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Bending stiffness of lipid bilayers. V. Comparison of two formulations

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Abstract. — Two formulations of the intrinsic bending elasticity of lipid bilayers are compared which are based on (A) the mean and the Gaussian curvature and (B) the mean and the deviatoric curvature. It is shown that (i) formulation A contains implicitly the elasticity associated with the deviatoric curvature even if the topology of the membrane does not change, (ii) the deviatoric elasticity is responsible for the change in elastic energy that comes with a change in membrane topology, (iii) the elastic constant associated with the Gaussian curvature is negative, and (iv) the spontaneous-curvature model may be extended by a curvature-independent term which becomes relevant when the membrane surface area changes. Formulation B is then (for the special case of symmetric bilayers) refined in that the curvatures of the two monolayers are accounted for separately. This leads to a curvature independent term and a correction of the elastic constants of the bilayer which both depend on the value of the monolayer-curvature. A continuum mechanical model for the bending elasticity of a lipid monolayer is developed. By comparison of this model to experimental data (i) the elastic constant associated with the deviatoric curvature is estimated and (ii) the decrease in experimentally determined elastic constants with increasing lipid unsaturation is traced back to the change in area compressibility and a trend in the spontaneous curvature of the monolayers. Finally it is shown that upon a change in temperature the change in elastic energy of a lipid vesicle due to the curvature-independent term is much larger than the concomitant change in elastic energy due to the shape change but only a small fraction of the total change in internal energy.

1. Introduction.

This is the last paper of a series. Papers number I and IV dealt with the distinction of bilayer-couple bending and single-layer bending elasticity of lipid vesicle and red cell membranes [1, 2]. Bilayer-couple bending arises from the 2D isotropic elasticity of the individual layers and their fixed distance. Single-layer bending denotes the intrinsic bending stiffness of each layer. Papers number II and III presented two concepts for an alternative formulation of single-layer bending elasticity. In number II the consequences of a separate account of the curvatures of the two monolayers were studied [3]. In number III it was suggested to use the deviatoric instead of the Gaussian curvature [4]. The treatment of these two concepts was incomplete in that:
(a) the second concept was not compared in a complete fashion to the traditional formulation based on the Gaussian curvature, and
(b) the second concept was not incorporated in the treatment of the first one.

The present paper removes these flaws. Point (a) is treated in sections 2.1 and 2.2. In section 2.3 the alternative and the traditional formulation are applied to budding of giant lipid vesicles in the spontaneous-curvature model. Point (b) is treated in section 3.1. In section 3.2 a continuum mechanical model is developed that describes the bending elasticity of a monolayer. By comparison with experimental data elastic constants in the alternative formulation are estimated. In section 3.3 the consequences arising for the bilayer-couple model from a change in membrane surface area are considered.

2. Consequences from the use of the deviatoric instead of the Gaussian curvature.

2.1 Zero spontaneous curvature. — In this section we consider the intrinsic (here called single layer) bending resistance of a bilayer. We adopt the traditional strategy by lumping together the properties of the two monolayers into a conceptual single layer. First we consider the case of zero spontaneous curvature. The energy density $e_{dl}$ can be written [4, 3]:

$$ e_{dl} = 2B_\gamma \bar{c}^2 + 2B_a \bar{\varepsilon}^2, \quad (1) $$

where $B_\gamma$ and $B_a$ are the elastic constants of the bilayer in isotropic and deviatoric bending, respectively. $\bar{c}$ denotes the local mean curvature. $\bar{\varepsilon}$, the deviator, is defined as follows:

$$ \bar{\varepsilon} = \left| \frac{c_1 - c_2}{2} \right|, \quad (2) $$

where $c_1$ and $c_2$ are the local principal curvatures. The curvatures $c_1$, $c_2$, $\bar{c}$, and $\bar{\varepsilon}$ are those of the neutral plane of the bilayer. Since any deviation from the unstrained (plane) configuration of the bilayer would increase its elastic energy both $B_\gamma$ and $B_a$ must be positive.

