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The effect of spontaneous curvature on a two-phase vesicle

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Abstract

Vesicles are membrane-bound structures commonly known for their roles in cellular transport and the shape of a vesicle is determined by its surrounding membrane (lipid bilayer). When the membrane is composed of different lipids, it is natural for the lipids of similar molecular structure to migrate towards one another (via spinodal decomposition), creating a multi-phase vesicle. In this article, we consider a two-phase vesicle model which is driven by nature's propensity to maintain a minimal state of elastic energy. The model assumes a continuum limit, thereby treating the membrane as a closed three-dimensional surface. The main purpose of this study is to reveal the complexity of the Helfrich two-phase vesicle model with non-zero spontaneous curvature and provide further evidence to support the relevance of spontaneous curvature as a modelling parameter.

In this paper, we illustrate the complexity of the Helfrich two-phase model by providing multiple examples of undocumented solutions and energy hysteresis. We also investigate the influence of spontaneous curvature on morphological effects and membrane phenomena such as budding and fusion.

Keywords

vesicles; membrane energy; spontaneous curvature; calculus of variation; nonlinear differential equations

1. Introduction

Vesicles are commonly known for their role as intracellular transport bodies, carrying molecules between organelles that cannot normally be absorbed through the membrane. Examples of such vesicles include cytoplasmic and synaptic vesicles. The former is involved with transporting proteins (via endocytosis and exocytosis) between organelles within a cell. The latter vesicles carry neurotransmitters into the synaptic region of nerve cells.

Synthesized vesicles, known as liposomes, have also been the focus of many pharmaceutical studies as potential drug carriers [1]. Liposomes have many qualities that make them perfect candidates for this purpose. They are non-toxic and biodegradable; they can be made into

various sizes, some small enough to fit through the tiniest of capillaries and they can be infused with homing proteins to target designated areas within the body.

Every vesicle is surrounded by a protective bio-membrane. The primary structural components of the membrane are polar molecules called lipids. In 1961, Bangham observed that when lipids are introduced to an aqueous solution, they spontaneously align themselves tail-to-tail so that only their heads are in contact with the internal and external surroundings [2]. This formation gives rise to an inner and outer lipid layer called a membrane bilayer. The matrix of lipids within a bilayer form a liquid crystal phase (an intermediate phase within the liquid and solid continuum), which endows all membrane-bound molecules with enough rigidity to withstand environmental stresses, while also adding flexibility to move throughout their surroundings. The material properties of liquid crystals permit the membrane to assume a variety of stable and complex morphologies. Moreover, significant morphological changes lead to a variety of membrane phenomena, such as budding, fusion and fingering. This is especially true for multi-phase membranes.

When a bilayer is composed of different lipids, it is natural for the lipids of similar molecular structure to migrate towards one another via spinodal decomposition and coarsening. This process produces a vesicle with distinct material phases and characterizes what is known as the strong segregation limit. Lipid phases are typically categorized as either ordered (more solid-like) or disordered (more fluid-like). Ordered lipids have more organizational structure and a higher density than their disordered counterparts. When a membrane contains both ordered and disordered lipids, the lipid phases separate creating the multi-phase vesicles.

Due to the properties of liquid crystals, there are multiple factors that influence membrane shape. In 1973, Helfrich modified the theory of liquid crystals to model two-phase vesicle membranes [3]. Helfrich reasoned that the membrane in each phase should be associated with bending stress, but not lateral shear stresses. The concept of spontaneous curvature was also incorporated into this model as the preferred curvature of a membrane induced from an asymmetry between the two layers within the bilayer.

Spontaneous curvature can arise from: (1) lipid composition; (2) the scaffolding of proteins onto the exterior monolayer; and (3) the reversible insertion of protein regions that act like wedges in membranes. Both internal and external membrane proteins are known to be related to vesicle budding and micro-domain formation [4]. Micro-domains (or rafts) are formed from the clustering of cholesterol and other molecules within the membrane. They are also thought to be thicker than the surrounding membrane. If so, monolayers should elastically deform so as to avoid exposure of hydrophobic surfaces to water at the raft boundary. The energy associated with this deformation is called line tension. It was found that this tension can be reduced by differences in spontaneous curvature between the two codomains [5].

True bio-membranes are composed of much more than lipids. They are known to contain cholesterol and proteins, as well as other components. While the intricate composition of membranes clearly influence its shape, it is not clear to what extent. A few studies have been

performed to investigate the role non-lipid molecules have on membrane curvature. It is believed that the presence of proteins in the membrane causes curvature deformations and particular budding [6,7]. In this case, proteins embedded in the membrane break the symmetry of the monolayers and a spontaneous curvature is induced. Ambient conditions such as sugar concentration in the surrounding solution are also known to alter the spontaneous curvature of a membrane [8].

There are many ways to approach bio-membrane modelling. One way is with a molecular model, which is based on the interaction between the lipids within the membrane [9, 10]. Alternatively, one may choose a macro-scale continuum model, where the membrane is treated as a surface and the lipids are globally classified [11–15]. Such a model is justified since a typical diameter of a vesicle is approximately on thousand times larger than the thickness of the membrane. Two popular, yet equivalent, continuum models are: (1) a direct mechanical approach using force and moment balancing [16–21]; (2) an energy variational approach [3, 8, 11–14, 22]. The models we consider in this paper will follow the latter format.

Jülicher [12] considered an energy analysis from the point of view of budding that was driven by a large line tension. His study showed that energy hysteresis surfaced during the early stages of budding and later when the bud approached a vanishing neck radius (fission) via increasing line tension. These results were very insightful for two-phase vesicle budding, but neglected the influence of spontaneous curvature.

For any model, it is important to verify its consistency with experimental data. Baumgart *et al* [16] provided a nice study in this direction. From photon microscopic images, they estimated the geometrical values of a number of two-phase experimental vesicles and using Jülicher's two-component bending model (without spontaneous curvature) [12], they matched the numerical solutions to their data. In this study, an inconsistency arose between experiment and numerics with respect to the geometrical behaviour in the neck region observed in experiment. This problem was addressed by the introduction of Gaussian curvature. The results from this adjustment proved to be more consistent with the vesicle shapes they observed. In a later study [23], Baumgart and Das, showed that the effect of Gaussian curvature is very similar to the effect of spontaneous curvature in one phase. Since so little is known about Gaussian curvature and it is not clear why it was employed over spontaneous curvature, however, it seems reasonable to believe that the inconsistencies in Baumgart's previous study could have also been addressed with spontaneous curvature.

