Diffusion of Macromolecules on Lipid Vesicles

W. T. Go´z´dz*

Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland

Received June 6, 2008. Revised Manuscript Received August 9, 2008

Diffusion of macromolecules on a surface of lipid vesicles of different reduced volume and geometry is investigated. It is assumed that the macromolecules deform the surface of the vesicles by inducing the spontaneous curvature which is proportional to their concentration. We study how nonuniform distribution of macromolecules is reflected in the shape of the vesicles and how the shape of the vesicles influences the diffusion process.

Introduction

Biological membranes are composed of many components such as lipids, proteins, and hydrocarbons. The membrane is fluid, and the components can freely diffuse. Proteins or polymers can deform in their neighborhood the shape of the membrane. When the macromolecules diffuse on the lipid vesicle, its shape is changed in time. The diffusion of the macromolecules influences the shape of the vesicle and vice versa. This phenomenon can be easily noticed when the membrane forms small necks which act as obstacles for the diffusing macromolecules, since the surface on which the macromolecules can diffuse is significantly decreased. Coupling the distribution of the membrane components with the shape of the vesicle is an important and interesting phenomenon present in biological systems. The macromolecules can influence the shape of the vesicles, but also the shape may lead to segregation of the macromolecules of different species. Here, we study the change of the vesicle shape induced by the macromolecules which diffuse on the surface of lipid vesicles. It is assumed that the macromolecules are attached to the surface of the vesicle and a domain of the macromolecules is created. The concentration of the macromolecules in the domain is larger than the concentration on the vesicle. The macromolecules diffuse from the domain to reduce the concentration gradient. Such phenomena have been observed in experiments where the polymers injected near the lipid vesicle were anchored to the membrane causing its deformation. The polymer which is attached to the membrane can increase its configurational entropy if the membrane is bent away from the polymer. Such mechanism leads to producing the spontaneous curvature. Not only polymers but also large macromolecules attached to the membrane may induce the spontaneous curvature. The free energy of the system is the sum of two terms \( F = U - TS \). For the studied phenomenon, the first term is related to the bending energy which is proportional to the bending rigidity and the second term is related to the entropy of mixing which is proportional to temperature. The bending energy is minimized for nonuniform distribution of components, and the entropy is maximized for the uniform distribution of the components. The calculations presented in the manuscript describe the situation where the term related to the entropy dominates and favors the uniform distribution of the components. It is assumed that the macromolecules diffuse until the concentration of the macromolecules on the vesicle is free of large concentration gradients. In the mathematical modeling, we assume that the concentration of the macromolecules tends to uniform distribution. Our main goal is to examine a general phenomenon of diffusion of macromolecules on a fluid membrane and in particular examining how diffusing macromolecules can influence the shape of the membrane and the shape of the membrane can influence the process of diffusion. We assume that the time scale of the diffusion is much larger than the time scale to reach the mechanical equilibrium for given distribution of macromolecules. Such assumption is consistent with recent reported experiments on diffusion of polymers on lipid vesicles. The studied phenomenon is nontrivial because the shape and diffusion are coupled in a complex way. In the proposed model, the macromolecules may change the shape of the surface when the distribution of the macromolecules is altered during the diffusion process. The macromolecules continue to diffuse on a membrane with a changed shape. Since the diffusion of the macromolecules on a lipid membrane is modeled by solving the diffusion equation on a membrane surface, not only is the shape of the membrane adjusted to the distribution of the molecules, but the diffusion is also modified by continuous change of the membrane shape. Such mutual dependence of the concentration of the macromolecules and the shape of the membrane is studied here for the first time. In similar work, the process of diffusion is modeled...
in such a way that only the shape of the membranes depends on the concentration of macromolecules but the change of the concentration profile of the macromolecules does not depend on the shape of the lipid membrane.\(^2\) The coupling of the concentration of membrane components with its shape may be used in biological systems to modify behavior of biological cells. For example, by slowing down the diffusion process or forming long lasting concentration gradients. For example, by slowing down the diffusion process or forming long lasting concentration gradients.

In the mathematical model, the shape of the vesicles is governed by the bending energy and the diffusion is described by the diffusion equation. The bending energy depends on the distribution of the macromolecules and is the sum of the integrals of the mean curvature and the Gaussian curvature over the surface of the vesicle.\(^2\)–\(^4\)

\[
\mathcal{F} = \frac{\kappa}{2} \int_S \left( \kappa \mathcal{C}_{1} + \kappa \mathcal{C}_{2} - 2\kappa_0 \right)^2 + \pi \int_S \mathcal{C}_{1} \mathcal{C}_{2}
\]

where \(\kappa\) and \(\kappa_0\) are the bending and Gaussian rigidity, \(\mathcal{C}_{1}\) and \(\mathcal{C}_{2}\) are the principal curvatures, \(\kappa_0\) is the spontaneous curvature, and the integral (1) is taken over the surface of a closed vesicle, \(S\). The first integral describes elastic energy of the membrane. The second integral describes topological changes. If the topology does not change, the second integral is constant according to the Gauss–Bonnet theorem. In general, if the Gaussian rigidity depends on the concentration of the macromolecules, then the Gaussian curvature cannot be neglected. It is assumed that the Gaussian rigidity and bending rigidity do not depend on the concentration; therefore, there are no boundary terms and the integral from the Gaussian curvature contributes a constant. In the future, the model can be easily extended and the influence of the concentration dependent Gaussian rigidity can be studied. The macromolecules induce the spontaneous curvature on the membrane, and it is assumed that the concentration of the macromolecules is coupled to the spontaneous curvature. Thus, the spontaneous curvature is the function of the local concentration of the macromolecules.