We compare equation (1) to the energy density $e_H$ introduced by Helfrich [5] for the case of zero spontaneous curvature:

$$ e_H = 2k_c \bar{c}^2 + \bar{k}_c c_1 c_2. \quad (3) $$

Here $k_c$ and $\bar{k}_c$ denote the elastic constants associated with the mean and the Gaussian curvature. Obviously there is no restriction on the sign of the elastic constants since the product $c_1 c_2$ may assume positive or negative values. Both notations (Eqs. (1) and (3)) are identical when

$$ k_c = B_\gamma + B_a, \quad (4) $$

and

$$ \bar{k}_c = -2B_a. \quad (5) $$

From equations (1) to (5) we conclude:

(a) For constant topology of a laterally uniform (for an explanation see next item) vesicle membrane it is sufficient to consider either $\bar{c}$ or $\bar{\varepsilon}$. The Helfrich notation uses $\bar{c}$, but note that $k_c$ as determined from fitting to experiments covers both types of bending stiffness (isotropic and deviatoric). If, in equation (3), $\bar{c}$ was replaced by $\bar{\varepsilon}$, equation (4) would still apply.

(b) $\bar{k}_c$ as defined in equation (3) is negative. Experimental and theoretical findings as to the
2.2 NON-ZERO SPONTANEOUS CURVATURE. — Second we treat the case that the spontaneous curvature assumes a non-zero value. This spontaneous curvature can also be decomposed in an isotropic and a deviatoric contribution. As suggested [4] we retain the old name spontaneous curvature for the isotropic contribution and call the deviatoric one « spontaneous warp ». Accounting for both quantities the energy density (stored in single layer bending) of the bilayer reads [4, 3]:

\[
E_{sl} = 2B_a(\bar{c} - \xi_{sl})^2 + 2B_a(\bar{c} - \phi)^2,
\]

(6)

where \(\xi_{sl}\) denotes the spontaneous curvature in single layer bending and \(\phi\) the spontaneous warp. Due to the rotational mobility of phospholipids, the spontaneous warp is free to orient with respect to \(\bar{c}\). For this reason \(\bar{c}\) and \(\phi\) are by definition positive [4]. \(\phi\) does not carry an index because there is no deviatoric contribution in bilayer-couple bending [4]. \(B_\xi\) and \(B_a\) are positive for the same reason as above.

Helfrich [5] accounted for the spontaneous curvature as follows:

\[
E_{H} = 2k_c \left( \bar{c} - \frac{c_0}{2} \right)^2 + \bar{k}_c c_1 c_2,
\]

(7)

where \(c_0\) was called spontaneous curvature. Equation (7) is the basis of the so-called spontaneous-curvature model. Equation (6) (for the special case : \(\phi = 0\)) and equation (7) are identical except for a term independent of curvatures when besides equations (4) and (5) the following holds:

\[
c_0 = 2\xi_{sl} \frac{B_a}{B_\xi + B_a}
\]

(8)

Adding the constant term and using \(b = B_a/B_\xi\), equation (6) (for \(\phi = 0\)) can be written alternatively:

\[
E_{sl} = 2k_c \left( \bar{c} - \frac{c_0}{2} \right)^2 + \bar{k}_c c_1 c_2 + 2b k_c \left( \frac{c_0}{2} \right)^2
\]

(9)

It is confusing that both \(\xi_{sl}\) and \(c_0\) are called spontaneous curvature. It has been shown [1] that
\(\xi_{sl}\) corresponds to what one would intuitively call the spontaneous curvature. We conclude that \(c_0\) is a phenomenological parameter. For \(b = 0\) equation (8) reduces to \(c_0/\xi_{sl} = 2\). With increasing \(b\) the ratio \(c_0/\xi_{sl}\) becomes larger reflecting the fact that equation (7) implicitly accounts for the elastic contribution of deviatoric bending. In section 3.2 the value of \(b\) will be estimated.

When different shapes with the same surface area and volume are compared with respect to their elastic energy the constant term in equation (9) drops out. This means minimum energy shapes predicted by equation (6) or equation (7) would be identical. If, however, the surface area is changing the constant term in equation (9) must not be omitted.