The consideration and study of spontaneous curvature has been done in various forms [7, 24–31]. These studies deal with the connection between spontaneous curvature and membrane phenomena such as budding, endocytosis and tubulation. For example, Zimmerman [7], illustrates how the embedding and scaffolding of proteins in and on the membrane influences membrane curvature and budding. Farge [31], on the other hand, attempts to explain endocytosis as a result of asymmetries in the plasma membrane. He asserts that transient asymmetries in lipid concentration (non-zero spontaneous curvature) in either the inner or outer membrane monolayers may contribute to the formation of endocytic vesicles. In particular, his study found that an increase of lipid concentration in the inner

layer enhances endocytosis, whereas an increase of the lipid concentration in the outer layer inhibits endocytosis.

Most of the studies referenced above do not address the direct morphological changes caused by fluctuating spontaneous curvature nor do they give any indication of the complexity of the model. This paper seeks to address the following three aspects of Jülicher's two-phase energy model: (1) to reveal the complexity of the two-phase model posed by Jülicher for non-zero spontaneous curvature. We accomplish this by uncovering multiple examples of energy hysteresis and a vast array of undocumented solutions; (2) to further illustrate the relevance of spontaneous curvature as a modelling parameter by using our simulations to describe budding, endocytosis, fusion and tubulation (fingering); and (3) to investigate the possible deformations that occur from natural or artificial fluctuations in bilayer asymmetry.

The rest of the manuscript is organized as follows: (1) introduction of the membrane energy model, assumed geometry, and system of nonlinear differential equations to be solved; (2) explanation of the numerical methods used; (3) summary of the effect of spontaneous curvature; (4) discussion and comparison of our results with experimental data; and (5) a summary with the conclusions of our study.

2. The membrane energy model

The general membrane energy

An entire vesicle membrane, Γ , is a closed surface composed of two sub-surfaces, $\Gamma^{(1)}$ and $\Gamma^{(2)}$, joined by a space curve $\Gamma^{(1)}$ (or equivalently $\Gamma^{(2)}$). Each surface contains a different material phase ((1) and (2)). Assuming that a vesicle membrane is influenced by surface tension and bending rigidity, one can express the total energy as the sum of the following energy components:

$$E = \sum_{i=1,2} (E_S^{(i)} + E_B^{(i)}) + E_L \quad (1)$$

where $E_S^{(i)}$ and $E_B^{(i)}$ are the surface and bending energies of $\Gamma^{(i)}$, respectively, and E_L is the line energy associated with $\Gamma^{(1)}$.

The surface energy, $E_S^{(i)}$, is expressed as

$$E_S^{(i)} = \int_{\Gamma^{(i)}} \tau^{(i)} dA, \quad i=1, 2 \quad (2)$$

with corresponding surface tensions $\tau^{(1)}$ and $\tau^{(2)}$.

The bending energy, $E_B^{(i)}$, can be further decomposed into its mean bending energy, $E_M^{(i)}$ and Gaussian bending energy, $E_G^{(i)}$ as shown below

$$E_B^{(i)} = E_M^{(i)} + E_G^{(i)} = \int_{\Gamma^{(i)}} \frac{1}{2} \kappa^{(i)} \left(2H - H_0^{(i)} \right)^2 dA + \int_{\Gamma^{(i)}} \kappa_G^{(i)} K dA, \quad i=1, 2 \quad (3)$$

where H and K denote the mean and Gaussian curvatures together with mean and Gaussian bending rigidities $\kappa^{(i)}$ and $\kappa_G^{(i)}$, respectively. $H_0^{(i)}$ represents the spontaneous curvature in phase- i and will serve as the critical parameter in this article. The expression for this bending energy was introduced by Helfrich [3] and was based on the Frank–Oseen theory of liquid crystals [32, 33].

Finally, we have the line energy, E_L

$$E_L = \int_{\partial\Gamma^{(1)}} \sigma dl \quad (4)$$

where σ is the line tension on the phase boundary, $\Gamma^{(1)}$. Note that line tension represents the surface tension restricted to the spatial curve $\Gamma^{(1)}$.

To account for physical constraints, Lagrange multipliers $\Sigma^{(1)}$, $\Sigma^{(2)}$ and P are introduced. $\Sigma^{(1)}$, $\Sigma^{(2)}$ constrain the surface areas of each phase and P constrains the total volume. The physical interpretation of P is the osmotic pressure difference between the interior and exterior of the vesicle. Therefore, the energy functional takes the form

$$E = \sum_{i=1,2} \int_{\Gamma^{(i)}} \left[\tau^{(i)} + \frac{1}{2} \kappa^{(i)} \left(H - H_0^{(i)} \right)^2 + \kappa_G^{(i)} K \right] dA + \int_{\partial\Gamma^{(1)}} \sigma dl + \int_{V_T} P dV. \quad (5)$$

The stretching of lipid bilayers is limited to relatively small deformations since they begin to rupture when approximately 1% of the area is changed [34]. Therefore, the total surface area is assumed constant.

Since both the surface tensions, $\tau^{(i)}$ and the Lagrange multipliers for the surface areas, $\Sigma^{(i)}$ contribute constant values under the integral, we write $\tau^{(i)}$ to mean $\tau^{(i)} + \Sigma^{(i)}$.