We restrict our attentions to axisymmetric vesicles with up and down symmetry and assume that symmetry is conserved during the process. The up–down symmetry is just used to make the numerical calculation as fast as possible and as accurate as possible. It is of course somewhat artificial, but the qualitative behavior for the diffusion of the macromolecules on vesicles with and without up–down symmetry should be the same. The shape profile is parametrized with the angle, \(\theta\), of the tangent to the profile with the symmetry plane, as a function of the area of the vesicle from the north pole of the vesicle to a given point on the shape profile, \(a\). It is schematically shown in Figure 1. The Cartesian coordinates \((x, y, z)\) of a point on the surface of the vesicle embedded in 3D Euclidean space are described by the following equations:

\[
x(a, \psi) = r(a) \cos(\psi) \\
y(a, \psi) = r(a) \sin(\psi) \\
z(a) = \frac{1}{2\pi} \int_0^a da' \sin(\theta(a')) r(a')
\]

where \(\psi\) is the rotation angle and \(r(a)\) is the distance between the point and the symmetry axis. This term is given by the following equation:

\[
r(a) = \sqrt{\int_0^a da' \cos(\theta(a')) / \pi}
\]

The bending energy for the shape eqs 2–4 is given by the following functional:

\[
F[\theta(a)] = \frac{\kappa}{2} \int_0^a \frac{\sin(\theta(a))}{d\theta} + 2\pi r(a) \frac{d\theta(a)}{da} - C_0(a) \right)^2
\]

where \(C_0(a)\) is the local spontaneous curvature and \(S\) is the total surface area of the vesicle. The derivation of the expressions for the principal curvatures is given in the Appendix. The spontaneous curvature is coupled to the concentration of the macromolecules \(\phi(a)\):

\[
C_0(a) = \phi(a) C_0
\]

where \(C_0\) is the spontaneous curvature induced by the macromolecules. In the mesoscopic description, the properties of the macromolecules are reflected in the value of the spontaneous curvature. In the mathematical model studied here, the detailed knowledge of the microscopic structure of the macromolecules and the bilayer is not necessary. In particular, it is irrelevant whether the macromolecules are embedded in the bilayer or they are attached to the one side of the bilayer. In the model, it is only considered how strong is the influence of the macromolecules on the spontaneous curvature.

We assume that the evolution of the concentration profile \(\phi(a, \tau)\) in time is governed by the diffusion equation:

\[
\frac{\partial}{\partial \tau} \phi = D \nabla^2 \phi
\]

where \(\nabla^2\) is surface Laplacian operator, \(D\) is the surface diffusion coefficient, and \(\tau\) is time. On the small time scale, the fluctuations of the membrane influence the diffusion coefficients for the membrane constituents which diffuse on its surface.\(^29\)–\(^30\) The curvature of the membrane may also influence the diffusion coefficient.\(^31\)–\(^33\) We assume that for the physical processes studied here \(D\) is independent of \(C_0\). In the case of the parametrization used for the shape profile given by eqs 2–4, the diffusion equation has the following form:

\[
\frac{\partial}{\partial \tau} \phi(a, \tau) = D 4 \pi \frac{\partial}{\partial a} \left( \frac{\pi^2(a) \partial}{\partial a} \phi(a, \tau) \right)
\]

The derivation of eq 9 is given in the Appendix. The symmetry and the physical situation which is studied imposes no flux.

---

boundary conditions at the poles of the vesicle and at the equator of the vesicle. The no flux boundary condition at the poles of vesicles comes from the fact that it is assumed that the macromolecules cannot be detach from the membrane and cannot be adsorbed on the membrane. So, the macromolecules cannot go beyond the poles of the vesicle. Of course, this is some model situation. The no flux boundary condition at the equator of the vesicle results from the symmetry of the problem. The diffusion equation (eq 9) is solved numerically for each shape profile calculated by minimization of the functional (eq 6). The total concentration of macromolecules \( \phi_{\text{tot}} \) is conserved during the diffusion process. \( \phi_{\text{tot}} \) can be easily calculated from the distribution of the macromolecules:

\[
\phi_{\text{tot}} = \int_0^S \, da \, \phi(a, \tau)/S
\]  

(10)

The experimental conditions, we would like to mimic, are such that the surface area \( S \) and the volume \( V \) of the vesicle are constant. The volume is calculated from the following formula:

\[
V = \frac{1}{2} \int_0^S \, da \, r'(a) \sin \theta(a)
\]  

(11)

The radius, \( R_0 \), and the volume, \( V_0 \), of the sphere which has the same surface area as the studied vesicle are chosen as the units of length and volume respectively. \( R_0 \) and \( V_0 \) are used to define the reduced volume and dimensionless spontaneous curvature:

\[
\nu = \frac{V}{V_0}
\]  

(12)

\[
c_0 = \frac{c_0}{R_0}
\]  

(13)

The time is reported in units defined by \( t = \tau DR^2 \). The control parameters for the studied process are the reduced volume, \( \nu \), the total concentration, \( \phi_{\text{tot}} \), and the spontaneous curvature induced by the macromolecules \( c_0 \). \( c_0 = 4 \) was chosen for all calculations performed in this article. If \( c_0 \) is small, the macromolecules have little influence on the shape profile. Therefore, the diffusion would not be interesting. Higher values of \( c_0 \) may lead to systems with many metastable states. If the shapes of vesicles for these metastable states are significantly different, then the studied model may take into account accurately the transition between such states.