2.3 APPLICATION TO MODEL CALCULATIONS. — We consider budding promoted by a spontaneous curvature (in single layer bending) when the surface area of an initial sphere (radius \(R\)) is increased at constant volume and a constant positive value for \(c_0\). Based on the first term of equation (7) the elastic energy decreases with increasing number of budds [10]. From equation (6), however, one would expect that above a threshold value for \(b\) the energy would increase because more elastic energy has to be paid for the formation of the necks than is released in the spherical portions of the membrane.

As an example we present approximate calculations following a case studied in detail by Miao et al. [10]. To ease comparison with their data we scale lengths by \(c_0\). In their figure 6 they start with a spherical vesicle of radius 4.0216. They find for distinguished values of the surface area the vesicle shape to consist of a number of spherical budds (with radius \(2/c_0\)) and the mother vesicle (spherical as well), all connected by microscopic necks.

We estimate the elastic energy at these distinguished values of surface area. To this end we assume (i) \(\bar{c} = 0\) in the neck region. Then according to equation (7) the contribution of the necks to the elastic energy is zero. We further neglect their surface area and calculate as if the mother vesicle and the budds were closed spheres. Then according to equation (7) the energy stored in the budds is zero as well. Energy is only stored in the mother vesicle. The values calculated according to the first term on the right hand side of equation (7) are shown in the second column of table I. Comparison with figure 6 of Miao et al. [10] shows that with increasing number of budds our estimate becomes increasingly larger than their precise values.

The values in the third and the fourth column of table I are calculated according to equation (9). The values for no budd are larger than in the second column due to the two additional terms. But note that the decrease in energy with increasing number of budds is much smaller for \(b = 0.2\) than in the second column. For \(b = 0.3\) the energies increase.

Table I. — Comparison of elastic energies calculated approximately (for details see text) according to equation (9) and the first term of equation (7). The surface area in each line is constant and chosen such that the mother vesicle as well as the budds are spherical. The volume of all shapes considered is the same.

<table>
<thead>
<tr>
<th>Number of budds</th>
<th>(E/2\pi k_c) acc. to first term of Eq. (7)</th>
<th>(E/2\pi k_c) acc. to Eq. (9) (b = 0.2)</th>
<th>(E/2\pi k_c) acc. to Eq. (9) (b = 0.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.09</td>
<td>6.65</td>
<td>8.02</td>
</tr>
<tr>
<td>1</td>
<td>3.42</td>
<td>6.52</td>
<td>8.14</td>
</tr>
<tr>
<td>2</td>
<td>2.76</td>
<td>6.37</td>
<td>8.25</td>
</tr>
<tr>
<td>3</td>
<td>2.10</td>
<td>6.21</td>
<td>8.35</td>
</tr>
</tbody>
</table>
Alternatively we use equation (6) to calculate the elastic energies. $\xi_{sl}$ is calculated from $c_0$ and $b$ according to equation (8). In this formulation energy is stored in the buds and necks as well. Under the same simplifying geometric assumptions as above the first term on the right hand side of equation (6) gives the contributions of the spherical portions whereas the second term that of the necks. The surface area of a neck is in good approximation inversely correlated to the square of its curvature. According to experimental evidence [4] we assume $\theta = 0$. The contribution of each neck to the elastic energy is then approximately constant irrespective of the size of the neck. If we take this contribution as $8 \pi B_4$ we get the same values for the total energy as in columns 3 and 4 of table I. The choice of $8 \pi$ as the constant of proportionality corresponds to a surface area of the neck of $4 \pi r^2_n$, where $r_n$ is half the neck diameter.


3.1 FORMULATION. — In this section we consider again single layer bending but this time we account for the two monolayers separately. We treat the case of a symmetric bilayer where the spontaneous curvatures of the individual monolayers have the same absolute value (different from zero) and have opposite sign [11]. The spontaneous curvature of the bilayer is zero. The spontaneous warp of the bilayer, on the other hand, has the same value as the spontaneous warp of the monolayers. This is due to the rotational mobility of phospholipids [4].