Model geometry and shape equations

Recall that the total membrane is denoted Γ and is composed of two distinct phase surfaces, $\Gamma^{(1)}$ and $\Gamma^{(2)}$. The surface areas of $\Gamma^{(1)}$ and $\Gamma^{(2)}$ and the volume enclosed by Γ are given by $A^{(1)}$, $A^{(2)}$ and V , respectively. For this model, we assume axisymmetric vesicle shapes with the vertical z -axis as the axis of rotation. All variables are parametrized with respect to the arc length, s , and the total arc length is denoted s_f . The planar profile is determined by the horizontal distance from the axis of rotation, $r(s)$, the vertical height of the vesicle, $z(s)$, and the tangent angle measured counter-clockwise with the horizontal, $\psi(s)$ and are defined on $[0, s_f]$ (figure 1). Values corresponding to $s = 0$, $s = s_f$ and $s = s_b$ will be referred to as the south pole, north pole and neck respectively. Phase-(1) (blue) coincides with $s \in [0, s_b]$ and phase-(2) (red) with $s \in [s_b, s_f]$. All 2D profile curves will be denoted with a subscript of p ,

(i.e. $\Gamma_p^{(1)}$ and $\Gamma_p^{(2)}$) and phase interface values will be denoted with a b -subscript (i.e. $r_b = r(s_b)$ is the neck radius) (figure 1).

Next, we present the total energy in these axisymmetric coordinates. The axisymmetric surface area element is $dA = 2\pi r ds$ and the volume element is $dV = \pi r^2 \sin \psi ds$. The principle curvatures are given by $d\psi/ds$, $\sin(\psi)/r$ (in-plane and azimuthal curvatures respectively) and therefore, the mean and Gaussian curvatures are

$$H(s) = \frac{1}{2} \left(\frac{d\psi}{ds} + \frac{\sin\psi}{r} \right), \quad K(s) = \frac{\sin\psi}{r} \frac{d\psi}{ds} \quad (6)$$

respectively. Using these axisymmetric variables, we obtain the axisymmetric expression for the energy

$$E = \sum_{i=1,2} \left[\int_{\Gamma^{(i)}} \frac{\kappa^{(i)}}{2} (2H - H_0^{(i)})^2 dA + 2\pi \kappa_G^{(i)} \cos\psi_b + \tau^{(i)} A^{(i)} \right] + 2\pi \sigma r_b + PV \quad (7)$$

where ψ_b and r_b are the tangent angle and radius at the phase boundary. All other parameters are as defined previously.

We non-dimensionalize the model with respect to the length scale R_0 (defined by $A^{(1)} + A^{(2)} := 4\pi R_0^2$) and energy scale σ . We define the length R_0 through the relationship $A^{(1)} + A^{(2)} := 4\pi R_0^2$. We motivate the non-dimensionalization by recalling the membrane energy E in equation (5) and scaling all quantities of length by R_0 and denote non-dimensional quantities with a hat, we obtain the reduced energy

$$\frac{E}{2\pi \sigma R_0} = \sum_{i=1,2} \int_{\Gamma^{(i)}} \hat{\kappa}^{(i)} \left(2\hat{H} - \hat{H}_0^{(i)} \right)^2 dx^{(i)} + 2\hat{\tau}^{(i)} x^{(i)} + \hat{r}_b + \hat{\kappa}_G \cos\psi_b + \frac{2}{3} \hat{P} v$$

where area and volume are now of the fractional form $x^{(i)} = A^{(i)} / 4\pi R_0^2$, $v = V / (4\pi R_0^3 / 3)$. We now update the system and boundary conditions while also suppressing the hats. The bulk phase-(i) is

$$\dot{Q} = -\kappa^{(i)} (2H - H_0^{(i)}) \left[2 \left(H - \frac{\sin\psi}{r} \right)^2 + H H_0^{(i)} \right] - Q \frac{\cos\psi}{r} + 2H \tau^{(i)} + P \quad (8)$$

$$\dot{H} = \frac{Q}{2\kappa^{(i)}} \quad (9)$$

$$\dot{\psi} = 2H - \frac{\sin\psi}{r} \quad (10)$$

$$\dot{r} = \cos\psi \quad (11)$$

$$\dot{z} = \sin\psi \quad (12)$$

$$\dot{\tau}^{(i)} = 0 \quad (13)$$

and the north and south pole boundary conditions

$$Q(0) = \psi(0) = r(0) = z(0) = s(0) = 0, \quad Q(s_f) = r(s_f) = 0, \quad \psi(s_f) = \pi \quad (14)$$

and matching conditions at the phase interface

$$\frac{\sin\psi_b}{r_b} = 2\kappa^{(2)} (H_b')^{(2)} - 2\kappa^{(1)} (H_b')^{(1)} \quad (15)$$

$$\begin{aligned} \kappa_G \frac{\sin\psi_b}{r_b} = & \kappa^{(1)} (2H_b^{(1)} - H_0^{(1)}) - \kappa^{(2)} (2H_b^{(2)} - H_0^{(2)}) \\ & - \frac{\cos\psi_b}{r_b} + \tau^{(2)} - \tau^{(1)} = \kappa^{(2)} (2H_b^{(2)} - H_0^{(2)}) \left[H_b^{(2)} - \frac{\sin\psi_b}{r_b} + \frac{H_0^{(2)}}{2} \right] \end{aligned} \quad (16)$$

$$- \kappa^{(1)} (2H_b^{(1)} - H_0^{(1)}) \left[H_b^{(1)} - \frac{\sin\psi_b}{r_b} + \frac{H_0^{(1)}}{2} \right]. \quad (17)$$

3. Methods

Equations (8)–(17) were solved using the MATLAB two-point boundary valued problem solver `bvp4c`. `bvp4c` is a finite difference code that utilizes the three-stage Lobatto IIIA collocation formula. The collocation polynomials provides a C_1 solution that is fourth-order accurate over the domain of integration.

Since our model produces a three-point BVP and `bvp4c` is a two-point BVP solver, we chose to adapt `bvp4c` into a three-point scheme by solving each set of phase equations over the common domain $[0, \pi]$. In particular, for a given area fraction, $x^{(1)}$, we determine S_b and simultaneously solve the equations of the phase-(1) over the interval $[0, S_b]$ and the equations for the phase-(2) over the interval $[S_b, \pi]$.

Singularities arise when $r = 0$, that is, at the north and south poles. Thus, when solving these ODEs, care must be taken near the poles. To account for this, we used implemented Taylor expansions near these boundaries for some of our variables.