The functional (eq 6) is minimized numerically. The function \( \theta(a) \) is expressed as a Fourier series:

\[
\theta(a) = \theta_0 \frac{2a}{S} + \sum_{i=1}^{N} b_i \sin \left( \frac{2\pi}{S} i a \right)
\]  

(14)

where \( N \) is the number of the Fourier modes, and \( b_i \) are the Fourier amplitudes. \( S \) is the surface area of the vesicle. When the function \( \theta(a) \), in the form of the Fourier series given by eq 14, is inserted into eqs 4–6, the functional minimization can be replaced by the minimization of the function of many variables. The functional (eq 6) becomes a function of the amplitudes \( b_i \). The minimization is performed for constant surface area \( S \) and under the constraint of constant volume \( V \). The constraint on the volume of the vesicle is implemented by means of the Lagrange multipliers. Numerical minimization of the bending energy is time-consuming. The accuracy of the calculation depends on the number of Fourier amplitudes used to parametrizes the shape profile. The evolution of the concentration profile depends on the shape profile. The shape profile may differ a little for the calculations performed for different numbers of Fourier amplitudes. The calculations presented in this article are performed for 80 Fourier amplitudes.

### Results and Discussion

The process of diffusion of macromolecules on the vesicles which is studied in this article mimics some nonequilibrium physical phenomenon. It is modeled by a series of mechanical equilibrium states. Each state of mechanical equilibrium is obtained by minimization of the bending energy (eq 6) for the concentration profile calculated from the diffusion equation (eq 9).

We study vesicles of different geometry (prolate, oblate) with different reduced volume and with the same surface area. We start from a given distribution of the macromolecules on the surface of the vesicle. It is assumed that in equilibrium the entropy of the system is maximized, which leads to the uniform distribution of the macromolecules for \( t \to \infty \). It is assumed that initially macromolecules form a circular domains on the north and south pole of the vesicle (Figure 2b and c) or a ring domain around the equator of the vesicle (Figure 2a). The diffusion of the macromolecules from the domains is studied until the concentration of the macromolecules is uniform on the surface of the vesicle. The initial distribution of the macromolecules for the ringlike domains located at the equator of the vesicle is set up by the following equation:

\[
\phi^{(c)}(a, t = 0; a_0; \xi) = \frac{1}{2}(\tanh(\xi(a - a_0)) + 1)
\]  

(15)

where the parameter \( a_0 \) determines the domain size and \( \xi \) determines how smooth is the concentration profile at the domain boundary. For the initial distribution of the macromolecules in the circular domains located on the poles, the following equation is used:

\[
\phi^{(c)}(a, t = 0; a_0; \xi) = \frac{1}{2}(\tanh(\xi(a - a_0 - S/2 + 6)) + 1)
\]  

(16)

where \( S \) is the total surface area of the whole vesicle. Equations 15 and 16 are constructed in such a way to guarantee that the amount of the macromolecules is the same for domains located at the equator and at the poles of the vesicle if the parameters \( \xi \) and \( a_0 \) are chosen appropriately. For example, with the choice of \( a_0 = 2 \) in eq 15 and \( a_0 = 4 \) in eq 16, the two domains contain the same amount of the macromolecules.

The evolution of the concentration of the macromolecules and the shape profile depends on the initial distribution of the macromolecules given by \( \phi^{(c)}(a, t = 0; a_0; \xi) \) or \( \phi^{(c)}(a, t = 0; a_0; \xi) \). The initial concentration profiles were set up with the

![Figure 2. Schematic illustration of different domains on vesicles. The dark gray color denotes the domains of macromolecules on the vesicle: (a) ring domain; (b) and (c) circular domains.](image-url)
described by the concentration profiles $\phi / \text{ksi}$; a macromolecules was chosen as column (c, g, e, and i). The profiles in the second row (b, c, d, and e) are for distribution of the macromolecules calculated at $t$ in the third row (f, g, h, and i) are for distribution of the macromolecules calculated at $t$.

The parameter $\phi$ is the volume of the vesicle is $V$.) The concentration profiles denoted by the dashed lines correspond to the shape $\phi_a$ which determines the total concentration of the macromolecules is the same for $\phi_a(t, \alpha_0 = 2; \xi = 50)$, $\phi^{(f)}(a, t; \alpha_0 = 4; \xi = 50)$. The total concentration of the macromolecules is $\phi^{(g)}(a, t; \alpha_0 = 4; \xi = 50)$ and for $\phi^{(h)}(a, t; \alpha_0 = 0; \xi = 50)$. When the macromolecules are distributed uniformly over the whole surface of the vesicle, the distribution profiles are given by $\phi(a, t = \infty) = 0.363 = \phi^{(j)}(a, t = \infty) = 0.682 = \phi^{(k)}(a, t = \infty)$. The calculations were performed in the following way: first, for the initial distribution of the macromolecules, $\phi(a, t = 0; \alpha_0; \xi)$, the shape of the vesicles was obtained by minimizing the bending energy functional (eq 6). Next, for the previously found shape of the vesicle, the diffusion equation was solved at the time $t_{i+1} = t_i + dt$, where $dt = 0.01$. The time step $dt$ has to be chosen in such a way to allow for adjustment of the shape to the distribution of the macromolecules which results from the solution of the diffusion equation (eq 9) on the surface of the vesicle. The new distribution of the macromolecules obtained from the diffusion equation (eq 9) was used in minimization of the bending energy functional (eq 6). The procedure was repeated until the macromolecules were distributed uniformly on the surface of the vesicle. We are quite convinced that a few cycles of solving parameter $\xi = 50$, which gives sharp domain boundary. The parameter $\alpha_0$ which determines the total concentration of the macromolecules was chosen as $\alpha_0 = 4$ and $\alpha_0 = 2$. It is assumed that the macromolecules may be deposited on the surface of the vesicle and soon after that they can form well-defined domains. It may be achieved by injecting the macromolecules near the vesicle and soon after that they can form well-defined domains. It is assumed that the macromolecules may be deposited on the surface of the vesicle and soon after that they can form well-defined domains. It is assumed that the macromolecules may be deposited on the surface of the vesicle and soon after that they can form well-defined domains.