Besides the individual spontaneous curvatures the analysis accounts for the slight difference in actual curvature between the two monolayers. From the sum of the two monolayer contributions the energy density (in single layer bending) of the bilayer is obtained by expansion in a Taylor series and collection of terms up to a power of 2 in $\delta$. The isotropic contribution, $\tilde{\varepsilon}$, reads [3]:

$$\tilde{\varepsilon} = 2 \tilde{B}_s \left\{ \tilde{c}^2 + \tilde{c}^2 + 2 \tilde{c} \delta (\tilde{c}^2 + \tilde{c}^2) + \frac{3}{4} \delta^2 (c_1^4 + c_2^4) + \frac{1}{2} \delta^2 c_1 c_2 (c_1^2 + c_1 c_2 + c_2^2) \right\} , \quad (10)$$

where $\tilde{c}$, $\tilde{c}$, $c_1$, and $c_2$ are as above the curvatures of the neutral plane of the bilayer. $\delta$ denotes the distance of the neutral plane of each monolayer from that of the bilayer. $\tilde{c}$ denotes the spontaneous curvature of the outer monolayer. $\tilde{c}$, and $\tilde{B}_s$ are quantities describing the bilayer. $\tilde{B}_s$ is positive since it is derived from the properties of the single layer. To distinguish the formulation in equation (10) from that in equation (6) a tilde is added to the respective symbols. $\tilde{c}$ is by definition a local quantity since it depends on the local composition of the lipid monolayer. Under most circumstances the thermal energy is, however, sufficient to randomize the distribution of different lipid species on the surface of the monolayer so that $\tilde{c}$ is then essentially uniform.

The first term in the curly brackets of equation (10) is a consequence of the opposite spontaneous curvatures of the two monolayers. It relates to the elastic energy stored in the bilayer when it is plane, i.e. when no external bending moments are acting on it.

The second and the third term give the increase in energy when the bilayer deviates from planarity due to the action of external bending moments. The second term is the standard one. It corresponds to the first term on the right hand side of equation (1).

The third term relates to the difference in the spontaneous curvatures and the difference in the actual curvatures of the two monolayers [3]. This term becomes relevant when $2 \tilde{c} \delta$ is comparable to unity. Using the estimates of $\tilde{c}$ presented in section 3.2 and taking $\delta = 1$ nm [1] we can appreciate that for the lipids listed in table II : $0 \approx 2 \tilde{c} \delta < - 1/3$. 
Table II. — Calculation (according to Eq. (25)) of the spontaneous monolayer-curvature \( \tilde{\xi} \) from the elastic constant \( k_c \) in single layer bending [16], the isotropic modulus \( K \) [16], and the thickness \( d \) of the bilayer [32] for four different pure lipids (DMPC = dimyristoyl-phosphatidylcholine, SOPC = stearoyl-oleoyl-phosphatidylcholine, DGDG = digalactosyldiacylglycerol, DAPC = diarachidonoyl-phosphatidylcholine) and a (1/1) lipid mixture with Cholesterol (= Chol) under the assumption \( \tilde{\xi} = 0 \) for DMPC. \( C = C \) denotes the number of carbon-carbon double-bonds per lipid molecule.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>( C = C )</th>
<th>( k_c ) ( \times 10^{-12} ) erg</th>
<th>( K ) dyn/cm</th>
<th>( d ) Å</th>
<th>( \frac{48 k_c}{Kd^2} )</th>
<th>( 1/\tilde{\xi} ) nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPC</td>
<td>0</td>
<td>0.56</td>
<td>145</td>
<td>35.1</td>
<td>1.50</td>
<td>-57.4</td>
</tr>
<tr>
<td>SOPC</td>
<td>1</td>
<td>0.90</td>
<td>190</td>
<td>39.8</td>
<td>1.44</td>
<td>-7.7</td>
</tr>
<tr>
<td>SOPC/Chol</td>
<td>6</td>
<td>2.46</td>
<td>640</td>
<td>44.9</td>
<td>0.92</td>
<td>-6.2</td>
</tr>
<tr>
<td>DGDG</td>
<td>8</td>
<td>0.44</td>
<td>160</td>
<td>38.7</td>
<td>0.88</td>
<td>-5.9</td>
</tr>
<tr>
<td>DAPC</td>
<td>6</td>
<td>0.44</td>
<td>135</td>
<td>47.3</td>
<td>0.70</td>
<td>-5.9</td>
</tr>
</tbody>
</table>

The last two terms contain higher order contributions which become relevant at radii of curvature comparable to the thickness of the bilayer [12].

