (*i.e.*, incompressibility is assumed). $\mu_i$ denotes the bulk chemical potential and $n_i$ the (rather minute) *excess* number of molecules or ions *outside* the volume $V$ of the vesicle. For a pure SDS vesicle, the last term in eq 6 vanishes, of course.

We can also write the corresponding overall $\Omega$-potential, here denoted by $\epsilon(R)$, for the formation of a (pure) spherical vesicle out of surfactant molecules in solution

$$\epsilon(R) = N_s(\hat{\mu}_v - \mu_s^-) - N_w\mu_w \qquad (7)$$

where, adhering to the notation employed by Hill,[13] $\hat{\mu}_v$ stands for the (Gibbs) free energy per aggregated surfactant molecule, $G/N_s$ and $N_w = N_w^e + N_w^i$. The radius $R$ at the bilayer midplane of the spherical vesicle is given by $N_s^i v_s + N_w^i v_w = 4\pi R^3/3$, where $v_w = 30$ Å$^2$ is the volume occupied by a water molecule.

Equation 7 may be rewritten as follows

$$\epsilon(R) = N_s(\hat{\mu}_v - \hat{\mu}_p) - (N_w - N_w^p)\mu_w + N_s(\hat{\mu}_p - \mu_s^-) - N_w^p\mu_w \qquad (8)$$

where $\hat{\mu}_p$ is the monomer free energy in a large planar bilayer kept at the same solution chemical potential, $\mu_s^-$, and $N_w^p$ is the number of water molecules which together with $N_s$ hydrocarbon chains form a full bilayer (*i.e.*, a bilayer plus adjacent water molecules) of half thickness $\tau$. This enables us to define three dimensionless parameters $\alpha$, $\beta$, and $\delta$, similarly as is usually done for rod-shaped micelles,[14] in accordance with the following relations

$$\alpha + \delta(R) = [N_s(\hat{\mu}_v - \hat{\mu}_p) - (N_w - N_w^p)\mu_w]/kT \qquad (9)$$

$$\alpha = \lim_{N_s \to \infty} [N_s(\hat{\mu}_v - \hat{\mu}_p) - (N_w - N_w^p)\mu_w]/kT \qquad (10)$$

$$\beta = (\hat{\mu}_p - \mu_s^- - x_w^p\mu_w)/kT \qquad (11)$$

where $x_w^p = N_w^p/N_s$.

$\alpha + \delta(R)$ can be interpreted as the work of bending a portion of an infinite planar bilayer into a geometrically closed vesicle and $\beta$ as the work per aggregated surfactant monomer of forming an *infinitely large planar* bilayer, in $kT$ units. Thus, for the full equilibrium case, when the vesicle is perfectly spherical and the packing densities in the outer and inner monolayer are optimal, we can write

$$\epsilon(R)/kT = \alpha + N_s\beta + \delta(R) \qquad (12)$$

The driving force for closing a bilayer disk and generating a vesicle is, of course, the annihilation of the unfavorable rim part. The minimum free energy required for the

(11) Eriksson, J. C.; Ljunggren, S.; Henriksson, U. *J. Chem. Soc., Faraday Trans. 2* **1985**, *81*, 833.
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(13) Hill, T. L. *Thermodynamics of Small Systems*; Benjamin: New York, 1963−64; Vols. I and II.
(14) Eriksson, J. C.; Ljunggren, S. *J. Chem. Soc., Faraday Trans. 2* **1985**, *81*, 1209.

overall formation process bilayer $\rightarrow$ disk $\rightarrow$ vesicle (whereby the rim energy is replaced by bending energy) can, according to our scheme, be divided into two terms: $\alpha kT$, which turns out to be independent of the vesicle size, and the repulsive branch part, $\delta(R)/kT$, which is important for very small aggregation numbers only, outside the range where vesicle formation tends to occur to some appreciable extent.

**Mixed Vesicle Case.** When a long-chain alcohol is mixed into the bilayer, the excess free energy becomes a function of the compositions in each of the two monolayers and eq 7 has to be replaced by the expression

$$\epsilon(R) = N[\hat{u}_v - (1 - x_{OH})\mu_s - x_{OH}\mu_{OH} - x_w\mu_w] \quad (13)$$

where the mole fraction of aggregated dodecanol is given by $x_{OH} = N_{OH}/N$ and the total number of monomers, $N$, is the sum of the number of surfactant (SDS) and alcohol monomers (DOH): $N = N^i + N^e$ with $N^i = N_s^i + N_{OH}^i$ and $N^e = N_s^e + N_{OH}^e$. Rewriting eq 13 gives

$$\epsilon(R) = N[\hat{u}_v - \hat{\mu}_p] + N^i[\hat{\mu}_p - (1 - x_{OH}^i)\mu_s^- - x_{OH}^i\mu_{OH} -$$
$$x_w^i\mu_w] + N^e[\hat{\mu}_p - (1 - x_{OH}^e)\mu_s^- - x_{OH}^e\mu_{OH} - x_w^e\mu_w]$$

$$= N[\hat{u}_v - \hat{\mu}_p] + [N^i(x_{OH}^i - x_{OH}^p) +$$
$$N^e(x_{OH}^e - x_{OH}^p)](\mu_s^- - \mu_{OH}) -$$
$$[N^i(x_w^i - x_w^p) + N^e(x_w^e - x_w^p)]\mu_w +$$
$$N[\hat{\mu}_p - (1 - x_{OH}^p)\mu_s^- - x_{OH}^p\mu_{OH} - x_w^p\mu_w] \quad (14)$$

and, hence, essentially as before,

$$\alpha = \lim_{N\to\infty} N[\hat{u}_v - \hat{\mu}_p] + [N^i(x_{OH}^i - x_{OH}^p) + N^e(x_{OH}^e -$$
$$x_{OH}^p)](\mu_s^- - \mu_{OH}) - [N^i(x_w^i - x_w^p) + N^e(x_w^e - x_w^p)]\mu_w \quad (15)$$

and

$$\beta = \hat{\mu}_p - (1 - x_{OH}^p)\mu_s^- - x_{OH}^p\mu_{OH} - x_w^p\mu_w \quad (16)$$

The additional terms in eq 15 as compared with eq 10, valid for the pure case, are due to the difference in composition in the inner and outer layer and are found to be virtually independent of $N$.

## The Curvature Dependence of the Bilayer Tension

For a certain solution state, *i.e.*, for some fixed chemical potentials, it is reasonable to assume a quadratic curvature dependence of the (monolayer) interfacial tension referred to the water/hydrocarbon dividing surface (located somewhat underneath the polar head-groups). Hence, in line with the conventional Helfrich expression for the bending free energy, we write

$$\gamma(H,K) = \gamma_0 + 2k_c(H - H_0)^2 + \bar{k}_c K \quad (17)$$

where $H \equiv \frac{1}{2}(c_1 + c_2)$ is the mean curvature, $K \equiv c_1 c_2$ is the Gaussian curvature, and $c_1$ and $c_2$ denote the principal curvatures which we take to be positive for a convex and negative for a concave surface. $k_c$ and $\bar{k}_c$ stand for two (state-dependent) bending constants (to use the conventional terminology "curvature elasticity constants" would be improper since we are dealing with an interface that is *open* in the thermodynamic sense).

Note that the spontaneous curvature $H_0$ for the monolayer is generally different from zero whereas the spontaneous curvature for the fully equilibrated bilayer is zero due to symmetry reasons. For a spherically shaped surface
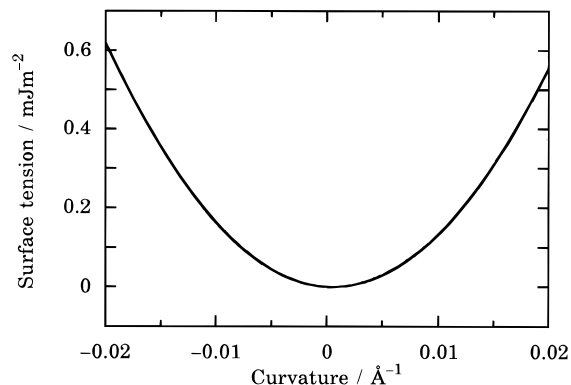


**Figure 2.** The surface tension of a pure SDS monolayer as a function of curvature at a monomer concentration in the bulk solution of $c_{surf} = 15.59$ mM and no added salt. The curvature-related parameters are $k_1 = -3.78 \times 10^{-3} kT\text{Å}^{-1}$ and $k_2 = 3.57 kT$.
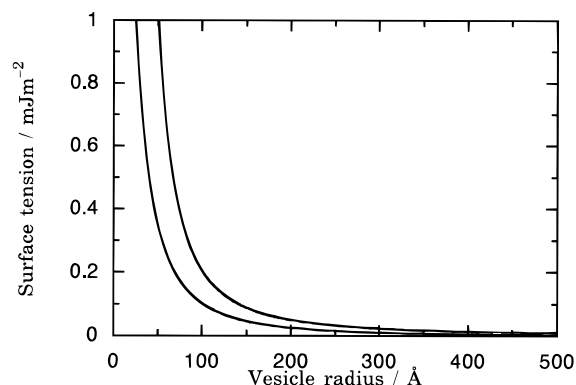


**Figure 3.** The surface tensions of the inner $\gamma_i$ (upper curve) and the outer monolayer $\gamma_e$ (lower curve), respectively, for a pure SDS vesicle plotted against the vesicle radius $R$. The solution state is identical to that in Figure 2.

of radius $r$ we have $H = 1/r$ and $K = 1/r^2$, and eq 17 can be written as

$$\gamma(r) = \gamma_\infty + \frac{k_1}{r} + \frac{k_2}{r^2} \quad (18)$$

with the identification relations $\gamma_\infty = \gamma_0 + 2k_c H_0^2$, $k_1 = -4k_c H_0$, and $k_2 = 2k_c + \bar{k}_c$. However, for our present purpose we only need to consider eq 18 as a Taylor expansion of the monolayer tension in curvature $H \equiv r^{-1}$.