Figure 3. Shape profiles for the oblate vesicles at the reduced volume $v = 0.6$ for different distribution of the macromolecules. The profile without the macromolecules was chosen as $c_0 = 0$ and in the first row (a). The profiles with the macromolecules distributed uniformly with the spontaneous curvature $c_{(0)} = c_0 \phi_{(0)}(a)$ are in the fourth row. The spontaneous curvature induced by the macromolecules is $c_0 = 4$. The total concentration of macromolecules for vesicles in the first and second column is $\phi^{(1)} = 0.682$ (b, c, f, and g) and in the third and fourth column is $\phi^{(2)} = 0.363$ (d, e, h, and i). The shape profiles for the vesicles with macromolecules distributed in ring domains are in the first and third column (b, d, and h). The shape profiles in Figure 3: (a) Figure 3b and f (first column); (b) Figure 3c and g (second column); (c) Figure 3d and h (third column); (d) Figure 3e and i (fourth column). Smoother concentration profiles are obtained at a later time.

Figure 4. Distribution of the macromolecules, $\phi(a, t)$, after 1, 2, 3, 4, 5, 7, 9, 13, 21, and 51 time steps. The time step is $dt = 0.01$. The reduced volume of the vesicle is $v = 0.6$. The spontaneous curvature induced by the macromolecules is $c_0 = 4.0$. The total concentration of macromolecules is $\phi^{(2)} = 0.682$ for parts a and b and $\phi^{(1)} = 0.363$ for parts c and d. The concentration profiles for the ring domains are in the left column (a and c), and those for circular domains are in the right column (b and d). The concentration profiles denoted by the dashed lines correspond to the shape profiles in Figure 3: (a) Figure 3b and f (first column); (b) Figure 3c and g (second column); (c) Figure 3d and h (third column); (d) Figure 3e and f (fourth column). Smoother concentration profiles are obtained at a later time.
the diffusion equation and minimization of the bending energy are sufficient to obtain realistic shape of the vesicle and concentration profile.

In the calculations at each time step, the bending energy functional is minimized for a given concentration profile of the macromolecules. Thus, the shape of vesicles are in mechanical equilibrium for a given set of constraints. The most important constraint is related to the shape of the concentration profile obtained from the solution of the diffusion equation. If the constraint on the concentration profile is relaxed, then the solution becomes unstable. However, the shape profiles presented in the manuscript can be observed in experiments as transient states.

Oblate Vesicles. We have examined how the size and the shape of the initial domains of the macromolecules attached to the vesicle determines their diffusion. The macromolecules were initially placed in ring and circular domains of different size. The evolution of the shape profiles for oblate vesicles with the reduced volume \( v = 0.6 \) is shown in Figure 3. Figure 3a (first row) shows the profile for the vesicle without the macromolecules, \( \phi(a, t) = 0 \), with the spontaneous curvature \( c_0 = 0 \). The profiles with the macromolecules located in ring domains at the equator of the vesicles are shown in Figure 3b and f (first column) and Figure 3d and h (third column). The profiles with the macromolecules located in circular domains at the poles of the vesicles are shown in Figure 3c and g (second column) and Figure 3e and i (fourth column). The size of the domains and therefore the total amount of the macromolecules on the vesicle is \( \phi_{\text{tot}}^{(2)} \) for two columns on the left and \( \phi_{\text{tot}}^{(1)} \) for two columns on the right. Figure 4a–d illustrates the evolution of the concentration profiles, \( \phi(a, t) \), for the configurations shown in Figure 3b, f, and j (first column), Figure 3c, g, and j (second column), Figure 3d, h, and k (third column), and Figure 3e, i, and k (fourth column), respectively. The shape profiles in Figure 3j and k (fourth row) are calculated for the uniform distribution of the macromolecules with \( \phi(a, t = \infty) = \phi_{\text{tot}}^{(2)} \) and \( \phi(a, t = \infty) = \phi_{\text{tot}}^{(1)} \), respectively. The effective spontaneous curvature which is induced by uniformly distributed macromolecules is \( c_0^{(2)} = c_0\phi_{\text{tot}}^{(2)} \) and \( c_0^{(1)} = c_0\phi_{\text{tot}}^{(1)} \), respectively, where \( c_0 = 4 \). From Figure 3b–e (second row), it can be inferred that the deformation of the oblate vesicle caused by the macromolecules attached to the membrane depends on the size and location of the domains of macromolecules. The initial deformation of the vesicle shape can be regarded as the deformation soon after the macromolecules begin to interact with the lipid vesicle. It very quickly disappears when the macromolecules diffuse on the membrane. It can be seen in Figure 3f, g, h, and i (third row), where the shape profiles,
calculated at time $t = 5\tau$, are similar despite quite different concentration profiles, $\phi(a, t)$, shown in Figure 4. The concentration profiles marked by the dashed lines in Figure 4 correspond to the shape profiles in Figure 3.