The deviatoric contribution \( \tilde{\varepsilon}_d \) to the energy density stored in the bilayer has as yet not been given accounting for the curvatures of the two monolayers individually. By an analogous calculation as presented by Fischer [3] for isotropic bending we obtain:

\[
\tilde{\varepsilon}_d = 2 \tilde{B}_s \left\{ (\tilde{c} - \vartheta)^2 - \vartheta \tilde{\xi} \tilde{\delta} \right\} c_1^2 c_2^2 + \frac{3}{4} \tilde{\delta}^2 (c_1^4 + c_2^4) - \frac{1}{2} \tilde{\delta}^2 c_1 c_2 (c_1^2 + c_1 c_2 + c_2^2) \right\}. \tag{11}
\]

\( \vartheta \) does not carry a tilde since the spontaneous warps in equations (6) and (11) are identical [13]. \( \tilde{B}_s \) is again a positive quantity. All terms in the curly brackets of equation (11) except for the first one become relevant only at small radii of curvature.

3.2 COMPARISON TO EXPERIMENTALLY DETERMINED ELASTIC CONSTANTS. — Adding equations (10) and (11) we obtain the total energy density stored in single layer bending:

\[
\tilde{\varepsilon}_{\text{el}} = 2 \tilde{B}_s (1 + 2 \tilde{\xi} \vartheta) \tilde{\varepsilon}^2 + 2 (\tilde{B}_d + 2 \tilde{B}_s \tilde{\xi} \tilde{\delta}) \tilde{\varepsilon}^2 + 2 \tilde{B}_s \left\{ \tilde{\xi}^2 + O(\tilde{\delta}^2 c^4) \right\}, \tag{12}
\]

where we have taken \( \vartheta = 0 \) according to experimental evidence [4]. Except for the higher order terms, equations (12) and (1) are identical when \( \tilde{\xi} = 0 \). This means in this case \( B_s = \tilde{B}_s \) and \( B_d = \tilde{B}_d \). If equation (1) is used when \( \tilde{\xi} \neq 0 \), \( B_s \) and \( B_d \) become effective elastic constants [14]. Equation (12) shows that these are the smaller the more negative \( \tilde{\xi} \). Lipids may exist so that the effective elastic constants and thus the bending energy become negative. Then the plane configuration of the bilayer would not be the one of lowest elastic energy. Giant vesicles would probably not form spontaneously in this case [15].

Experiments [16] with vesicles prepared (via spontaneous formation) from various lipid species showed that \( k_c \) [17] decreases with increasing degree of unsaturation of the hydrocarbon chains. The 2D isotropic modulus which was measured in the same experiments decreased as well. It was, however, noted [3, 16] that besides the influence of \( K \) there is an additional influence on the trend of \( k_c \). To trace it back on a trend in \( \tilde{\xi} \) we rewrite...
Comparison with equation (3) shows that the expression within brackets in the first term on the right hand side of equation (13) corresponds to \( k \). To this end a lipid monolayer is modelled as a thin plate of a linearly elastic 3D material with in-plane \((x, y)\) isotropy. The material is assumed to resist volumetric changes with a 3D isotropic modulus \( k \), transverse shear strains with a 3D shear modulus \( G \), and in-plane shear strains with a 3D shear modulus \( g \). From the symmetry properties of the material it follows \([18]\) that 5 elastic constants are needed for its description. The respective stress-strain relations are:

\[
\begin{align*}
\sigma_{11} &= a_1 u_{11} + (a_1 - 2 a_2) u_{11} + a_2 u_{11}, \\
\sigma_{22} &= (a_1 - 2 a_2) u_{11} + a_1 u_{22} + a_2 u_{11}, \\
\sigma_{33} &= a_2 u_{11} + a_2 u_{33} + a_3 u_{11}, \\
\sigma_{12} &= a_4 2 u_{12}, \\
\sigma_{13} &= a_4 2 u_{13}, \\
\sigma_{23} &= a_5 2 u_{23},
\end{align*}
\]