For a spherical vesicle, setting $r = -R_i$ and $r = R_e$ for the inner and outer monolayer tensions, respectively, we hence get

$$\gamma_i = \gamma_\infty - \frac{k_1}{R_i} + \frac{k_2}{R_i^2} \quad (19)$$

$$\gamma_e = \gamma_\infty + \frac{k_1}{R_e} + \frac{k_2}{R_e^2} \quad (20)$$

where $\gamma_\infty$ is the equilibrium interfacial tension of the planar monolayer. Typical examples of the functions $\gamma_i(R_i)$, $\gamma_e(R_e)$, and $\gamma(H)$ are displayed in Figures 2–5. They were obtained by combining eqs 1 and 2 and using eq 40 below. We note that the magnitude of the monolayer tension can greatly exceed $\gamma_\infty$ and, furthermore, for the mixed case, it becomes negative for negative curvatures, *i.e.*, $\gamma_i < 0$, corresponding to a *surface pressure* rather than a tension. Moreover, for this case no minimum is observed upon varying the curvature. However, it is worth stressing that $\gamma$ is the *thermodynamic* surface tension associated
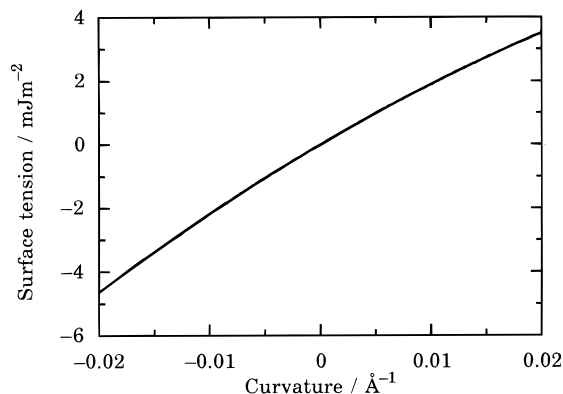
**Figure 4.** The surface tension of a mixed SDS/DOH monolayer as a function of curvature. SDS monomer concentration $c_{surf}$ = 8.78 mM, mole fraction of alcohol $x_{alc} = 5 \times 10^{-8}$, which gives a mole fraction of dodecanol $x_{OH}^p = 0.52$, aggregated in the large vesicles. The curvature-related parameters are $k_1 = 0.495 kT$ Å$^{-1}$ and $k_2 = -3.43 kT$.

with the hydrocarbon/water dividing surface implying that $\gamma$ has no immediate mechanical significance [cf. ref 11].

When combined, eqs 19 and 20 yield the excess free energy of a spherically shaped bilayer or, more precisely, of a spherical vesicle which is fully equilibrated with the surrounding solution:

$$\epsilon(R) = 4\pi(R_i^2\gamma_i + R_e^2\gamma_e)$$

$$= 4\pi[\gamma_\infty(\xi_i^2 + \xi_e^2) - 2\gamma_\infty R(\xi_i - \xi_e) + k_1(\xi_i + \xi_e) + 2k_2] + 8\pi R^2 \gamma_\infty \quad (21)$$

where, as before, $\xi_i = R - R_i$ and $\xi_e = R_e - R$ denote the thicknesses of the hydrocarbon parts of the monolayers. Now, it turns out that the first two terms within the square brackets are, as a rule (using SI units), $10^{-18} - 10^{-19}$ times $\gamma_\infty$, which amounts to $10^{-6} - 10^{-10}$ in regimes where vesicles tend to form, whereas the last two terms are of the order of $10^{-19} - 10^{-20}$. Hence, for all practical purposes we can replace eq 21 by the simpler expression

$$\epsilon(R) = 8\pi(\xi_p k_1 + k_2 + \gamma_\infty R^2) = 4\pi R^2 \times 2[(\xi_p k_1 + k_2)/R^2 + \gamma_\infty] \quad (22)$$

where, in addition, we have introduced the approximate, but yet rather precise, relation for comparatively large $R$, $\xi_p \approx (\xi_i + \xi_e)/2$, $\xi_p$ denoting the thickness of the planar (hydrocarbon) monolayer. Hence, we obtain the somewhat surprising result that $d\epsilon/dA = \gamma_\infty$, where $A = A_i + A_e \approx 8\pi R^2$, i.e., the work of increasing the size of a spherical vesicle equals just $\gamma_\infty \Delta A$.

According to the above eq 22, the *bilayer tension*, $\gamma_{bi}$, is given by

$$\gamma_{bi} = 2\left(\gamma_\infty + \frac{\xi_p k_1 + k_2}{R^2}\right) = 2\gamma_\infty + \frac{k_{bi}}{R^2} \quad (23)$$

implying that the spontaneous curvature of the bilayer is zero, as it must be because of symmetry reasons. Referring back to the previous eq 12 it also appears that we can make the identifications $\alpha = 4\pi k_{bi}/kT = 8\pi(\xi_p k_1 + k_2)/kT$ and $\beta = a_p\gamma_\infty/kT$. In other words, $\alpha$ is related to the *bending* of the vesicle midplane whereas $\beta$ accounts for the *stretching* of the same plane (*note bene* at constant chemical potentials). Moreover, a significant part of $k_{bi}$ is due to the $\xi_p k_1$ contribution and, in principle, we can have $k_{bi} > 0$ even when the bending constant $k_2$ for the monolayer
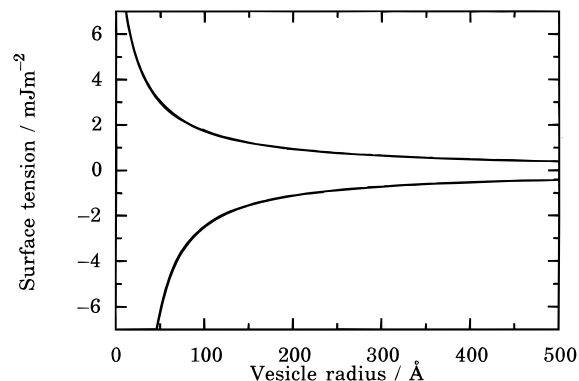
**Figure 5.** The surface tensions of the inner $\gamma_i$ (lower curve) and the outer monolayer $\gamma_e$ (upper curve), respectively, for a mixed SDS/DOH vesicle plotted against the vesicle radius $R$. The solution state is identical to that in Figure 4.

is less than zero. A positive $k_{bi}$ is necessary for obtaining entropically stabilized vesicles (*cf.* eq 5).

By extending the curvature expansion of eq 18 to higher orders it can be shown that terms proportional to $R^{-2}$, $R^{-4}$, and so on arise in the resulting expression for $\epsilon(R)$, usually yielding a minimum for some very small value of $R$ ($\sim 20$ Å). However, vesicle formation generally occurs for relatively large $R$-values ($\geq 100$ Å), on the slowly rising branch of the $\epsilon(R)$-function, determined mainly by the value of $\gamma_\infty$, and far from the ascending branch at very small radii. Thus, to account for ordinary vesicles the simple free energy function given by eq 22 is sufficiently exact even if it does not reproduce the $\epsilon(R)$-function very precisely for small values of $R$.

It may be illuminating to dwell somewhat longer on the combination of the monolayer tension expressions eqs 19 and 20 to yield eq 21. The contributions to $\epsilon(R)/4\pi$ from the monolayers are

$$R_i^2\gamma_i = \gamma_\infty R_i^2 - k_1 R_i + k_2 \approx \gamma_\infty R^2 - k_1 R + k_1 \xi_i + k_2 \quad (24)$$

and

$$R_e^2\gamma_e = \gamma_\infty R_e^2 + k_1 R_e + k_2 \approx \gamma_\infty R^2 + k_1 R + k_1 \xi_e + k_2 \quad (25)$$

respectively. Since $\gamma_\infty$ is a rather small quantity, when plotted against $R$ the two functions will be represented by straight lines with approximately the same intercept $k_{bi}/2 = k_1\xi_p + k_2$ and with slopes of same magnitude, $k_1$, but of opposite sign. However, upon summing the two contributions, the $k_1 R$ terms cancel exactly and the only $R$-dependent term left is $2\gamma_\infty R^2$.