Figure 7. Distribution of the macromolecules $\phi(a, t)$ after 1, 2, 3, 4, 5, 7, 9, 13, 21, and 51 time steps for oblate vesicles. The time step $\tau = 0.01$. The reduced volume of the vesicle is $v = 0.8$. The spontaneous curvature induced by the macromolecules is $c_0 = 4.0$. In the first row are the concentration profiles for the total concentration of macromolecules $\phi_{\text{tot}}^{(2)} = 0.682$ (a), and in the second row are the concentration profiles for the total concentration of macromolecules $\phi_{\text{tot}}^{(1)} = 0.363$ (b and c). The concentration profiles for the macromolecules located in the ring domains are in the first column (a and b), and the concentration profiles for the macromolecules located in the circular domains are in the second column (c). The concentration profiles denoted by dashed lines correspond to the shape profiles in Figure 6: (a) Figure 6b and e (first column); (b) Figure 6c and f (second column); (c) Figure 6d and g (third column). Smoother concentration profiles are obtained at later time.

Figure 8. Value of the concentration of macromolecules at the poles $\phi(a = 0, t)$ and at the equator $\phi(a = S/2, t)$ for the oblate vesicles. The reduced volume of the vesicle is $v = 0.8$. The spontaneous curvature induced by the macromolecules is $c_0 = 4.0$. The total concentration of macromolecules is $\phi_{\text{tot}}^{(2)} = 0.682$ for part a (first row) and $\phi_{\text{tot}}^{(1)} = 0.363$ for parts b and c (second row). The concentration for the macromolecules located in the ring domains are in the first column (a and b), and the concentration for the macromolecules located in the circular domains are in the second column (c).
The concentration of the macromolecules is \( \phi \). The initial distribution with large energy (eq 6) can be minimized for ring configurations both for \( h, j, l, n, p, s, \) and \( v \) and macromolecules at the poles, also the plots which illustrate the change of concentration of the concentration profiles at different time steps. Therefore, we present the distribution of the macromolecules presented as a set of the macromolecules calculated at \( t = 1dt \) (second row) for \( d, f, \) \( h, j, l, n, p, s, \) and \( v \) and \( \phi_{tot}^{(i)} \) for \( e, g, i, k, m, o, r, t, u, \) and \( w \). The profiles in the second row (\( d-i \)) are for the distribution of the macromolecules calculated at \( t = 1dt \), and those in the third row (\( j-o \)) are for the distribution of the macromolecules calculated at \( t = 21dt, dt = 0.01 \). The profiles in the fourth row (\( p-w \)) are for uniform distribution of the macromolecules.

It may be inconvenient to analyze the evolution of the distribution of the macromolecules presented as a set of concentration profiles at different time steps. Therefore, we present also the plots which illustrate the change of concentration of the macromolecules at the poles, \( \phi(a = 0, i) \), and the equator, \( \phi(a = S/2, i) \), of the vesicle; see Figure 5. These plots demonstrate that it takes comparable amount of time, for the studied vesicles with \( \nu = 0.6 \), to reach the state where the macromolecules are uniformly distributed on the whole vesicle. The macromolecules are uniformly distributed when the concentration at the poles is equal to the concentration at the equator.

The reduced volume is an important factor determining the shape of the vesicle. The larger the reduced volume the more spherical is the vesicle. In order to examine the influence of the reduced volume on the diffusion process, similar calculations have been performed for oblate vesicles with \( \nu = 0.8 \). The minimization of the bending energy (eq 6) for oblate vesicles with the reduced volume \( \nu = 0.8 \) and the initial distribution of the macromolecules in the circular domains gives a stable configuration for the distribution with the smaller amount of the macromolecules, \( \phi_{tot}^{(1)} \). For larger amount of the macromolecules, \( \phi_{tot}^{(2)} \), the stable configuration is a prolate vesicle. The bending energy (eq 6) can be minimized for ring configurations both for the initial distribution with large \( \phi_{tot}^{(2)} \) and small \( \phi_{tot}^{(1)} \) amount of the macromolecules. Figure 6b, e, and h (first column) shows the evolution of the profiles for larger amount, \( \phi_{tot}^{(2)} \), of macromolecules for ring domains. Figure 6c, f, and i and Figure 6d, g, and i (second and third column) show the configuration for the smaller amount, \( \phi_{tot}^{(1)} \), of macromolecules for ring and circular domains respectively. The evolution of the shape profile, Figure 6, and the concentration profile, Figure 7, for oblate vesicles with the reduced volume \( \nu = 0.8 \) is very similar to the evolution of the shape, Figure 3, and the concentration, Figure 4, for \( \nu = 0.6 \). The shape of the vesicles becomes quickly almost identical, despite the fact that they are minimized for quite different concentration profiles. It is interesting that even for macromolecules distributed initially in ring and circular domains the shape of the vesicles does not differ noticeably, as shown in Figure 6c–g (third row). The time needed to reach the uniform distribution of macromolecules for the configuration with the reduced volume \( \nu = 0.8 \) and for the configurations with \( \nu = 0.6 \) is almost the same; compare Figures 5 and 8. It may be expected that for the same surface area of oblate vesicles the diffusion should proceed in a similar way. However, the geometry of the vesicle may make the difference. The differences of shapes for the studied oblate vesicles are not big enough to change the process of diffusion of the macromolecules in such a way to make difference in the time to reach the uniform distribution.