To obtain many of the equilibrium shapes for non-zero spontaneous curvature, it was necessary to write an adaptive algorithm which had the following properties: (i) the step size could be adjusted automatically to accommodate the extreme sensitivity of parameter

changes; and (ii) spontaneous curvature could be varied in such a way that multiple equilibrium solutions could be revealed.

We accomplished the first goal of the algorithm by first computing the residual of the solution and if it exceeded a specified tolerance (usually 10^{-6}) we would decrease the step size of the parameter we were varying. To keep the algorithm efficient, step sizes would be periodically increased as long as the residual tolerance was satisfied. The second quality was ensured by approaching the same set of parameters from eight different directions (figure 1(b)).

4. Results and discussion

Vastness of solutions from non-zero spontaneous curvature

Our simulations produced equilibrium shapes that were numerical solutions to a third-order nonlinear differential equation. By the procedure described in the methods section, we varied both spontaneous curvature parameters along eight different paths. As we obtained new solutions, the algorithm was reset to vary the spontaneous curvature on these new morphologies. The algorithm was designed to back-track to previous parameter states from different directions. In some cases we received the solution originally found at that state, however, it was also common to observe new solutions for the same set of parameters. In fact, for a few cases we discovered eight different solutions (one for each variation direction). figure 2 illustrates the distribution of solutions for 50%, 60%, 75% and 90% area fractions. Each solution is represented by a circle around it corresponding to the spontaneous curvature integer node ($H_0^{(1)}, H_0^{(2)}$). For example, in the 50% area fraction plane, the spontaneous curvature pair (1, 0) is surrounded by five concentric circles and therefore has five different solutions.

The complexity and vastness of unique solutions is clearly demonstrated in figure 2, especially in the 50% area fraction case. Considering how many solutions were found for non-zero spontaneous curvature, it was interesting that we were only able to obtain the three well-known morphologies (figure 3(b) - *the 3rd and 4th figures are the same*) for the zero spontaneous curvature case. If nothing else, this shows just how mathematically rich the model can become in the presence of spontaneous curvature.

In addition, figure 2 also demonstrates: (1) that the further the area fraction deviates from 50%, the number of solutions obtained is significantly reduced; and (2) for spontaneous curvature pairs that are large and of opposite signs the number of solutions also decrease (this can be seen in the second and fourth quadrants of the $H_0^{(1)}, H_0^{(2)}$ -phase plane). In contrast, we were able to discover the majority of our solutions in a region surrounding the diagonal line, $H_0^{(1)} = H_0^{(2)}$ (i.e. when jumps in spontaneous curvatures were relatively small).

Shape change

When the area fraction, bending rigidity, and Gaussian curvature of each phase match (i.e. $x^{(1)} = 0.5$, $\kappa^{(1)} = \kappa^{(2)}$, and $\kappa_G = 0$), the model assumes a state of symmetry about the line $H_0^{(1)} = H_0^{(2)}$. Since this symmetric case yields the highest number of solutions (as seen in

figure 2), it will serve as the starting point for our study. In this setting, it can be shown that the matching conditions, equations (15)–(17), reduce to

$$\frac{\sin\psi_b}{r_b} = 2\kappa \left((H'_b)^{(2)} - (H'_b)^{(1)} \right) \quad (18)$$

$$H_0^{(2)} - H_0^{(1)} = 2 \left(H_b^{(2)} - H_b^{(1)} \right) \quad (19)$$

$$-\frac{\cos\psi_b}{r_b} = \kappa \left(2H_b^{(2)} - H_0^{(2)} \right) \left(H_0^{(2)} - H_0^{(1)} \right) - \tau^{(2)} + \tau^{(1)}. \quad (20)$$

Moreover, by the fact that $2H = \psi' + \sin(\psi)/r$ and the continuity of ψ , we also have

$$(\psi'_b)^{(2)} - (\psi'_b)^{(1)} = H_0^{(2)} - H_0^{(1)}. \quad (21)$$

Notice, the expression in equation (19) indicates that the mean curvature is proportional to the jump in spontaneous curvature between the two phases and therefore, the mean curvature is continuous everywhere if $(H_0^{(1)} = H_0^{(2)})$. This fact is true independent of the other parameter values. The direct impact of spontaneous curvature in the interface curvature can be seen in figure 3(a).

As a starting point for the investigation of shape change from fluctuating spontaneous curvature, we begin with the common prolate solution (leftmost shape in figure 3(b)) where $(H_0^{(1)}, H_0^{(2)}) = (0, 0)$ and allow the spontaneous curvature in each phase to vary in eight directions. The directions will be referenced using the following convention: 1 = west (increasing $H_0^{(1)}$, fixed $H_0^{(2)}$), 2 = east (decreasing $H_0^{(1)}$, fixed $H_0^{(2)}$), 3 = north (fixed $H_0^{(1)}$, increasing $H_0^{(2)}$), 4 = south (fixed $H_0^{(1)}$, decreasing $H_0^{(2)}$), 5 = northwest (increasing $H_0^{(1)}$, increasing $H_0^{(2)}$), 6 = southeast (decreasing $H_0^{(1)}$, decreasing $H_0^{(2)}$), 7 = southwest (increasing $H_0^{(1)}$, decreasing $H_0^{(2)}$), and 8 = northeast (decreasing $H_0^{(1)}$, increasing $H_0^{(2)}$). Due to the assumed symmetry, it is enough to consider directions 1, 2, 5, 6 and 7 since the shape change in directions 2, 3 and 8 are reflected versions of directions 1, 2 and 7 respectively.

Direction 1 in figure 4 shows that an increase in $H_0^{(1)}$ immediately results in an increase of $H_b^{(1)}$ and decrease of $H_b^{(2)}$. As $H_0^{(1)}$ is increased, the larger the jump in in-plane curvature becomes. To accommodate a fixed volume and area, the increasing jump in curvature at the neck, the negative curvature that began in at the phase interface, is forced into the bulk of the opposite phase. We also note that the morphology of the bulk region of phase-(1) (same phase) is significantly changed. As expected, the decrease of $H_0^{(1)}$ (direction 2) produces an opposite effect to direction 1 where $H_b^{(1)}$ decreases while $H_b^{(2)}$ increases and the bulk morphology of phase-(2) (opposite phase) significantly changes. However, in both

directions 1 and 2, the negative interface curvature migrates into phase-(2). The mirrored effect of directions 3 and 4 are also provided in figure 4 for the reader's observation.