By combining eqs 19 and 20, we can also write the bilayer bending parameter $k_{bi}$ as follows,

$$k_{bi} \equiv 2(\xi_p k_1 + k_2) \approx 2R(\xi_e\gamma_e - \xi_i\gamma_i) + R^2(\gamma_e + \gamma_i - 2\gamma_\infty) \quad (26)$$

Hence, the total bending work divided by $4\pi$ may be considered as a sum of two contributions: the work of changing the bilayer tension from $2\gamma_\infty$ to $(\gamma_e + \gamma_i)$ at a constant surface area, $R^2(\gamma_e + \gamma_i - 2\gamma_\infty)$, and the work of changing the hydrocarbon/water contact area from that corresponding to the midplane of the bilayer into what we
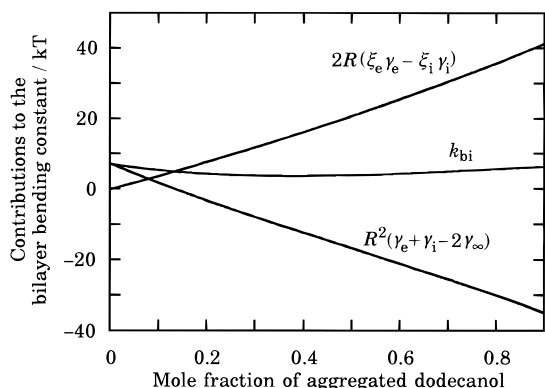
**Figure 6.** $2R(\xi_e\gamma_e - \xi_i\gamma_i)$ and $R^2(\gamma_e + \gamma_i - 2\gamma_\infty)$ plotted against the mole fraction of dodecanol in a planar mixed SDS/DOH bilayer, $x_{OH}^p$. The bilayer bending constant $k_{bi}$ equals the sum of the two contributions.
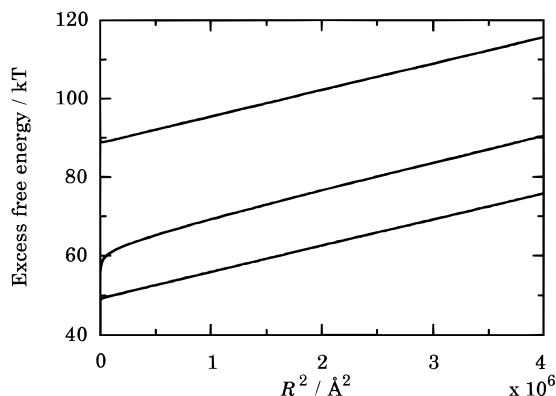


**Figure 7.** The excess free energy of pure SDS (upper curve) and a mixed SDS/DOH vesicle (middle curve), $\epsilon$, plotted against the square of the radius $R^2$. The bending constant for the pure SDS case, with a solution state identical to that in Figure 2, is $k_{bi} = 7.06kT$ and for the mixed case, with a solution state identical to that in Figure 4, is $k_{bi} = 3.92kT$. For the mixed case the free energy function $\epsilon^*$ (eq 42) is plotted as well (lower curve).

have for outer and inner monolayers together, *i.e.*

$$(R_e^2 - R^2)\gamma_e + (R_i^2 - R^2)\gamma_i = 2R(\xi_e\gamma_e - \xi_i\gamma_i) + (\xi_e^2\gamma_e + \xi_i^2\gamma_i) \quad (27)$$

where the last term is negligible. In Figure 6 $k_{bi}$ is plotted jointly with its two contributions according to eq 26, against the alcohol mole fraction in the planar bilayer. Below we will further discuss the trends of these two contributions and their roles as to favoring the formation of mixed vesicles.

In Figure 7 we have plotted the excess free energy $\epsilon$ against $R^2$ for a pure SDS and a mixed SDS/DOH case, respectively. These functions were generated on the basis of the calculation scheme which we will present in detail below, and they verify that eq 22 constitutes quite a satisfactory approximation for $R \geq 10$ nm.

### The Various Contributions to the Free Energy of a SDS/DOH Vesicle

The free energy change per aggregated monomer associated with bringing a $C_{12}$ chain from a water solution to a pure hydrocarbon bulk phase was estimated by Tanford[15] on the basis of solubility data. Accordingly, in the present case we have

(15) Tanford, C. *The Hydrophobic Effect, Chapter 7*; Wiley: New York, 1980.

$$\frac{\epsilon_{tan}}{kT} = -[19.960 + (1 - x_{OH})\ln x_{surf} + x_{OH}\ln x_{alc}] \quad (28)$$

per chain where $x_{surf}$ and $x_{alc}$ are the monomer mole fractions of SDS and DOH in the surrounding solution. This free energy contribution, which to a large extent is of entropic origin, is the well-known driving force of amphiphile aggregation and is entirely independent of the detailed vesicle characteristics.

A counteracting effect arises due to the accumulation of charged head-groups and counterions in geometrically restricted volumes at the hydrocarbon/water interfaces of the vesicle. The corresponding electrostatic free energy to second order in curvature for a spherical surface has been calculated, independently, by Mitchell and Ninham[16] and Lekkerkerker[17] as a function of surface charge density, salt concentration, and curvature, using the Poisson–Boltzmann approximation. Using notations of the former authors it is given by the expression (per each charged head-group)

$$\frac{\epsilon_{el}}{kT} = 2\left[\ln(S + \sqrt{S^2 + 1}) - \frac{\sqrt{S^2 + 1} - 1}{S}\right] - \frac{4}{\kappa SR_{el}} \times$$

$$\ln\left(\frac{1 + \sqrt{S^2 + 1}}{2}\right) + \frac{2}{S\kappa^2 R_{el}^2}\left[1 - \frac{2}{S^2} + \frac{2}{S^2\sqrt{S^2 + 1}} - D_1\left(\ln\left[\frac{1 + \sqrt{S^2 + 1}}{2}\right]\right)\right] \quad (29)$$

where in the last (second order in curvature) term, $D_1$ stands for the Debye function

$$D_1(x) = \int_0^x \frac{t\,dt}{e^t - 1} \quad (30)$$

The radial distance to the smeared-out surface charges of the sulfate head-groups was assumed to be

$$R_{el} = R_e + 3.0 \times 10^{-10} \text{ m} \quad (31)$$

for the outer convex surface and

$$R_{el} = -(R_i - 3.0 \times 10^{-10}) \text{ m} \quad (32)$$

for the inner concave surface. In other words, we have assumed that the sulfate groups protrude a small distance (3 Å) from the hydrocarbon/water dividing surface.

The dimensionless reduced charge parameter $S$ employed above is defined by the expression

$$S = \frac{\sigma}{\sqrt{8(c_{surf} + c_{salt})\epsilon_0\epsilon_r RT}} \quad (33)$$

whereas $\kappa$, the inverse of the Debye length, is given by

$$\kappa = \sqrt{\frac{2(c_{surf} + c_{salt})N_A^2 e^2}{\epsilon_0\epsilon_r RT}} \quad (34)$$

$N_A$ denoting the Avogadro number and $e$ the elementary charge. $c_{surf}$ is the SDS monomer concentration in solution and $c_{salt}$ the concentration of added monovalent salt. The

(16) Mitchell, D. J.; Ninham, B. W. *Langmuir* **1989**, *5*, 1121.
(17) Lekkerkerker, H. N. W. *Physica A* **1989**, *159*, 319.

surface charge density is $\sigma = e/a_{el}$, where $a_{el} = 4\pi R_{el}^2/N_s$ denotes the surface area per charge.

The hydrocarbon/water contact is, of course, unfavorable from a thermodynamic point of view. Our previous calculations indicate a curvature dependence of the corresponding interfacial tension $\gamma_{hc/w}$ that is roughly reproduced by the relation

$$\gamma_{hc/w} = \gamma_{hc/w}^p\left(1 - \frac{2}{r}\right) \tag{35}$$

where $\gamma_{hc/w}^p = 50.7$ mJ/m$^2$ denotes the macroscopic (planar) hydrocarbon/water interfacial tension, at 25 °C, and $r$ is given in angstrom units.

Inside the hydrocarbon core, the aggregated chains are conformationally restricted compared to a hydrocarbon bulk phase. This tends to raise their free energy. A quantitative mean field theory of this contribution was elaborated by Gruen and de Lacey,[18] and in the present calculations, we have used the following expression valid for a planar bilayer

$$\frac{\epsilon_{conf}}{kT} = 3.12630 - 1.15534\xi + 0.18273\xi^2 - 1.40763 \times$$
$$10^{-2}\xi^3 + 4.77423 \times 10^{-4}\xi^4 \tag{36}$$

where $\xi$ is the monolayer thickness, and $\epsilon_{conf}$ is the conformational free energy per chain. Note that the conformational free energies will vary with curvature since $\xi = \xi(H)$.