For the studied examples of oblate vesicles, their shape without the macromolecules and with the uniform distribution of the macromolecules with the total concentration \( \phi_{tot}^{(1)} \) and \( \phi_{tot}^{(2)} \) is almost the same—compare the configurations in the first and the last row in Figures 3 and 6. The effective spontaneous curvature for the uniform distribution is \( c_{0}^{(1)} = 2c_{0}\phi_{tot}^{(1)} \) or \( c_{0}^{(2)} = 2c_{0}\phi_{tot}^{(2)} \), where the spontaneous curvature is \( c_{0} = 4 \). The vesicle is deformed when the domain of macromolecules with the spontaneous curvature is created; see the configurations in the second row in Figures 3 and 6. The shape of the initial deformation depends on the size and the location of the domain.

Since the shapes of the vesicles without the macromolecules and with the uniform distribution are similar, the initial shape deformation does not propagate but decays during the diffusion process. The deformation disappears very quickly for the studied oblate vesicles. The size and the location of the initial domain weakly influence the time in which the deformation disappears. It is quite surprising that the shape of the vesicles becomes so quickly similar to the shape of the vesicles without the macromolecules or with uniform distribution of the macromolecules, despite still nonuniform concentration profiles. Thus, one can obtain only a very limited knowledge about the distribution of the macromolecules from the shape of the vesicles.

**Prolate Vesicles.** The diffusion process on prolate vesicles is much more interesting than the diffusion on oblate vesicles. Prolate vesicles exist for a wide range of the reduced volume. Moreover, the shapes of prolate vesicles differ significantly for different values of the reduced volume. We have examined how the initial size of the domains determines the diffusion of the macromolecules on vesicles with different reduced volume and the same surface area. For each vesicle, we have studied the diffusion of macromolecules from domains of two sizes. The shape profiles shown in Figure 9a–c (first row) are examples of prolate vesicles without the macromolecules, calculated for the spontaneous curvature \( c_{0} = 0 \) and the reduced volume \( \nu = 0.4, 0.6, \) and \( 0.8 \), respectively. The attachment of macromolecules to the poles of the vesicles changes their shape, as can be seen in Figure 9d–i (second row). The shape deformation induced by the macromolecules is observed over much longer period of time for prolate vesicles than for oblate ones. Figure 9j–o (third row) shows the shape profiles after 21 time steps \( dt = 0.01 \). In general, the uniform distribution of macromolecules is also

---

reached after much longer time for prolate than for oblate vesicles. The shape profiles with the uniform distribution of macromolecules are presented in Figure 9p and r (fourth row).

The time which is necessary to reach the uniform distribution of the macromolecules on prolate vesicles can be read off from Figure 10. This is the time when the concentration of the macromolecules at the poles is the same as the concentration at the equator. The evolution of the shape and concentration for prolate vesicle with the reduced volume \( V = 0.8 \) is similar to the evolution for oblate vesicles. The changes of the shape are not pronounced, but the geometry of the prolate vesicle makes the diffusion slower and the time to reach the uniform distribution of macromolecules longer, compare Figure 10e and f and Figure 8. Much more interesting is the diffusion on prolate vesicles for the reduced volume \( V = 0.4 \) and 0.6. The time to reach the uniform distribution is significantly larger than the time for the oblate vesicles and even for prolate vesicles with \( V = 0.8 \). Figure 10a and b (first row) and Figure 10c and d (second row) show the evolution of the concentration of macromolecules at the poles and at the equator on prolate vesicles with the reduced volume \( V = 0.4 \) and 0.6, respectively. For prolate vesicles with \( V = 0.4 \), the time to reach the uniform distribution is similar for the diffusion from domains with different amount of macromolecules; see Figure 10a and b. For prolate vesicles with \( V = 0.6 \), the time to reach the uniform distribution is larger for the diffusion of macromolecules from the bigger domain; compare Figure 10c and d.

When the prolate vesicle is approximately cylindrical, the smaller the radius of the cylinder the slower is the diffusion. It can be observed by analyzing the evolution of the concentration at the poles and at the equator in Figure 10a, c, and e (first column) or the concentration profiles, shown in Figure 11a, c, and e (first column). The shape profiles at a few time steps for the vesicle studied in Figure 10a are shown in Figure 9d, j, and p (first column); for Figure 10c, they are shown in Figure 9f, l, and s (third column); and for Figure 10e, they are shown in Figure 9h, n, and v (fifth column). The time to reach the uniform distribution of the macromolecules doubles when the reduced volume is increased from \( V = 0.4 \) to \( V = 0.6 \) and from \( V = 0.6 \) to \( V = 0.8 \); compare Figure 10a, c, and e.