where \( u_{ij} \) and \( \sigma_{ij} \) \((i, j = x, y, z)\) are the components of the strain and stress tensor in linear elasticity. Trivially it follows \( a_4 = G \) and \( a_5 = g \). Deviatoric bending leads to in-plane shear strains which are not uniform in thickness direction of the plate \([4]\). The respective distribution is characterized by a rotary-reflection symmetry of order 4 around an axis normal to the plate. Such strains are resisted by a lipid monolayer because they change the shape of individual lipid molecules. In-plane shear strains which are uniform normal to a lipid monolayer are, of course, not resisted elastically because the lipids can flow past each other. We account for these properties by restricting ourselves in the following to boundary conditions which are characterized by an absence of in-plane shear strain that is uniform in thickness direction.

To express \( a_1, a_2, \) and \( a_3 \) by \( G, g, \) and \( k \) we require that the stress-strain relations (Eqs. (14-19)) must be identical to those of an isotropic 3D material (characterized by a shear modulus \( \mu \) and an isotropic modulus \( k \)) under the following two boundary conditions. The first one is unilateral compression \((u_{zz} \neq 0; \text{all other } u_{ij} = 0)\). With \( k = \kappa \) and \( G = \mu \) we obtain by comparison: \( a_2 = k - \frac{2}{3} G \) and \( a_3 = k + \frac{4}{3} G \). The second boundary condition is isotropic compression. With \( k = \kappa \) we obtain: \( a_1 = k + \frac{1}{3} G + g \). With these coefficients we obtain for the free energy \((f)\) per volume:

\[
f = \frac{k}{2} (u_{11} + u_{22} + u_{33})^2 + \frac{G}{6} \left\{ (u_{11} + u_{22})^2 + 4 u_{11} (u_{zz} - u_{11} - u_{11}) + 12 (u_{zz}^2 + u_{11}^2) \right\} + \frac{g}{2} \left\{ (u_{11} - u_{11})^2 + 4 u_{11}^2 \right\}.
\]

\( K \) is calculated subjecting the plate to isotropic in-plane stress \((\sigma_{11} = \sigma_{22} \neq 0, \text{all other } \sigma_{ij} = 0)\). Using equations (14), (15), and (16) we obtain \((u_{11} + u_{11})\) which is equal to the relative change in surface area of the plate. From the definition of \( K \) \([16]\) we obtain:

\[
\frac{K}{2} = \frac{18 k G}{3 k + 4 G} m,
\]

where \( m \) is the thickness of the plate which in turn corresponds to the thickness of a lipid...
monolayer. To obtain \( \tilde{B}_c \) and \( \tilde{B}_a \) we follow the standard treatment [19] and calculate the bending energy per surface area \( (e_p) \) stored in a thin plate. Using equations (14-16, 19) we obtain:

\[
e_p = \frac{m^3}{4} \left\{ \frac{3}{k + 4} \frac{kG}{3} \left( \frac{\partial^2 \xi}{\partial x^2} + \frac{\partial^2 \xi}{\partial y^2} \right) + \frac{g}{3} \left[ \left( \frac{\partial^2 \xi}{\partial x^2} \right)^2 + 4 \left( \frac{\partial^2 \xi}{\partial x \partial y} \right)^2 \right] \right\},
\]

(22)

where \( \xi \) denotes the displacement of the neutral fiber normal to the plane. In principal axes the mixed derivative vanishes, the other derivatives are replaced by \( (\bar{c} - \bar{\xi}) \) and \( (\bar{c} - \bar{\theta}) \).