Direction 5 in figure 4 corresponds to the simultaneous increase of both $H_0^{(1)}$ and $H_0^{(2)}$ in the northeast direction. Recall that when $H_0^{(1)} = H_0^{(2)}$, the boundary conditions require the continuity of the mean curvature and $\psi_b = \pi/2$. As you can see from the figure, the vesicle shape appears to take a spherical shape in each phase. In fact, we have also verified that for large (and equal) values of $H_0^{(1)}$ and $H_0^{(2)}$, the bulks of phase (1) and (2) approach constant mean curvature. The only constraint preventing full convergence is the continuity of ψ (which approaches a delta function). It turns out that these shapes are precisely the solutions of the two-phase surface energy problem (Helfrich model with $\kappa^{(1)} = \kappa^{(2)} = 0$ and no continuity of ψ).

On the other hand, for negatively decreasing $H_0^{(1)}$ and $H_0^{(2)}$ values (direction 6), another interesting shape change occurs. In this case, the vesicles appear to approach a cylindrical profile, but again full convergence of such a shape is impossible by the continuity of ψ . What makes this interesting is that cylinders are also shapes with constant mean curvature. This motivates one to consider what the asymptotic effect of large spontaneous curvature has on the Helfrich model. Perhaps it acts to suppress the bending energy in a similar way that small values of κ does. A formal study of these behaviours has not yet been performed and it is still unclear as to whether this is a generic behaviour of the model or just a consequence of this symmetric case³.

Finally, we look at direction 7 and remind the reader that direction 8 is equivalent by symmetry. Along this southeast path the $H_0^{(1)}$ and $H_0^{(2)}$ values are equal but opposite and therefore the jump in spontaneous curvature is doubled at each iteration. As a result, we found this direction to be computationally very sensitive and much smaller step sizes were necessary to resolve any solutions. As before, the neck region is always directly affected, followed by a relatively large morphological change in the bulk. In contrast to directions 1 and 2, the phase with the larger H_0 value assumes the bulk negative curvature as well as the significant morphological change.

Membrane energy

Considering the large number of solutions observed for the same set of parameters, we seek to investigate what distinguishes one shape from another. Since our model is based on minimization of the membrane energy, it is natural to consider the energy states of these solutions.

We begin our investigation by considering the four shapes given in figure 3(b), which all correspond to zero spontaneous curvature in both phases and are listed in order of lowest energy (*left*) to highest (*right*). Given a suitable initial solution, our solver easily produced the prolate shape (1) and the biconcave shape (2), however, the trilobal shapes (3) and (4)

³This limiting behaviour has been initially verified through numerical simulations and will serve as a topic of future work

were only obtained through the variation of non-zero spontaneous curvature. In the symmetric setting, shapes (3) and (4) are just reflections of one another and have the same energy value. Of the four shapes, the prolate (1) is the most common profile of experimental vesicles. Shape (2) resembles the red blood cell and, to our knowledge, shapes (3) and (4) have not been observed experimentally.

Since most observed vesicles tend to assume a prolate shape, the fact that the prolate shape produced the lowest energy could be an example of the model's consistency. However, we also found that the prolate shape does not necessarily correspond to the lowest energy in general. For other sets of parameters, our results show that non-prolate shapes have lower energy values than prolate shapes. One of many examples of this can be seen on the left and right of the intersection point in figure 6(a). To the right of the intersection point the prolate has the lower energy while to the left, it has a higher energy state.

The shapes and energy states become much more interesting with the introduction of spontaneous curvature. figure 5 captures this fact by giving multiple examples of energy hysteresis and non-unique solutions. Before summarizing the details of figure 5 we should clarify that each of the displayed spontaneous curvature planes are the same, but we separate them in order to give the reader a sampling of the solutions that are linked through a direct variation of spontaneous curvature. In other words, all shapes that are on the same plane can be obtained by appropriately changing the spontaneous curvature in the direction of the desired shape. Once an overlapping of shapes occur, the new shape is placed on a new plane along with all new shapes that spawn that shape. For example, if we start with the prolate shape in the right plane of figure 5(c) and vary the spontaneous curvature east to the shape at (5, 0), then vary south to the shape at (5, -9), and finally west to (0, -9) we would be at the shape displayed in the left plane of figure 5(c). Notice that if you continue varying north the shape will become the biconcave shape (originally introduced in figure 3(b)).

The middle section of figure 5(c) shows the collection of all equilibrium solutions corresponding to four arbitrary spontaneous curvature pairs, $(H_0^{(1)}, H_0^{(2)})$. The leftmost shape in each box is the solution with the lowest energy and the right-most shape has the highest energy. Note that each collection connects to the $(H_0^{(1)}, H_0^{(2)})$ pair in the left and right planes of figure 5(c), but could also be extended to the plane in figure 5(a). Keep in mind that all of the shapes in each of these four collections have the exact same parameter values. The collection corresponding to $(H_0^{(1)}, H_0^{(2)}) = (2, -2)$ displays eight unique solutions. These collections are by no means exhaustive and there may very well be additional solutions not discovered by our algorithm. In fact, the greatest number of solutions we could find at any given $(H_0^{(1)}, H_0^{(2)})$ pair was eight.

Considering that all the shapes in every possible collection have the exact same parameter values, but different energy states, we were driven to investigate the energy plots as a function of spontaneous curvature. In doing so, we discovered many occurrences of energy hysteresis. One example is presented in figure 5(b) which also shows a morphological and energetic link between the familiar zero spontaneous curvature shapes (2) and (4) from figure 5. The vertical thick line seen in figure 5(a) and the left plane of figure 5(c) also

denotes the $H_0^{(2)}$ hysteresis path in figure 5(b). We can view this particular hysteresis as starting with shapes (2) and (4) and increasing $H_0^{(2)}$ until a critical value ($H_0^{(2)} \approx 5.1$) is reached where the variation of $H_0^{(2)}$ is no longer possible. As the figure shows, the two shapes converge to the same morphology shown on the far right of figure 5(b).