When the contributions associated more specifically with the sulfate head-groups of the DS$^-$ monomers are considered, several less known effects have to be accounted for: (i) shielding of the hydrocarbon/water contact by the comparatively large sulfate head-group, (ii) asymmetric hydration of the ionic species in close proximity to the hydrocarbon core, and (iii) repulsive local interactions between the hydrated head-groups which have not been included in the electrostatic free energy as the PB approximation assumes a smeared-out surface charge. Unfortunately, there are no reliable quantitative estimates of these effects available at the present time. We have therefore in our calculations introduced a constant (planar) head-group parameter, $\epsilon_{pg}^p = 0.5962kT$. This is supported by similar calculations on the surface tension of dodecylammonium chloride solutions and comparison with experimental data.[8,19]

The shielding due to the head-group of the hydrocarbon/water contact alters the curvature-dependent part of $\gamma_{hc/w}$ and adds a second term to the head-group parameter

$$\epsilon_{pg} = \epsilon_{pg}^p + \frac{2a_{pg}\gamma_{hc/w}^p}{r} \tag{37}$$

The corresponding parameter associated with the alcohol head-group becomes

$$\epsilon_{OH} = \epsilon_{OH}^p + \frac{2a_{OH}\gamma_{hc/w}^p}{r} \tag{38}$$

where we have inserted $\epsilon_{OH}^p \approx 0$ in view of the small size of the head-group and its similarity with a water molecule and the fact that it spends an appreciable fraction of time somewhat inside the hydrocarbon core.[20]

(18) Gruen, D. W. R.; Lacey, E. H. B. In *Surfactants in Solution*; Mittal, K., Lindman, B., Eds.; Plenum: New York, 1984; Vol. I; p 279.
(19) Eriksson, J. C..; Ljunggren, S. *Colloids Surf.* **1989**, *38*, 179.
(20) Gruen, D. W. R. *J. Phys. Chem.* **1985**, *89*, 153.

The free energy of mixing the different monomers in a vesicle monolayer was obtained by means of the standard combinatorial expression

$$\frac{\epsilon_{mix}}{kT} = -\ln\left(\frac{N}{N_s}\right) = N[(1 - x_{OH})\ln(1 - x_{OH}) +$$
$$x_{OH}\ln x_{OH}] + \ln\sqrt{2\pi N(1 - x_{OH})x_{OH}} \tag{39}$$

where $N = N_s + N_{OH}$, and the last equality is obtained upon inserting Stirling's approximation in the form $N! \approx (2\pi N)^{1/2}(N/e)^N$. The factor $(2\pi N)^{1/2}$ has to be included because after all we are dealing with aggregates of limited size.

Adding all these various contributions yields the following final overall free energy expression for each monolayer of a spherical vesicle

$$\epsilon(r,a,x_{OH},\text{solution state}) = N[\epsilon_{tan} + \epsilon_{conf} + a\gamma_{hc/w} +$$
$$(1 - x_{OH})[\epsilon_{el} + \epsilon_{pg} + kT\ln(1 - x_{OH})] + x_{OH}(\epsilon_{OH} +$$
$$kT\ln x_{OH})] + kT\ln\sqrt{2\pi N(1 - x_{OH})x_{OH}} \tag{40}$$

The excess free energy functions for the inner, $\epsilon_i \equiv \epsilon(R_i,a_i,x_{OH}^i)$, and the outer, $\epsilon_e \equiv \epsilon(R_e,a_e,x_{OH}^e)$, monolayers were minimized at constant chemical potentials in the surrounding bulk solution and under the geometrical constraints of eqs 1 and 2, for various radii $R$ of the midplane surface in between the two monolayers. The sum of the two (minimized) monolayer free energies gives the free energy function $\epsilon(R)$ for the bilayer [*cf.* Figure 7].

The last logarithmic term of eq 40, originating from the combinatorial expression of eq 39, is entirely due to the finite size of a vesicle and does not depend on the curvature or bending *per se*. It turns out to be convenient to omit this term when evaluating the bending parameters of the monolayer. Hence, we introduce a modified free energy function, $\epsilon^*(r)$, for a mixed vesicle monolayer (in principle, infinite extension)

$$\epsilon^*(r) = \epsilon(r) - kT\ln\sqrt{N(1 - x_{OH})x_{OH}} \tag{41}$$

with this size dependence removed. Comparing with Figure 7 we conclude that the corresponding $\epsilon^*(r)$-function for the whole vesicle can be represented by an expression which is linear in $R^2$ of the standard form

$$\epsilon^*(R)/4\pi = k_{bi}^* + 2\gamma_\infty R^2 \tag{42}$$

For $\epsilon(R)$, this is not the case to an equally good approximation. When treating the size distribution of mixed vesicles, the above $\epsilon^*(R)$ function will serve as the starting point (*cf.* ref 10). For simplicity, in the following we will omit the asterisk for the bilayer bending constant of mixed vesicles.

By minimizing the free energy expression given by eq 40, *i.e.*, by setting the chemical potentials equal in the two monolayers and in the solution, we can calculate equilibrium values of the monolayer surface tensions (Figures 2−5), monolayer thicknesses, surface areas per hydrocarbon chain, and alcohol mole fractions for the inner and outer monolayers, respectively, for given values of the vesicle radius $R$ and at some chosen solution state. It turns out that for small and moderate curvatures all these properties can be rather accurately represented by quadratic functions of curvature.
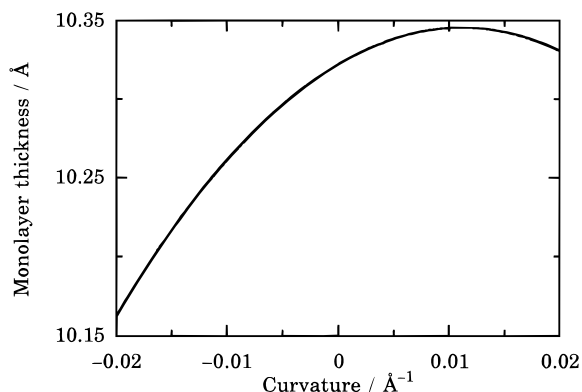
## Contributions to the Bending Constants for Equilibrated Spherical Vesicles

In Figures 2 and 4 the monolayer tensions $\gamma_i = \epsilon_i/4\pi R_i^2$ and $\gamma_e = \epsilon_e/4\pi R_e^2$ have been plotted against curvature for
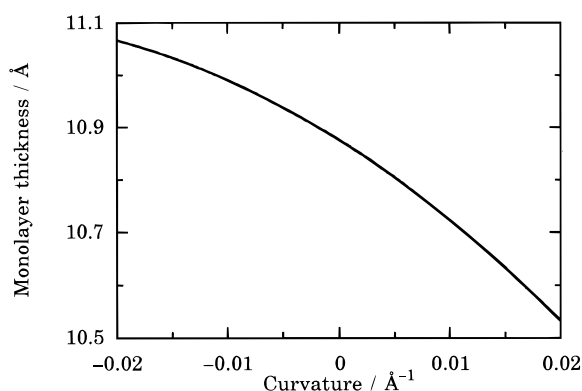
**Figure 8.** The thickness of a pure SDS monolayer, $\xi$, as a function of curvature at the same solution state as in Figure 2. The function can, to a very good approximation, be written as $\xi = \xi_p (1 + 0.4079/r - 18.25/r^2)$ in the curvature interval of interest, where $\xi_p = 10.322$ Å. A maximum of $\xi$ is reached at $r = 89$ Å.



**Figure 9.** The thickness of a mixed SDS/dodecanol monolayer, $\xi$, as a function of curvature at the same solution state as in Figure 4. The function can, to a very good approximation, be written as $\xi = \xi_p(1 - 1.228/r - 17.58/r^2)$ in the curvature interval of interest, where $\xi_p = 10.876$ Å.

a pure SDS bilayer and a mixed SDS/DOH bilayer containing 52 mol % alcohol. For the pure SDS bilayer a minimum is reached very close to $H = 0$ as $k_1 \approx 0$. However, the monolayer bending constants $k_1$ and $k_2$ change considerably upon adding alcohol and at higher dodecanol mole fractions no minimum occurs. This comes about because the reduction of the electrostatic free energy due to adding alcohol lowers $\gamma_i$ substantially for the inner monolayer where the head-groups are comparatively closely packed.

The thicknesses of the monolayers are plotted against curvature in Figures 8 and 9 for the same solution states. As a rule, $\xi$ decreases for large positive values of the curvature. The process of inserting an extra monomer in the outer positively curved monolayer at a fixed value of $R$, and thereby increasing the thickness of the hydrocarbon layer, is accompanied by an unfavorable *increase* of the overall hydrocarbon/water contact area. This yields a positive contribution to the chemical potential that in turn gives rise to a trend toward smaller $\xi$ as the monolayer becomes more curved. On the other hand, inserting an additional monomer in the inner monolayer will *decrease* the overall inner hydrocarbon/water surface, resulting *per se* in a larger monolayer thickness. However, for the highly charged inner monolayer of a pure SDS vesicle the electrostatic repulsion takes over, resulting in the end in a positive monolayer tension and a decreasing monolayer thickness as the hydrocarbon/water interface gets more strongly curved on the negative side. Eventually, a maximum is reached as shown in Figure 8.
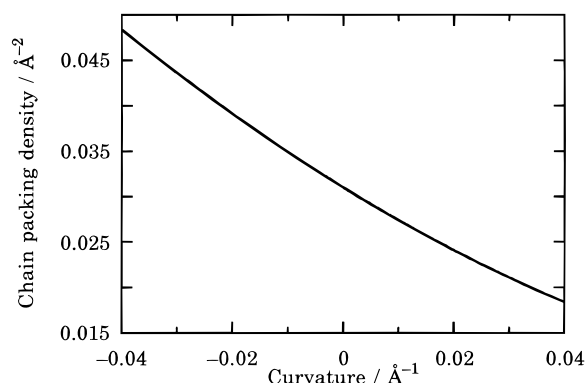


**Figure 10.** The chain packing density, $\Gamma = 1/a$, in the hydrocarbon/water contact interface of a mixed SDS/dodecanol monolayer plotted against the curvature at a solution state identical to that in Figure 4. The function can be written in the form $\Gamma = \Gamma_p(1 - 12.10/r + 48.57/r^2)$, where the area per aggregated monomer for a planar layer at same solution state is $a_p = 1/\Gamma_p = 32.27$ Å².