The energy density of a monolayer is given by equation (6) with \( B_c, B_a, \) and \( \xi_{\perp} \) replaced by \( \tilde{B}_c/2, \tilde{B}_a/2, \) and \( \tilde{\xi} \). Comparison with equation (22) results in:

\[
\frac{\tilde{B}_c}{2} = \frac{3}{2} \frac{kG}{3 k + 4 G} m^3
\]

(23)

and

\[
\frac{\tilde{B}_a}{2} = \frac{1}{6} g m^3
\]

(24)

From equations (21) and (23) we obtain \( \tilde{B}_c = Km^2/12. \) \( \tilde{B}_a \) as determined in equation (24) cannot be expressed by \( K. \) By analogy to 3D elasticity we assume \( \tilde{B}_a = b\tilde{B}_c, \) where \( b \) is a constant. The expression for \( k_c \) as obtained from equation (13) is then:

\[
k_c = \frac{K d^2}{48} (1 + b + \tilde{\xi} d),
\]

(25)

where we have used \( d = 2m = 4 \delta, \) \( d \) being the bilayer thickness. Table II shows the numbers calculated for the expression \( 1 + b + \tilde{\xi} d \) from the experimental data of Evans and Rawicz [16].

To determine the unknown parameter \( b \) we assume \( \tilde{\xi} = 0 \) for the lipid with saturated hydrocarbon chains [20]. We get \( b = 0.5 \) and values for \( \tilde{\xi} \) which decrease with increasing unsaturation (Tab. II).

The trend in the values of \( \tilde{\xi} \) is in qualitative agreement with the observed structures of lipids in water upon increasing the degree of unsaturation and/or upon addition of cholesterol [20]. Independent determinations of \( \tilde{\xi} \) are available from the radius of stress-free cylinders of the inverted hexagonal phase [21]. For phospholipids with two monounsaturated hydrocarbon chains this radius has been measured to be on the order of \( -3 \) nm [21]. Taking the mean curvature of the cylinders as an estimate for \( \tilde{\xi} \) we obtain \( 1/\tilde{\xi} = -6 \) nm, which is comparable to the value given in Table II for polyunsaturated hydrocarbon chains. To explain this discrepancy we note that (a) the methods to determine \( \tilde{\xi} \) are different and (b) the headgroup size of the lipids used [21, 16] differs markedly.

The main uncertainty in our determination of \( b \) is the large variation in the value of \( k_c \) reported by different laboratories. We used the values of Evans and Rawicz [16] because their study is the only one in which \( K \) and \( k_c \) were determined at the same preparation of vesicles. We conclude that it is likely that \( b \) is appreciably greater than zero. This means the conclusions reached in section 2 have a realistic foundation.

From data on microemulsions Farago [7] concluded: \( \bar{k}_c/k_c \approx -2. \) By comparison of the coefficients of equations (3) and (13) we obtain:

\[
\frac{\bar{k}_c}{k_c} = \frac{-2 b + \tilde{\xi} d}{1 + b + \tilde{\xi} d}
\]

(26)
Figure 1 shows $\tilde{k}_c/k_c$ as a function of the monolayer curvature for $b = 0.5$ and $d = 4$ nm. The strong dependence on $\tilde{\xi}$ indicates that $\tilde{k}_c/k_c$ is not a well-behaving indicator of the ratio of deviatoric and isotropic elasticity. The values of $\tilde{\xi}$ in table II correspond to values of $\tilde{k}_c/k_c$ between $-0.67$ and $-0.39$. $\tilde{k}_c/k_c = -2$ as found by Farago [7] corresponds to $1/\tilde{\xi} = -2$ nm a value which is not unreasonable for the surfactant used in the study of this author.

![Graph showing $\tilde{k}_c/k_c$ as a function of the monolayer curvature $\tilde{\xi}$](image)

Fig. 1. — $\tilde{k}_c/k_c$ (according to Eq. (26) for $b = 0.5$ and $d = 4$ nm) as a function of the monolayer curvature $\tilde{\xi}$.

3.3 CONSEQUENCES FOR THE BILAYER-COUPLE MODEL. — The so-called bilayer-couple model [9] accounts for single layer bending by using equation (3) and for bilayer-couple bending by using a boundary condition on the difference in surface area of the two leaflets. However, when the surface area of the membrane changes, the constant term in equation (13) predicts a contribution to the elastic energy not contained in equation (3). In contrast to the spontaneous-curvature model the constant term in equation (13) has a straightforward physical interpretation: it accounts for the energy stored in the balance of bending moments of the two monolayers with opposite spontaneous curvature.