![Figure 10](image-url). Value of the concentration of macromolecules at the poles \( \phi(a = 0, t) \) (solid line) and at the equator \( \phi(a = S/2, t) \) (dashed line) of the prolate vesicles. The reduced volume of the vesicle is (a and b) \( V = 0.4 \) (first row); (c and d) \( V = 0.6 \) (second row); (e and f) \( V = 0.8 \) (third row); the spontaneous curvature induced by the macromolecules is \( c_0 = 4.0 \). In the first column, the total concentration of the macromolecules is \( \phi^{(1)}_{\text{tot}} = 0.363 \) (a, c, and e) and in the second column is \( \phi^{(2)}_{\text{tot}} = 0.682 \) (b, d, and f).
The diffusion on prolate vesicles with the reduced volume \( V = 0.6 \) is qualitatively different than the diffusion in all other cases studied here. The initial configurations for the domains with small, Figure 9f, and large, Figure 9g, amount of macromolecules differ significantly. The presence of large amount of macromolecules causes budding of the vesicle.\(^{38-40,13}\) The evolution of the shape profile due to diffusion for the vesicle with initial configuration presented in Figure 9g leads to the metastable configuration shown in Figure 9t. For prolate vesicles with high spontaneous curvature, there may exist many stable solutions for the same value of the reduced volume.\(^{34,35}\) Figure 9t and u shows two stable prolate vesicle with the same uniform distribution of the macromolecules. The vesicle in Figure 9t is metastable, but since the initial configuration for the diffusion was similar to the metastable configuration, the diffusion process led to this one rather than to the stable one. Thus, such mechanism may be used for preparation of vesicles in a metastable state.

The formation of a bud on the vesicle results in an increase of the time to reach the uniform distribution. This is shown in Figure 10d, which presents the change in time the concentration of the macromolecules at the poles and at the equator. Slowing down of the diffusion is caused by small neck of the bud, which acts as an obstacle for the diffusing macromolecules. The neck of the bud divides the vesicle into two regions. The concentration of the macromolecules in each region becomes very quickly approximately uniform as can be observed on concentration profiles \( \phi(a, t) \) in Figure 11d. This is possible since the diffusion is much faster in each region than the diffusion through the neck. Small necks do not stop the diffusion and the uniform distribution is always reached, but it takes the more time the more narrow are the necks. The size of the bud

Figure 11. Distribution of the macromolecules \( \phi(t, a) \) after 1, 11, 21, 31, 41, 51, and 200 time steps for prolate vesicles. The time step \( dt = 0.01 \). The reduced volume of the vesicle is (a and b) \( V = 0.4 \) (first row); (c and d) \( V = 0.6 \) (second row); (e and f) \( V = 0.8 \) (third row). The spontaneous curvature induced by the macromolecules is \( c_0 = 4.0 \). In the first column, the total concentration of macromolecules is \( \phi_{tot}^{(1)} = 0.363 \) (a, c, and e), and that in the second column \( \phi_{tot}^{(2)} = 0.682 \) (b, d, and f). The concentration profiles denoted by the dashed lines correspond to the shape profiles in Figure 9: (a) Figure 9d and j (first column); (b) Figure 9e and k (second column); (c) Figure 9f and l (third column); (d) Figure 9g and m (fourth column); (e) Figure 9h and n (fifth column); (f) Figure 9i and o (sixth column). Smoother concentration profiles are obtained at later time.

\(^{38}\) Lipowsky, R. J. Phys. II Fr. 1992, 2, 1825–1840.
\(^{39}\) Lipowsky, R. Biophys. J. 1993, 64, 1133–1138.
increases slightly when the concentration of the macromolecules in the bud decreases. This can be understood since the effective spontaneous curvature decreases in time due to decreasing concentration of the macromolecules on the surface of the bud.

The diffusion on prolate vesicles is nontrivial due to existence of narrow necks. The necks can be wide like the ones in Figure 9d–f or quite narrow like the one in Figure 9g. When the macromolecules diffuse on the prolate vesicles with necks, the diffusion process slows down significantly if the necks are narrow. By increasing or decreasing the neck radius, it is possible to speed up or slow down the diffusion of the macromolecules from one part of the vesicle to the other.

It has to be noted that in all the cases presented here the equilibrium shape of the vesicles are consistent with former calculations of vesicle shapes with uniform composition. The diffusion usually leads to the stable shapes but it is also possible that a metastable shape is obtained as it has been shown for the prolate vesicle with the reduced volume $v = 0.6$.

**Summary and Conclusions**

The process of diffusion of macromolecules on the surface of the vesicle has been investigated. The diffusion of macromolecules on lipid membranes is an important phenomenon present in biological systems. This phenomenon is difficult to study, because the macromolecules can deform the lipid membranes while they diffuse. We have examined how nonuniform distribution of the macromolecules is reflected in the shape of the vesicles. It has been observed that the shape deformation caused by the macromolecules disappears after longer time for prolate than for oblate vesicles. Creation of a bud due to the attached macromolecules changes the diffusion process significantly. The bud is separated from the vesicle by small necks. We can speculate that the phenomena described in this paper can be used in many processes in biological systems. We hope that this work will help in analysis and interpretation of experimental results concerning the diffusion of macromolecules in biological systems.

**Acknowledgment**. The author would like to acknowledge the support from the Polish Ministry of Science and Higher Education, Grant No. N N204 240534. I would like to thank D. Kandel, J. Stavans, and I. Tsafir for attracting my attention to the problem of diffusion of macromolecules on lipid membranes and for many discussions on mathematical modeling of the problem.