For comparatively large vesicles we can expand the monolayer thickness with respect to curvature and neglect terms of third order and higher

$$\xi = \xi_p\left(1 + \frac{k_\xi'}{r} + \frac{k_\xi''}{r^2}\right) \qquad (43)$$

Here $k_\xi'$ and $k_\xi''$ are constants accounting for the effect of bending a monolayer at constant chemical potentials. Inserting eq 43 in eqs 3 and 4 and omitting terms of higher order we can write down the curvature dependence of the chain packing density as

$$\Gamma = \Gamma_p\left(1 + \frac{k_a'}{r} + \frac{k_a''}{r^2}\right) \qquad (44)$$

where $\Gamma_p = 1/a_p$ is the chain packing density of a planar layer and the curvature related constants become $k_a' = k_\xi' - \xi_p$ and $k_a'' = \xi_p^2/3 - 2k_\xi'\xi_p + k_\xi''$. Since $\xi_p > k_\xi'$ and $\xi_p^2/3 > 2k_\xi'\xi_p - k_\xi''$ for all solution states we generally have $k_a' < 0$ and $k_a'' > 0$ and since the first order term in eq 44 always predominates over the second order term, $a$ increases montonously with curvature and, hence, $a_e > a_i$ generally holds true. The function given by eq 44 is plotted in Figure 10.

To realize the equilibrium condition of constant chemical potentials a larger fraction of alcohol will aggregate as the monolayer becomes more negatively curved and the charged head-groups tend to become more densely packed. In Figure 11 the monotonously decreasing alcohol mole fraction, $x_{OH}$, is plotted *vs* curvature. For small deviations from a planar geometry we can represent $x_{OH}$ as well be a quadratic function of curvature,

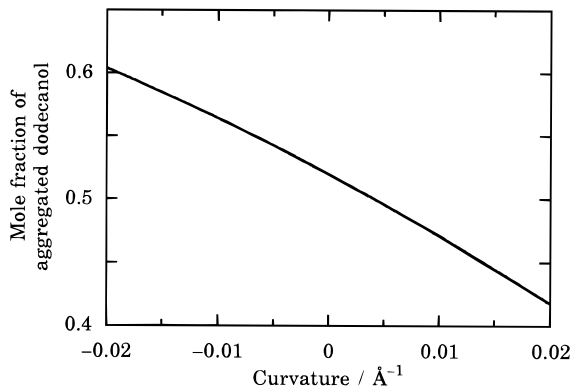$$x_{OH} = x_{OH}^p\left(1 + \frac{k_x'}{r} + \frac{k_x''}{r^2}\right) \qquad (45)$$

where $k_x'$ is negative for all $x_{OH}^p$.

Combining eq 44 with the geometrical constraints given by eqs 1 and 2 yields the relations
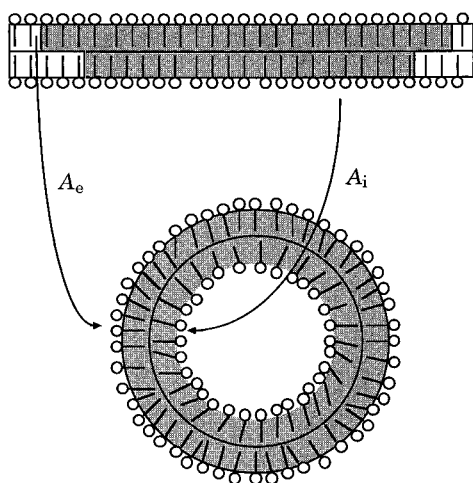
$$\frac{R_e^3 - R^3}{3v_sR_e^2} = \frac{1}{a_p}\left(1 + \frac{k_a'}{R_e} + \frac{k_a''}{R_e^2}\right) \qquad (46)$$

and

**Figure 11.** The dodecanol mole fraction, $x_{OH}$, in a mixed SDS/dodecanol monolayer as a function of the curvature at an identical solution state as in Figure 4. The function can to a very good approximation be written in the form $x_{OH} = x_{OH}^p (1 - 8.964/r - 42.74/r^2)$, where the planar alcohol mole fraction $x_{OH}^p = 0.5196$.



**Figure 12.** Schematic sketch illustrating the formation of a bilayer vesicle out of monomers from a planar bilayer at a fixed hydrocarbon/water contact area $A = A_i + A_e$.

$$\frac{R^3 - R_i^3}{3 v_s R_i^2} = \frac{1}{a_p}\left(1 - \frac{k_a'}{R_i} + \frac{k_a''}{R_i^2}\right) \qquad (47)$$

When the product of eq 46 and $R_e^2$ is subtracted from the product of eq 47 and $R_i^2$ we eventually obtain

$$k_a' = \xi_i + \xi_e - \frac{\xi_i^2 + \xi_e^2}{2\xi_p} - \frac{R}{2\xi_p}(\xi_e - \xi_i) \approx \xi_p -$$
$$\frac{R}{2\xi_p}(\xi_e - \xi_i), \quad (48)$$

where we have set $\xi_p \approx (\xi_i \xi_e)^{1/2}$ which, according to eq 43, is a very good approximation in the interval of interest. Likewise, we get

$$k_a'' = \frac{\xi_e^3 + \xi_i^3}{6\xi_p} - \frac{R(\xi_e - \xi_i)}{2} + \frac{R^2}{2\xi_p}(\xi_e + \xi_i - 2\xi_p) \approx$$
$$\frac{\xi_p^2}{3} - \frac{R(\xi_e - \xi_i)}{2} + \frac{R^2}{2\xi_p}(\xi_e + \xi_i - 2\xi_p) \quad (49)$$

where we have, in addition to $\xi_p \approx (\xi_i \xi_e)^{1/2}$, assumed that $(\xi_e - \xi_i)^2 \approx 0$. Equations 48 and 49 will be of use below when considering the volume effect connected with forming a closed vesicle bilayer shell out of planar monolayers.
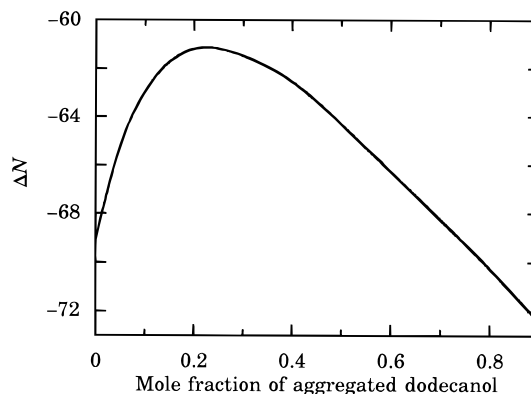


**Figure 13.** The change in the number of aggregated monomers upon forming a spherical vesicle at constant hydrocarbon/water contact surface area out of the corresponding monolayer parts, $\Delta N$, as a function of the dodecanol mole fraction, $x_{OH}^p$.

In a similar way, eq 45 gives

$$k_x' = 2\xi_p - [R(x_{OH}^e - x_{OH}^i) + 2(\xi_e x_{OH}^e + \xi_e x_{OH}^e + \xi_i x_{OH}^i)]/2x_{OH}^e \quad (50)$$

and

$$k_x'' = \left[R^2(x_{OH}^e + x_{OH}^i - 2x_{OH}^p) + \frac{R}{2}[(3\xi_e - \xi_i)x_{OH}^e - (3\xi_i - \xi_e)x_{OH}^i]\right]/2x_{OH}^p - R(\xi_e - \xi_i) \quad (51)$$

Now, turning back to eqs 40 and 41, the ("macroscopic") monolayer tension is given by

$$\gamma^*(r) \equiv \frac{\epsilon^*(r)}{A} = \frac{1}{a}[-19.960kT + \epsilon_{conf} + (1 - x_{OH})[\epsilon_{el} + \epsilon_{pg} - kT\ln x_{surf} + kT\ln(1 - x_{OH})] + x_{OH}[kT\ln x_{alc} + \epsilon_{OH} + kT\ln x_{OH}]] + \gamma_{hc/w}^p\left(1 - \frac{2}{r}\right) \quad (52)$$

where the appropriate (equilibrium) $a$ and $x_{OH}$ functions have to be inserted. The rather minor (bending-independent) finite-size correction contribution given by eq 41 will be taken care of separately later on when we derive the size distribution function. That is why $\epsilon^*(r)$ is really the free energy function we need to consider for the time being.