To get an idea on the magnitude of the increase in this energy during a thermal increase in surface area we compare it to a typical change in elastic energy occurring in parallel due to the shape change, on one hand, and to the energy calculated from the heat capacity of the membrane, on the other. Typical changes in elastic energy are obtained by comparing the experimental data of Käs and Sackmann [22], in particular the change from picture (3) to (5) in their figure 3 with figures 4 and 5 of Seifert et al. [23]. We get $1.6 \pi k_c$ for the increment in elastic energy due to the change in curvature. With an increase in surface area of 60 $\mu$m$^2$ [22] and from equation (13) we obtain for the additional energy stored in the balance of opposite
bending moments $2 k_c \xi^2 = 60 \mu m^2/(1 + b + \xi d)$. Using the data in Table II we get $2.5 \times 10^4 k_c$ and $5 \times 10^6 k_c$ for SOPC and DAPC, respectively. This is several orders of magnitude greater than the increase in elastic energy due to the shape change. It is, however, only a small fraction of the change in total internal energy of the membrane. For a rough estimate we use the heat capacity of glycerin, a temperature change of 3.5 °C [22], and an average membrane surface area of 2 800 $\mu m^2$ [22] and get an increase in energy of $10^9 k_c$.

4. Summary.

Two formulations of the elastic energy (of a bilayer) stored in single-layer bending were compared: the traditional formulation which is based on the mean and the Gaussian curvature and an alternative formulation which is based on the mean and the deviatoric curvature. A further distinction is that the traditional formulation does not account for the monolayers individually, in contrast to the alternative formulation.

The advantage of the traditional formulation is that — as long as the topology remains unchanged and in the absence of lateral segregation of lipid species — it requires one stiffness parameter and one type of curvature less than the alternative formulation. Calculations are therefore simpler. The advantage of the alternative formulation is that it allows a straightforward interpretation of its stiffness parameters and spontaneous curvatures. Their use facilitates physical thinking. An example would be two buds connected by a microscopic neck having essentially the same energy as the buds after fission.

To describe a symmetric bilayer the alternative formulation uses $\tilde{B}_a$ and $\tilde{B}_s$, the elastic constants for isotropic and, respectively, deviatoric bending and $\tilde{\xi}$, the spontaneous monolayer-curvature. The traditional formulation uses the elastic constants $k_c$ and $\bar{k}_c$. $\tilde{B}_s$, $\tilde{B}_a$ and $\tilde{\xi}$ are independent of each other whereas both $k_c$ and $\bar{k}_c$ depend on $\tilde{B}_s$, $\tilde{B}_a$ and $\tilde{\xi}$.

At first sight it appears that the alternative formulation needs one parameter more than the traditional formulation. However, for a complete description of the elastic properties of a symmetric bilayer we have to add in both formulations the 2D isotropic modulus ($\bar{K}$) to account for isotropic tension and the distance ($2 \delta$) of the neutral surfaces of the monolayers to account for bilayer-couple bending. Since $\tilde{B}_s$ can be expressed by $\bar{K}$ and $\delta$ the total number of parameters is the same in both formulations.

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    structure and conformation of amphiphilic membranes (Springer-Verlag, Berlin, 1992).

[8] This is a correction of what I suggested earlier, reference [4].

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[11] Curvatures are defined as positive in a spherical vesicle for both the inner and the outer monolayer.

[12] These terms are a by-product of our analysis. For a better account of the energy density at large

[13] For the same reason equation (11) is very similar to the corresponding expression for isotropic
    bending in an asymmetric bilayer (see Ref. [3], Eq. (7)).

[14] There is no dependence of these effective constants on the shape of the vesicle as I conjectured
    earlier, reference [3].

[15] A way to produce bilayers from such lipids may be to start from spontaneously formed vesicles and
    to reduce $\tilde{\xi}$ by a chemical modification of its lipids or a change in the ionic environment while
    the membrane is under isotropic tension. Upon release of this tension such membranes would
    be unstable and bend until higher order terms would limit the curvature and/or until the excess
    surface area would be used up and isotropic tension would stabilize again the vesicle shape.


[17] It has been shown that bilayer-couple bending does not appreciably contribute to the total bending
    stiffness in thermally excited shape fluctuations [1]. For this reason the values determined by
    Evans and Rawicz [16] correspond to $k_o$.