Another interesting example of hysteresis is shown in figure 6. In this case, we see that the energy plots of two distinct paths intersect. This result is surprising since it is tempting for one to deduce that two energy minimization solutions with the same parameters and energy values would be the same. Therefore, this provides evidence that this is not necessarily the case. Upon further investigation of this special behaviour, the only quantity that we found to differentiate these two solutions was the osmotic pressure P as seen in figure 6(b).

The hystereses described above are just two examples of the many we encountered. To get an idea of how intricate the energy landscape over the spontaneous curvature plane is, we have provided dashed lines in the planes of figure 5 that give locations of the critical pairs where different shapes converge to one. The arrows indicate the direction in which the critical pair were approached. Based on our results, it is evident that this behaviour is a generic property of the model in the presence of non-zero spontaneous curvature. A few more examples of this behaviour are provided in the appendix.

To complete this discussion of the how spontaneous curvature effects the membrane energy of a vesicle, we present the reader with a collection of all the lowest energy shapes obtained for the area fractions $x^{(1)} = 0.50, 0.60, 0.75, 0.90$ in figure 7. The shapes given in each of the spontaneous curvature planes represent the equilibrium solution corresponding to the lowest energy from all solutions with the same set of parameters. With the exception of the shapes in the lower left quadrant, many of the shapes given in this figure resemble experimental shapes that we have seen. It is not yet known which of the shapes provided in figure 7 are stable equilibrium solutions, but we plan to investigate this in a future study.

A few other observations that we gathered from the information in figure 7 is that adjacent shapes that have a significantly different morphological type indicate additional examples of energy hysteresis described earlier where there must be a spontaneous curvature pair that yields different shapes with the exact same energy value. It is also apparent that there is no one shape-type that consistently has the lowest energy state, however, this question should be revisited once the stability of the solutions is known. Finally, we mention that lowest energy shapes exhibiting endocytosis (inward budding) tend to occur at higher area fractions, as figure 7 shows.

Membrane phenomena

There exists a number of morphological mechanisms of biomembranes that have been widely observed and documented. In particular, membranes commonly experience transformations such as endocytosis and exocytosis [31]; budding and fusion [35,36]; tubulation and beading [37], etc—all of which are crucial to cell function and survival. Therefore, it is desirable to have a vesicle model which can account for most of the major morphological changes experienced by a vesicle membrane. Assuming non-zero

spontaneous curvature, the two-phase Helfrich model that we consider in this paper appears to satisfy this quality very well. In order to help illustrate spontaneous curvature as a relevant model parameter, we will show that its presence can yield solutions which exhibit budding, fusion, endocytosis and tubulation.

We begin by directly comparing spontaneous curvature solutions with experimentally observed vesicles. figure 8 shows two comparisons with the observed vesicle on the left, the two-dimensional profile in the centre and the three-dimensional axisymmetric shape on the right. The area and volume fractions were visually estimated and the exact values used are given below each figure. In each case, the Gaussian curvature was set to zero ($\kappa_G = 0$). It may also be worth mentioning that in order to accurately model any experimental shape, we consistently needed to utilize a small bending rigidity (we used $\kappa = 0.02$, & 0.06 in figures 8(a) and 8(b), respectively). This may not be so surprising considering that most observed multi-phase vesicles appear to have constant mean curvature away from the phase interface and shapes of this type are solutions of a surface energy model (i.e. take $\kappa \rightarrow 0$ in the equation (3)). The location of a multi-phase vesicle that is influenced by bending is usually near the phase interface or at a budding site in the bulk of each phase.

Membrane budding occurs when a section of a membrane extends towards the exterior of the parent structure. Analytically, this can be characterized by large spikes of negative curvature surrounded by areas of positive curvature (figure 9). The increased negative curvature forces the membrane to pinch in a very small neck region and if conditions permit, the neck will break allowing the two structures to separate [40]. This physical separation is called fission. Although these two processes are directly related, the biological mechanism that drives fission may be different from the force that drives the budding [41, 42].

During our simulations, we observed many occurrences of ‘near’ budding from fluctuations in spontaneous curvature. figure 9 shows a sampling of our results. An interesting observation in many of our simulations was the fact that budding in one phase can be associated with changes in spontaneous curvature in the opposite phase. As one example, we start with a vesicle corresponding to the parameters $(-1.1, -3.0, 0.75, 0.72, 0, 1, 1)$ and vary $H_0^{(1)}$ from -1.1 to -8.46 (figure 10(b)). As the negative magnitude of $H_0^{(1)}$ increases, the neck curvature of the blue phase increases in (negative) magnitude producing an increased positive curvature in the neck region of the red phase. This shift in curvature induces the bud shown in figure 10(a).

figure 11 shows how budding typically occurs in the bulk rather than the phase interface and is dependent on the fixed value of the spontaneous curvature of the opposite phase. The top diagram in figure 11 shows the resulting equilibrium solution we obtain by starting with $(H_0^{(1)}, H_0^{(2)}) = (0, 0)$ and increasing $H_0^{(1)}$, whereas the bottom diagram starts with $(H_0^{(1)}, H_0^{(2)}) = (0, -2)$ for increasing $H_0^{(1)}$. The final shapes (right) shown in figure 11 correspond to the exact same spontaneous curvature difference. Therefore, if spontaneous curvature fluctuation occurs in one phase, budding may depend on the presence of spontaneous curvature in the opposite phase. figure 11 also helps to illustrate how so many solutions for the same set of parameters can be obtained just by varying the spontaneous

curvatures and why multiple solutions for a given set of parameters can consist of both budding and non-budding shapes.

Membrane fusion is the reverse effect of budding and endocytosis where two membrane-bound structures come into contact and join together, creating a neck. The neck then expands and the two membranes become part of the same structure. In nature, the fusion of membrane-bound structures is commonly assisted by ligand proteins [43], however, an interesting study has shown that fusion can be induced by electrically charged membranes [44].