**Appendix**

**Derivation of the Curvature Energy**

The principal curvatures can be derived from the equations describing the surface of the vesicle. In the three-dimensional Euclidean space, the vector $\mathbf{R} = \{x(\psi, a), y(\psi, a), z(\psi, a)\}$ describing the points on a surface of revolution, parametrized with the function $\theta(a)$, is given by

$$\mathbf{R} = \{\cos(\psi) r(a), \sin(\psi) r(a), z(a)\}$$

The angle of rotation $\psi$ and the area $a$ are coordinates on the surface. $x, y,$ and $z$ are coordinates in three-dimensional Euclidean space. In order to calculate the principal curvatures $\mathcal{C}_1$ and $\mathcal{C}_2$ on the surface, the metric tensor $g_{ij}$ is calculated in the following way:

$$g_{ij} = \begin{pmatrix} \frac{\partial \mathbf{R}}{\partial a} & \frac{\partial \mathbf{R}}{\partial \psi} \\ \frac{\partial \mathbf{R}}{\partial \psi} & \frac{\partial \mathbf{R}}{\partial \psi} \end{pmatrix} = \begin{pmatrix} 1/(4\pi r^2(a)) & 0 \\ 0 & r^2(a) \end{pmatrix}$$

where

$$\frac{\partial \mathbf{R}}{\partial a} = \{\cos(\psi) \cos(\theta(a))/(2\pi r(a)), \cos(\theta(a)) \sin(\psi)/(2\pi r(a))\}$$

$$\frac{\partial \mathbf{R}}{\partial \psi} = \{-\sin(\psi)/(r(a)), \cos(\psi)/(r(a))\}$$

The unit normal $\mathbf{n}$ can be calculated from

$$\mathbf{n} = (\partial \mathbf{R}/\partial \psi \times \partial \mathbf{R}/\partial a)/\sqrt{\det(g_{ij})} = \{-\sin(\psi)/(r(a)), -\cos(\psi)/(r(a)), \cos(\psi)/(r(a))\}$$

Next, $\mathbf{Y}$ and $L_{ij}$ are defined as

$$\mathbf{Y} = \begin{pmatrix} \frac{\partial \mathbf{R}}{\partial a} & \frac{\partial \mathbf{R}}{\partial \psi} \\ \frac{\partial \mathbf{R}}{\partial \psi} & \frac{\partial \mathbf{R}}{\partial \psi} \end{pmatrix}$$

$$L_{ij} = \mathbf{Y} \cdot \mathbf{n}$$

The $H_{ij}$ tensor is then

$$H_{ij} = g_{ij}^{-1} L_{ij} = \begin{pmatrix} 2\pi r(a) & 0 \\ 0 & \sin(\theta(a))/r(a) \end{pmatrix}$$

Thus, $\mathcal{C}_1$ and $\mathcal{C}_2$ are

$$\mathcal{C}_1 = 2\pi r(a) \frac{d\theta(a)}{da}$$

$$\mathcal{C}_2 = \sin(\theta(a))/r(a)$$

The bending energy $\mathcal{J}$ for the profile parametrized with the function $\theta(a)$ is given by the following formula

$$\mathcal{J}[\theta(a)] = \frac{\kappa}{2} \int_0^\psi da \left( \frac{\sin(\theta(a))}{r(a)} + 2\pi r(a) \frac{d\theta(a)}{da} - C_0(a) \right)^2$$

where $\psi$ is the angle of rotation.

**Derivation of the Diffusion Equation**

The general form of the surface Laplacian is defined by

$$\nabla_s^2 \phi = \frac{1}{\sqrt{g(a, \psi)}} \left\{ \frac{\partial}{\partial a} \left[ \frac{1}{\sqrt{g(a, \psi)}} \left( g_{\psi\psi} \frac{\partial \phi}{\partial a} - g_{\theta\theta} \frac{\partial \phi}{\partial \psi} \right) \right] + \frac{\partial}{\partial \psi} \left[ \frac{1}{\sqrt{g(a, \psi)}} \left( g_{\psi\psi} \frac{\partial \phi}{\partial \psi} - g_{\theta\theta} \frac{\partial \phi}{\partial a} \right) \right] \right\}$$

where $a$ and $\psi$ are local coordinates on the surface, $g(a, \psi)$ is the determinant of the metric tensor, and $g_{\psi\psi}, g_{\theta\theta},$ and $g_{\psi\theta}$ are the metric tensor coefficients calculated in eq 18. After substituting appropriate metric tensor coefficients to eq 28 and performing

differentiation, one obtains the diffusion equation on the curved surface:

\[
\frac{\partial}{\partial \tau} \phi(a, \tau) = 4D \pi \left( \cos(\theta(a)) \frac{\partial}{\partial a} \phi(a, \tau) + \int_0^\pi \cos(\theta(a')) da' \frac{\partial^2}{\partial a^2} \phi(a, \tau) \right)
\]

(29)

which can be transformed to a more compact form

\[
\frac{\partial}{\partial \tau} \phi(a, \tau) = D 4\pi \frac{\partial}{\partial a} \left( \left( \int_0^\pi da' \cos(\theta(a')) \right) \frac{\partial}{\partial a} \phi(a, \tau) \right)
\]

(30)

When we use the definition of \( r(a) \), eq 30 can be written even in a more compact way:

\[
\frac{\partial}{\partial \tau} \phi(a, \tau) = D 4\pi \frac{\partial}{\partial a} \left( \pi r^2(a) \frac{\partial}{\partial a} \phi(a, \tau) \right)
\]

(31)