The electrostatic free energy expression of eq 29 has an explicit curvature dependence in the last two terms. However, since the surface charge density depends on curvature, the first term of eq 29 also has an implicit curvature dependence and, hence, we can write the electrostatic free energy contribution as an expansion in curvature

$$\epsilon_{el} = \epsilon_{el}^p\left(1 + \frac{k_{el}'}{r} + \frac{k_{el}''}{r^2}\right) \qquad (53)$$

Likewise, the conformational excess free energy contribution of eq 36 has an implicit curvature dependence because the hydrocarbon chain packing density varies with curvature, and it can be represented by the second order expression

$$\epsilon_{conf} = \epsilon_{conf}^p\left(1 + \frac{k_{conf}'}{r} + \frac{k_{conf}''}{r^2}\right) \qquad (54)$$

Inserting eqs 44, 45, 53, and 54 into eq 52 gives the monolayer tension in terms of a power series in curvature. Collecting all first and second order terms, we obtain explicit expressions for the two bending constants $k_1$ and $k_2$,

$$k_1 = [k_a{}'\epsilon' + k_{conf}{}'\epsilon_{conf}^p + x_{OH}^p k_x{}'\epsilon'' + 2\gamma_{hc/w}^p[(1 - x_{OH}^p)a_{pg} + x_{OH}^p a_{OH} - a_p] + (1 - x_{OH}^p)k_{el}{}'\epsilon_{el}^p]/a_p \quad (55)$$

and

$$k_2 = [k_a{}''\epsilon' + x_{OH}^p(k_a{}'k_x{}' + k_x{}'')\epsilon'' + (k_{conf}{}'' +$$
$$k_a{}'k_{conf}{}')\epsilon_{conf}^p]/a_p + \frac{2\gamma_{hc/w}^p}{a_p}[k_a{}'a_{pg} - x_{OH}^p(k_x{}' + k_a{}')(a_{pg} -$$
$$a_{OH})] + \frac{\epsilon_{el}^p}{a_p}[(1 - x_{OH}^p)(k_a{}'k_{el}{}' + k_{el}{}'') - x_{OH}^p k_x{}'k_{el}{}'] +$$
$$\frac{x_{OH}^p k_x{}'^2 kT}{2a_p(1 - x_{OH}^p)} \quad (56)$$

where the last term of eq 56 originates from the curvature dependence of the free energy of mixing as calculated in the Appendix. The first two terms in each of eqs 55 and 56 invoke the curvature-independent free energy contributions

$$\epsilon' = \epsilon_{tan} + \epsilon_{conf}^p + (1 - x_{OH}^p)[\epsilon_{pg}^p + \epsilon_{el}^p + kT \times \ln(1 - x_{OH}^p)] + x_{OH}^p[\epsilon_{OH}^p + kT\ln x_{OH}^p] \quad (57)$$

and

$$\epsilon'' = kT\ln\left(\frac{x_{surf}}{x_{alc}}\right) - \epsilon_{el}^p + \epsilon_{OH}^p - \epsilon_{pg}^p + kT\ln\left(\frac{x_{OH}^p}{1 - x_{OH}^p}\right) \quad (58)$$

Comparing with eq 40 we observe that eq 57 is just the free energy per monomer of an infinite planar monolayer *minus* the contribution from the hydrocarbon/water interfacial tension. This comes about because dividing by $a$ eliminates the curvature dependence of $\gamma_{hc/w}^p a$.

To attain a better understanding of the largest contribution, which is associated with the $\epsilon'$ term, we may consider the process of bending the monolayers so as to form a vesicle while keeping the hydrocarbon/water contact area, $A = A_e + A_i$, constant, *i.e.*, without stretching the monolayers (Figure 12). A change in the number of aggregated monomers, $\Delta N = \Delta N_i + \Delta N_e$, will occur because of the new geometry. There is less space inside the hydrocarbon core for a certain overall hydrocarbon/water contact area of a vesicle bilayer than in the form of planar monolayers. For $\Delta N$ we can write

$$\Delta N = \frac{A_e}{a_e} + \frac{A_i}{a_i} - \frac{(A_e + A_i)}{a_p} = A_e\left(\frac{1}{a_e} - \frac{1}{a_p}\right) + A_i\left(\frac{1}{a_i} - \frac{1}{a_p}\right) \quad (59)$$

Inserting eq 44 with $r = -R_i$ and $r = R_e$ in eq 59 gives

$$\Delta N = 4\pi[(\xi_e + \xi_i)k_a{}' + 2k_a{}'']/a_p \approx 8\pi(\xi_p k_a{}' + k_a{}'')/a_p \quad (60)$$

showing that, surprisingly enough, $\Delta N$ is independent of the vesicle size. There is, however, a dependence of the solution state as is seen in Figure 13.
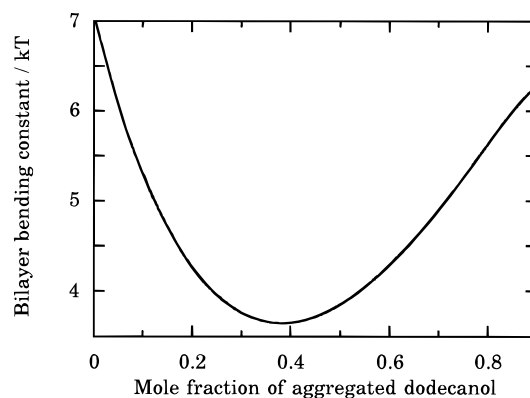


**Figure 14.** The bilayer bending constant, $k_{bi}$, plotted against the mole fraction of dodecanol in the mixed SDS/DOH bilayer, $x_{OH}^p$. The reduction of the bending free energy upon mixing a surfactant solution with a long-chain alcohol promotes the spontaneous formation of vesicles. Note the difference in scale compared with Figure 6.

We also note that $\Delta N$ generally assumes negative values corresponding to a loss of aggregated monomers upon bending the two monolayer parts into a closed vesicle while keeping the hydrocarbon/water surface area fixed. In other words, the average area per monomer at the hydrocarbon/water interface is larger for a spherical vesicle than for the corresponding planar bilayer.

Finally, by making use of eqs 48 and 49 we can eliminate $k_a{}'$ and $k_a{}''$

$$\Delta N = \frac{4\pi}{v_s}\left[R^2(\xi_i + \xi_e - 2\xi_p) - \frac{4}{3}\xi_p^3\right] \quad (61)$$

where the most significant term is the second one which is the contribution to $\Delta N$ if the bilayer half-thickness were the same for the planar monolayers and the vesicle. However, the dynamic monomer exchange between the vesicle bilayer and the bulk solution at equilibrium gives rise to a difference in bilayer half thicknesses compared with the planar monolayer which shows up in the first term of eq 61. By the way, this important relation can, of course, also be derived more directly by straightforward geometrical considerations.

The free energy change due to the loss of aggregated monomers is obtained simply by multiplying $\Delta N$ by $\epsilon'$, giving $\epsilon'\Delta N = 8\pi(\xi_p k_a{}' + k_a{}'')\epsilon'/a_p$. We find this to be the predominant contribution to the bending work, $\alpha \equiv 8\pi(\xi_p k_1 + k_2)/kT = 4\pi k_{bi}/kT$, with $k_1$ and $k_2$ given by eqs 55 and 56. The free energy change per molecule $\epsilon'$ is always negative since $\epsilon' + a_p\gamma_{hc/w}^p = \beta \approx 0$ (*cf.* eqs 40 and 57 resulting in a positive overall contribution to the bending free energy. Hence, this largely geometrical contribution equals the work of diminishing the hydrocarbon core of the bilayer while keeping the hydrocarbon/water interface fixed and it is the main contribution resulting in a net positive work of bending two monolayer portions into a geometrically closed vesicle.

## Discussion

In Figure 14 we have plotted the bilayer bending constant $k_{bi}$ against the alcohol mole fraction in the planar bilayer, $x_{OH}^p$. Let us next analyze this dependence in terms of the two (bending and stretching) contributions to $k_{bi}$ according to eq 26. Since the total surface area of the outer monolayer is larger than the surface area of the inner monolayer, there is a contribution to the bending work, explicitly given by eq 27, to replace the two surface areas of the same extension as the midplane with the

inner and outer hydrocarbon/water contact areas, respectively. This contribution is represented by the term $2R(\xi_e\gamma_e - \xi_i\gamma_i)$ and is a monotonously growing function of the planar alcohol mole fraction. Figure 3 shows that for a pure SDS bilayer, $\gamma_i$ is somewhat larger than $\gamma_e$, which is a consequence of the higher electrostatic free energy associated with the more closely located charged head-groups in the inner layer. Upon adding a long-chain alcohol the surface charge density is diluted and $\gamma_i$ diminishes as shown by comparing Figures 3 and 5, and consequently, $2R(\xi_e\gamma_e - \xi_i\gamma_i)$ increases (*cf.* Figure 6).

Furthermore, as the electrostatic free energy is lowered, the hydrocarbon chains are able to pack more densely ($\xi_i$ and $\xi_e$ increase) and, according to eqs 3 and 4, $a_i$ decreases more rapidly than $a_e$ and the difference between the outer and inner monolayer tensions becomes larger. This is also seen from the expressions for $\gamma_i$ and $\gamma_e$ in eqs 19 and 20, according to which the difference between the monolayer tensions increases as the difference between $R_e$ and $R_i$ increases.

From the above considerations we also conclude that the reduction of $\gamma_i$ due to adding alcohol occurs more rapidly than the increase of $\gamma_e$. This gives rise to the second contribution to $k_{bi}$ in eq 26, $R^2(\gamma_e + \gamma_i - 2\gamma_\infty)$, which decreases monotonously with the planar alcohol mole fraction. The two contributions are plotted in Figure 6. The latter contribution predominates at low alcohol concentrations when $\gamma_e$ and $\gamma_i$ are of comparable magnitude giving rise to the descending branch in Figure 14, while the former predominates at high alcohol concentrations yielding the ascending branch. As a result $k_{bi}$ passes through a distinct minimum implying that mixed vesicles are generally favored as has, indeed, been observed in a number of experiments.[21-29]

To sum up, we have shown that the monolayer tensions of a geometrically closed, spherically shaped bilayer, *i.e.*, a vesicle, can be calculated as a sum of several contributions, each of which can be written in the same form as eq 18. The monolayer bending constants, $k_1$ and $k_2$, can then be derived as a sum of such contributions.