Membrane fusion is a critical biological process. Examples of its significance are observed in cellular secretion via exocytosis, signalling between nerve cells, and virus infection. In both the life sciences and bioengineering, controlled membrane fusion has many possible applications, such as drug delivery, gene transfer, chemical microreactors, or synthesis of nanomaterials.

A relatively recent study shows how vesicles can be fused through electrofusion [44,45]. As two vesicle membranes come into close proximity of one another, an electrical pulse near the contact region forces a pore to open in both membranes where the now open membranes join, fusing the adjacent vesicles. When these membranes join there is an extremely rapid expansion of the neck connecting the two vesicles. At this point, the vesicle is free to deform into its stable state of minimum energy. Assuming the pre-fused vesicles have homogeneous membranes, then the resulting body is a fully phase-separated two-component vesicle. An example of this phenomenon is presented in figure 12(a). The newly fused vesicle immediately begins a budding process followed by complete fission. The result is one smaller homogeneous vesicle and a larger two-phase vesicle. To reference a previous result, notice that the budding does not occur at the phase interface, but in the bulk region of one of the phases.

Attempting to give insight into this process, we present the following possible scenario for spontaneous curvature as the main driving force for this biological mechanism. Suppose we have two single-component approximately spherical vesicles of nearly equal surface area as demonstrated in figure 12(a). Suppose also that the spontaneous curvature in each vesicle is given by $(H_0^{(1)}, H_0^{(2)})$. Now, when these vesicles fuse the new vesicle will have known volume and surface area fractions. These parameters must accommodate the following two important outcomes: (1) they must have an equilibrium shape which corresponds to a complete or nearly complete bud in one of the phases and (2) the post-fission vesicles with their new area and volume fraction must produce equilibrium shapes similar to those observed in experiment.

Let the pre-fusion vesicles be denoted by v_1 and v_2 , the post-fusion vesicle by v , and the post-fission vesicles by v_1^- and v_2^- . Suppose v_1 begins with parameters $(H_0^{(1)}, x^{(1)}, v, \kappa^{(1)}) = (2.64, 1, 1, 0.15)$ and v_2 with $(H_0^{(2)}, x^{(2)}, v, \kappa^{(2)}) = (-2.64, 1, 1, 0.30)$. After a simple geometrical calculation, v will have approximate parameters $(H_0^{(1)}, H_0^{(2)}, x^{(1)}, v, \kappa_G, \kappa^{(1)}, \kappa^{(2)}) = (2.64, -2.64, 0.50, 0.71, 0.00, 0.15, 0.30)$. A numerical simulation of this scenario

was performed with the parameters of ν and resulting the shape is shown as the second shape from the right. Now, assuming that fission takes place from the presence of such a small neck, ν will separate into the two vesicles, ν_1 and ν_2 . ν_1 is a two-component vesicle while ν_2 is single component. In terms of modelling non-dimensional vesicles, ν_1 and ν_2 are equivalent and the sphere is the expected equilibrium shape. ν_1 , on the other hand, should have a more complex equilibrium shape. Approximating the radius of ν_2 we may recover the area and volume fraction of ν_1 . The parameters used to approximate equilibrium shape of ν_1 are given by (2.64, -2.64, 0.58, 0.82, 0.00, 0.15, 0.30). The numerical result is given in the right-most shape in figure 12(b) labelled ν_1 .

The resulting parent and child vesicles show remarkable consistency with the experimental result. It is worth noting that higher values of spontaneous curvature would still result in budding, but since our simulations cannot accommodate topological changes, the magnitude of our values were limited. Without this restriction, we would be able to simulate this electrofusion process with greater accuracy.

We complete our discussion of membrane phenomena by showing some examples of how changes in spontaneous curvature can also produce membrane endocytosis and tubulation. These mechanisms are similar to budding except with endocytosis the membrane deforms inward instead protruding outward and with tubulation the outward protrusion becomes elongated rather than immediately pinching off. See figure 13.

In nature, endocytosis is associated with a section of the parent membrane bending inward in order to encapsulate exterior particles and allow them inside the interior. Neural synaptic transfer [46] is one example of this essential mechanism. Endocytosis is characterized by a spike in positive curvature, rather than negative curvature. This behaviour is clearly illustrated in figure 13(a) for three arbitrarily chosen solutions.

In contrast to endocytosis, membrane tubulation naturally occurs from the body of the Golgi apparatus and endosomes (organelles involved in intracellular trafficking) [47], but are also observed using microtubules [48–51]. Research shows that the changes to the spontaneous curvature can induce membrane tubulation. By adding polymers to the outer layer of the membrane one can alter the spontaneous curvature. When done properly, this variation in the spontaneous curvature causes the membrane to elongate into tubular structures [52]. It has also been shown that length of the tubular protrusions are directly dependent on the amount of spontaneous curvature within the membrane [37]. This was confirmed by our simulations by increasing the spontaneous curvature of one of the phases. figure 13 shows a sample of our results. For these solutions, we have allowed the volume to vary according to the osmotic pressure. Our results suggest that the length of the protrusion depends not only on the spontaneous curvature, but on the area fraction as well.

5. Conclusion

In this article, we focused on the spontaneous curvature parameter of the Helfrich two-phase vesicle model. Our simulations produced a huge collection of equilibrium solutions and many examples of energy hysteresis never before documented. figure 5(c) alone shows eight unique solutions for the same set of parameters. By comparing the energy values of these

solutions, we were surprised to also find examples of different shapes with the same parameters and the same energy value. Further testing showed that this behaviour was not isolated and it appeared to be a generic characteristic for non-zero spontaneous curvature where we commented that the ambient pressure (solved as a Lagrange multiplier) was the only difference between the solutions. Considering the fact that our search algorithm approached each integer node of the $H_0^{(1)}, H_0^{(2)}$ -plane, it is reasonable to assume that we only discovered a subset of all the possible solutions. Clearly, this rich and intricate nonlinear system is ripe for further investigation.