(i) There is first of all an important change of the water contact area/hydrocarbon chain volume ratio upon passing from planar to spherical shell geometry,

$$k_{bi}^{geom} = \frac{2\epsilon'}{a_p}(\xi_p k_a' + k_a'') = \frac{\Delta N\epsilon'}{4\pi} \qquad (62)$$

(ii) For mixed vesicles the monolayer compositions depend on curvature, resulting in

$$k_{bi}^{comp} = \frac{2x_{OH}^p \epsilon''}{a_p}(\xi_p k_x' + k_a'k_x' + k_x'') \qquad (63)$$

(iii) The electrostatic free energy depends on curvature *per se*, yielding

$$k_{bi}^{el} = \frac{2\epsilon_{el}^p}{a_p}[(1 - x_{OH}^p)(\xi_p k_{el}' + k_a'k_{el}' + k_{el}'') - x_{OH}^p k_x'k_{el}'] \qquad (64)$$

(iv) The hydrocarbon/water contact free energy and the related head-group shielding are curvature-dependent and contribute with

$$k_{bi}^{hc/w} = \frac{4\gamma_{hc/w}^p}{a_p}[\xi_p((1 - x_{OH}^p)a_{pg} + x_{OH}^p a_{OH} - a_p) + k_a'a_{pg} - x_{OH}^p(k_a' + k_x')(a_{pg} - a_{OH})] \qquad (65)$$

(v) The conformational free energy of the hydrocarbon chains contributes with

$$k_{bi}^{conf} = \frac{2\epsilon_{conf}^p}{a_p}(\xi_p k_{conf}' + k_a'k_{conf}' + k_{conf}'') \qquad (66)$$

(vi) Finally, there is a contribution due to the curvature dependence of the free energy of mixing:

$$k_{bi}^{mix} = \frac{x_{OH}^p k_x'^2 kT}{a_p(1 - x_{OH}^p)} \qquad (67)$$

Note that the last four contributions arise merely due to the intrinsic curvature dependence of the separate *free energy* contributions as given previously. All these different contributions are listed in Table 1 for a pure SDS vesicle, for a mixed vesicle with $x_{OH}^p = 0.52$, and in addition, for a pure SDS vesicle with added salt in the surrounding solution.

The major contribution to the bending free energy of a vesicle, $\Delta N\epsilon'$, common to all equilibrated, geometrically closed, amphiphilic bilayers, regardless of the kind of aggregting monomers, is an immediate consequence of the hydrocarbon volume change upon switching from a planar to a spherical shell bilayer geometry. The largest single contribution to $\epsilon'$, the hydrophobic (Tanford) free energy given by eq 28, is $\sim -7kT$ per aggregated chain, which yields a positive contribution to the bilayer bending constant larger than about $30kT$. This is compensated mainly by the electrostatic and chain conformational contributions which are negative.

Equation 61 accounts for the change in aggregation number of the (incompressible) hydrocarbon chains. The half-thickness of the planar bilayer $\xi_p$ increases as the aggregated monomers become more packed at higher alcohol mole fractions, giving rise to a contribution to $\Delta N$ equal to $-16\pi\xi_p^3/3v_s$ which is predominant at high DOH concentrations. However, for the highly charged, SDS-rich vesicles the bilayer thickness decreases upon bending as the charged head-groups repel each other more strongly in the inner monolayer giving rise to a negative contribution, $4\pi R^2(\xi_i + \xi_e - 2\xi_p)/v_s$, which decreases in magnitude with the alcohol mole fraction and, consequently, is responsible for the decrease of $k_{bi}^{geom}$ when adding a long-chain alcohol below about $x_{OH}^p \approx 0.5$ (*cf.* Table 1).

From Table 1 it is seen that the geometrical contribution to the bending work alone is much too large to permit the formation of SDS/DOH vesicles at all. Actually, it turns out that a bending parameter $k_{bi}$ less than about $6kT$ is required to obtain vesicles of reasonable size. The main contribution to lowering $k_{bi}$ is due to electrostatics. Relatively speaking, the lower charge density in the outer monolayer means a lower, and the higher charge density in the inner monolayer means a higher, electrostatic free energy per charge. Since the number of surface charges

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**Table 1. The Different Contributions to the Bilayer Bending Constant $k_{bi}$ in $kT$ Units[a]**

|  | $c_{salt} = 0$ $x_{OH}^p = 0$ | $c_{salt} = 2.0$ M $x_{OH}^p = 0$ | $c_{salt} = 0$ $x_{OH}^p = 0.52$ |
|---|---|---|---|
| $a_p/\text{Å}^2$ | 34.0 | 33.1 | 32.3 |
| $\Delta N$ | −69 | −77 | −65 |
| $k_{bi}^{el}$(PB) | −6.05 | −1.30 | −5.68 |
| $k_{bi}^{geom}$ | 23.04 | 25.10 | 20.44 |
| $k_{bi}^{comp}$ |  |  | 1.96 |
| $k_{bi}^{el}$ | −8.68 | 0.99 | −10.44 |
| $k_{bi}^{hc/w}$ | −4.96 | −5.22 | −4.48 |
| $k_{bi}^{conf}$ | −2.34 | −4.29 | −2.36 |
| $k_{bi}^{mix}$ |  |  | 2.70 |
| $\Sigma = k_{bi}$ | 7.06 | 16.58 | 3.90 |

[a] See text for details. We have also included the planar surface area per aggregated monomer, the difference in aggregation number between a vesicle and a planar bilayer of same surface area, and the electrostatic contribution to $k_{bi}$ calculated at constant surface charge density, $k_{bi}^{el}$(PB). The molecular free energy $\epsilon'$ defined by eq 57 is to a very good approximation obtained by multiplying $a_p$ by $-\gamma_{hc/w}^p = -50.7$ mJ m$^{-2}$.

in the outer monolayer exceeds that in the inner layer the net contribution to $k_{bi}$ becomes negative.

The curvature dependence of $\gamma_{hc/w}$ right away gives a contribution to $k_1$ equal to $-2\gamma_{hc/w}^p$ and, hence, to $k_{bi}$ which equals $-4\gamma_{hc/w}^p \xi_p \approx -5.2kT$. Note that the factor 2, which originates from the curvature dependence of $\gamma_{hc/w}$ according to eq 35, is given in angstrom units. Shielding due to the head-groups gives a contribution which depends on the cross section of the head-group. Because more monomers are aggregated in the outer monolayer $k_{bi}$ increases as the size of the head-group increases. The difference in magnitude between the constant head-group terms enters the expression of $\epsilon''$ in eq 57. The larger this difference the smaller the bending work.

It is well established that pure SDS forms spherical micelles in water solution and that transitions to other micellar shapes occur when adding salt. By previous calculations we have theoretically accounted for the gradual favoring, due to electrostatic reasons, of the less curved aggregate surfaces as salt is added. Spherical, rod-, and disk-shaped micelles predominate in succession at salt concentrations lower than about 2.0 M.[8] Shielding the surface charges by means of adding salt makes the negative electrostatic contribution to the bending constant less in magnitude (see Table 1). For a pure SDS vesicle at $c_{salt} = 2.0$ M our calculations yield a bending parameter equaling $k_{bi} = 16.6kT$ at a total volume fraction of $\phi_{tot} = 0.1$. This would result in a vesicle size distribution with a most probable radius of about 3 km, *i.e.*, in this case $k_{bi}$ is much too large to enable the formation of pure SDS vesicles. This suggests that SDS in water, at salt concentrations favoring planar geometry, would tend to form large lamellar bilayer membranes rather than vesicles. This is in qualitative agreement with experimental results obtained by Hoffmann *et al.*,[28] where destabilization of a phase of liposomes mixed by a neutral and an ionic amphiphile was observed when salt was added to the system.

On the other hand, making the electrostatic free energy contribution even more negative by adding a long-chain alcohol, *i.e.*, to DOH, to the SDS/water solution brings about a reduction of the bending free energy, thus facilitating the formation of mixed SDS/DOH vesicles.

## Comparison with Earlier Calculations of the Electrostatic and Chain Conformational Contributions to the Bilayer Bending Constant $k_{bi}$

Amphiphilic bilayer films were first treated in terms of curvature elasticity by Helfrich in his celebrated paper from 1973.[30] Later on, the electrostatic and chain conformational contributions to the bending constants were considered in a number of papers.

The electrostatic contributions to the bending constants were calculated in the Debye−Hückel approximation by Winterhalter and Helfrich[31] for the case of constant surface charge density. For monolayers, Lekkerkerker[17] applied nonlinearized Poisson−Boltzmann theory and so did Mitchell and Ninham[16] for symmetric bilayers.

For our three main calculation examples, Poisson−Boltzmann theory (assuming a constant surface charge density) yields the (negative!) contributions included in Table 1. As a matter of fact, Mitchell and Ninham showed that $k_{bi}$ is negative for large values of the reduced charge parameter $S$ (*i.e.*, high surface charge density or low salt concentration) and for small $S$ (*i.e.*, low surface charge density or high salt concentration) if the Debye length is smaller than the half-thickness of the bilayer. When no salt is added these contributions are less negative than those we obtain in our present calculations at constant chemical potentials. This is what might be anticipated because the net work of decreasing the surface charge density in the larger outer monolayer and increasing it in the smaller inner layer is negative insofar as the effect of electrostatic shielding is moderate. In addition, Winterhalter and Helfrich[32] have calculated the bending constants using Poisson−Boltzmann theory, allowing a difference in charge density between the outer and inner layers while keeping the overall surface charge of the bilayer fixed.

Szleifer *et al.*[33] argue that the main contribution to the bending free energy is rather related to the hydrocarbon chain conformations. However, our calculations show that this contribution is comparatively small for the constant chemical potential case. On the other hand, for a thermodynamically closed bilayer it may be quite large ($\sim 100kT$) but the calculations of Szleifer *et al.* also show that an exchange of monomers between the two monolayers can bring it down about 2 orders of magnitudes. A fairly minor contribution of this kind is in agreement with what we have estimated elsewhere.[34]

In two recent papers Kumaran[6] and Porte and Ligoure[7] investigate the effect of mixing a neutral lipid membrane with an ionic surfactant and its effect on the stability of a dilute phase of equilibrated vesicles. The former author performs his calculations using the Debye−Hückel approximation and he concludes that vesicles do not form when the aggregated monomers are allowed to "flip-flop" between the two monolayers.

On the basis of the Poisson−Boltzmann equation Porte and Ligoure[7] calculate the electrostatic contribution to the bilayer bending constant at constant chemical potentials for an *infinitely thin* membrane. In other words, they only consider the contribution to $k_{bi} = 2(\xi_p k_1 + k_2)$ from $k_2$ whereas the contribution from $k_1$ is neglected. They derive an expression for the electrostatic bending constant as a function of the Debye length and the reduced

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charge parameter $S$ and obtain positive values for $S > 0.1$ and negative values when $S > 3.4$. The trends and signs agree with our results with $S$ typically being 10 or more in regions where vesicles actually form. However, our calculations for a bilayer of finite thickness generally gives a electrostatic contribution of larger magnitude.

## Concluding Remarks

To a good approximation, one can write the bending free energy of forming a spherical vesicle at constant chemical potentials out of the corresponding planar monolayer as $\alpha = 4\pi k_{bi}/kT$, *i.e.*, independent of vesicle size. The overall excess free energy of an equilibrated spherical vesicle can thus be written as the sum of the bending work and the stretching work, $\epsilon(R) = 4\pi(k_{bi} + 2\gamma_\infty R^2)$, *i.e.*, essentially in the same mathematical form as has frequently been employed for rod-shaped micelles.[14]

For given overall amphiphilic concentrations, the bilayer bending constant $k_{bi}$ determines the bilayer tension $2\gamma_\infty$ indirectly through the size distribution which is largely geared by the factors $\exp(-\epsilon(R)/kT)$ (*cf.* eq 5 and ref 10). The larger $k_{bi}$ is, the smaller the value of $\gamma_\infty$ which is needed to have a fixed total volume fraction of vesicles $\phi_{tot}$ is. Furthermore, from the size distribution we can derive a relation between the most probable radius of the vesicle and the bilayer tension to, $R_{max} \sim 1/(\gamma_\infty)^{1/2}$ and, hence, the size of the vesicles is bound to increase with the bending constant $k_{bi}$.[10] To obtain vesicles with average radii in the range 100 Å−10 $\mu$m the planar bilayer tension has to be small enough ($5 \times 10^{-3} - 5 \times 10^{-11}$ mJ/m$^2$) and the bilayer bending constant $k_{bi}$ should fall in the range $2-7kT$.[10] The corresponding monolayer tension can generally be represented as a quadratic function of curvature which does not necessarily pass through a minimum.

In a forthcoming paper[10] we demonstrate how the product of the Boltzmann factor $\exp(-\epsilon(R)/kT)$ and a fluctuation-related size-dependent statistical-mechanical factor $S(R)$ determines the size distribution $\phi(R)$ of spontaneously formed bilayer vesicles.

The (positive) contribution to $k_{bi}$, which by and large is due to the spherical shell geometry ($\sim 20kT$), is partly compensated by a large negative contribution caused by the curvature dependence of the electrostatic free energy ($\sim 10kT$). The latter contribution vanishes as salt is added, resulting in a very significant increase of $k_{bi}$. Hence, we infer that vesicle formation cannot be promoted by just adding salt; the proper thing to do is to add an additional, uncharged or oppositely charged, amphiphilic compound.

An appreciable amount of dodecanol has to be added to a pure SDS water solution to promote bilayer vesicles to form. Hargreaves *et al.*[35] found large liposomes for a 50 mol % SDS/DOH mixture. They increased in size upon adding more dodecanol. This is in good agreement with our present calculations which yield a most probable vesicle radius of about 0.3 $\mu$m at $x_{OH}^p = 0.5$ and show that $k_{bi}$ increases with the alcohol mole fraction above this value.

Finally, our present as well as previous calculations,[8,11,12,14] carried out for constant chemical potentials, show that all of the various contributions to the free energy of amphiphilic aggregates have to be invoked. In other words, to make realistic estimates, one cannot focus on just one particular contribution as has frequently been done in the past.

## Appendix

The ordinary expression for the ideal free energy of

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mixing of two aggregated monomers in a mixed monolayer is

$$\frac{\epsilon_{mix}^{id}}{kT} = x_{OH} \ln x_{OH} + (1 - x_{OH}) \ln(1 - x_{OH}) \quad (A1)$$

is part of the monolayer tension expression of eq 52, and contributes to the bending free energy parameters $k_1$ and $k_2$. Inserting the curvature dependence of the alcohol mole fraction given by eq 45 in eq A1 we get

$$\frac{\epsilon_{mix}^{id}}{kT} = x_{OH} \ln x_{OH}^p + (1 - x_{OH}) \ln(1 - x_{OH}^p) +$$
$$x_{OH} \ln\left(1 + \frac{k_x'}{r} + \frac{k_x''}{r^2}\right) + (1 - x_{OH}) \times$$
$$\ln\left[1 - \frac{x_{OH}^p}{(1 - x_{OH}^p)}\left(\frac{k_x'}{r} + \frac{k_x''}{r^2}\right)\right] \quad (A2)$$

The first two terms are included in the expressions of $\epsilon'$ and $\epsilon''$ in eqs 57 and 58 so from now on we can concentrate on the last two terms of eq A2. Expansion of the logarithms gives

$$\frac{\epsilon_{mix}'}{kT} \equiv x_{OH} \ln\left(1 + \frac{k_x'}{r} + \frac{k_x''}{r^2}\right) + (1 - x_{OH}) \times$$
$$\ln\left[1 - \frac{x_{OH}^p}{(1 - x_{OH}^p)}\left(\frac{k_x'}{r} + \frac{k_2''}{r^2}\right)\right] \times$$
$$= x_{OH}\left[\frac{k_x'}{r} + \frac{k_x''}{r^2} - \frac{1}{2}\left(\frac{k_x'}{r} + \frac{k_x''}{r^2}\right)^2\right] +$$
$$(1 - x_{OH})\left[-\frac{x_{OH}^p}{(1 - x_{OH}^p)}\left(\frac{k_x'}{r} + \frac{k_x''}{r^2}\right) -\right.$$
$$\left.\frac{x_{OH}^{p2}}{2(1 - x_{OH}^p)^2}\left(\frac{k_x'}{r} + \frac{k_x''}{r^2}\right)^2\right] \quad (A3)$$

Inserting the $x_{OH}$-function of eq 45 and neglecting terms of third order, we can write eq A3 as

$$\frac{\epsilon_{mix}'}{kT} = x_{OH}^p\left(\frac{k_x'}{r} + \frac{k_x''}{r^2}\right) - x_{OH}^p\left(\frac{k_x'}{r} + \frac{k_x''}{r^2}\right) +$$
$$\frac{x_{OH}^{p\,2}}{2(1 - x_{OH}^p)}\left(\frac{k_x'}{r} + \frac{k_x''}{r^2}\right)^2 + \frac{x_{OH}^p}{2}\left(\frac{k_x'}{r} + \frac{k_x''}{r^2}\right)^2$$
$$= \frac{x_{OH}^p}{2(1 - x_{OH}^p)}\left(\frac{k_x'}{r} + \frac{k_x''}{r^2}\right)^2 \quad (A4)$$

Multiplying eq A4 with $\Gamma$, given by eq 44, we see that there are no terms proportional to $1/r$ and, hence, we do not obtain any contribution to $k_1$. One of the terms is proportional to $1/r^2$, and we obtain the mixing free energy contribution to $k_2$ as the coefficient of this term

$$k_2^{mix} = \frac{x_{OH}^p k_x'^2 kT}{2 a_p (1 - x_{OH}^p)} \quad (A5)$$

which is identical to the expression for $k_{bi}^{mix}$ in eq 67 and the last term in the overall expression for $k_2$ in eq 56.