Photographs of multi-phase vesicles are ubiquitous and observations of these structures all exhibit a few common characteristics. First the bulk shape of the coexisting phases appear to have nearly spherical profiles (constant mean curvature). In fact, most of the simulated vesicles required a small bending rigidity to maintain accuracy. This is not exactly surprising since negligible or no bending rigidity reduces equation (1) to

$E = E_s^{(1)} + E_s^{(2)} + E_v$. Dropping the continuity of the tangent angle, ψ , this model produces solutions with piecewise constant mean curvature which is also consistent with experimental vesicles. We also noted that small bending rigidities in each phase ($\kappa^{(i)}$ near zero) seem to provide the most ideal setting for replicating experimental shapes. However, we also discovered that in some cases, solutions with large spontaneous curvature appear to approach solutions with constant mean curvature, indicating that the surface energy model could be a limiting case of this model through large spontaneous curvature.

Another interesting feature of spontaneous curvature is its impact on membrane morphology. It was shown to directly control jumps in interface curvature and induce major morphological changes, particularly in the bulk of the opposite phase. Additionally, non-zero spontaneous curvature accounted for budding, endocytosis, tubulation, and fusion which our simulations we able to reproduce. The fact that bilayer asymmetry is a well-known property of membranes, we believe that spontaneous curvature is a relevant membrane modelling parameter that should be further studied.

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Appendix

This appendix gives a few more figures similar to the ones shown in figure 14, but for different area fractions. These figures are provided to give the reader an idea of the variety of shapes and energy hysteresis that occurs for 75% and 90% area fractions and connect well with the lowest energy figures shown in figure 15.

Figure 1.

(a) A 2D axisymmetric profile of a fully separated two-phase vesicle. The profile is parametrized with respect to arc length s . The south pole, north pole and neck can be identified by $s = 0$, $s = s_f$ and $s = s_b$ respectively. We define phase-(1) on $[0, s_b]$ and phase-(2) on $[s_b, s_f]$. (b) Directional illustration of our multiple solution search algorithm at each spontaneous curvature node.

Figure 2.

Distribution of solutions for different area fractions. Each ring represents one solution. The area fractions used to generate these figures were $x^{(1)} = 0.50$ (left) and $x^{(1)} = 0.75$ (right). The other parameters are $(\nu, \kappa_G, \kappa^{(1)}, \kappa^{(2)}) = (0.72, 0, 1, 1)$.

Figure 3.

(a) The effect spontaneous curvature has on the neck region. $(x^{(1)}, \nu, \kappa_G, \kappa^{(1)}, \kappa^{(2)}) = (0.50, 0.72, 0, 1, 1)$ and $H_0^{(1)}=0$, $H_0^{(2)}=6$. This equilibrium solution was arbitrarily chosen and is merely intended to illustrate the jump properties of the solutions. (b) Zero spontaneous curvature equilibrium shapes. The energy values of each shape increase from left to right.

Figure 4.

Shape progression with azimuthal curvature, $\sin \psi/r$, (light) and mean curvature, H , (dark) versus arc length, s .

Figure 5.

(a) Spontaneous curvature plane with solutions that can be directly linked through a variation of spontaneous curvature. (b) Energy hysteresis and energetic link between two of the zero spontaneous curvature shapes (figure 3(b)). (c) Additional spontaneous curvature plane solutions and a sampling of multiple solutions for arbitrarily chosen spontaneous curvature pairs. More examples of hysteresis can be discerned by superimposing all three planes in this figure.

Figure 6.

More examples of energy hysteresis. (a) shows there exist multiple equilibrium solutions for the same parameters and energies. (b) is the pressure, which is the only distinguishing factor between the solutions that correspond to the intersection of (a).

Figure 7.

(Horizontal axis: $H_0^{(1)}$, vertical axis: $H_0^{(2)}$.) Lowest energy shapes for $x^{(1)} = 0.5$ (upper-left), 0.60 (upper-right), 0.75 (lower-left) and 0.90 (lower-right). No solutions were found on nodes with no shape.

Figure 8.

Experiment versus numerical comparison. In the left column are the experimental shapes, the middle and right column are the numerical cross-section and 3D axisymmetric solutions with the parameter values specified by $(H_0^{(1)}, H_0^{(2)}, x^{(1)}, \nu, \kappa_G, \kappa^{(1)}, \kappa^{(2)})$. (a) Image reproduced with permission from [38], Copyright 2006 World Scientific Publishing, using the parameters $(10, 2, 0.50, 0.72, 0, 0.13, 0.02)$. (b) Image reproduced with permission from [39], Copyright 2007 The American Society for Biochemistry Molecular Biology, using the parameters $(6, -7, 0.60, 0.95, 0, 0.01, 0.06)$.

Figure 9.

Examples of budding with non-zero spontaneous curvature. The large spikes of in-plane curvature characterize nearly complete buds.

Figure 10.

Budding by varying spontaneous curvature. (a) Close-up of the neck region (blue–red phase interface). (b) Varying spontaneous curvature from -1.1 (right) to -8.46 (left). The in-plane curvature for each solution is given below their shape.

Figure 11.

Dependence on the initial spontaneous curvature values for budding to take place. The numbered pairs above each shape are the spontaneous curvature values $(H_0^{(1)}, H_0^{(2)})$. Each of the graphs next to the shape are of the in-plane curvature (vertical) versus arc length (horizontal).

Figure 12.

(a) Vesicle fusion followed immediately by membrane fission; (b) simulation of the mechanism in (a). The different shapes of ν are obtained by varying spontaneous curvature in a southeast direction starting at 0 such that $H_0^{(1)}/H_0^{(2)} = -1$. Reproduced with permission from [38], Copyright 2006 World Scientific Publishing.

Figure 13.

(a) Examples of endocytosis with non-zero spontaneous curvature. (b) Membrane tubulation from increasing spontaneous curvature (in the red phase) for $x^{(1)} = 0.50$; and (c) $x^{(1)} = 0.75$.

Figure 14.
More examples of shape change and energy hysteresis for 75% area fraction.

Figure 15.
More examples of shape change and energy hysteresis for 90% area fraction.