Multidomain and ground state configurations of two-phase vesicles

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A simple model is used to study the equilibrium of lipid domains on two-phase vesicles. Two classes of configurations are considered: multidomain and ground state configurations. For multidomain configurations, the vesicle has a finite number of identical lipid domains. For ground state configurations, the vesicle is fully phase separated into two coexisting domains. Whereas the volume enclosed by a vesicle with multidomains is fixed, the volume enclosed by a vesicle in a ground state is allowed to vary with the osmotic pressure. Guided by experimental observations, all domains are assumed to be spherical caps. In a multidomain configuration, the line tension is found to decrease with the number of domains present, with possible exceptions when the number of domains is very small. The importance of a critical osmotic pressure and a critical excess radius on ground state configurations is explored. Emphasis is placed on understanding the variations of these critical quantities with relevant parameters.

1. Introduction

It is widely believed that lipids and proteins on the cell membrane interact to form very small stable regions called rafts [1]. Usually in the size range of 10–200 nm, rafts are domains in the liquid-ordered phase, enriched by saturated lipids and cholesterol, and surrounded by the background cell membrane in the liquid-disordered phase [2]. Rafts are crucial in protein sorting [3,4]. They also coalesce to form larger regions that serve as platforms for cellular functions such as signaling, trafficking, endocytosis and immune responses [1,3,4]. Moreover, it has been shown that pathogens such as viruses use rafts as gateways during attacks on cells [1,3–5]. Despite many experimental and theoretical investigations, a definitive understanding of the size, stability and lifetime of rafts remains elusive [2].

A major difficulty encountered in studying rafts is their observation. Nanoscopic rafts on the cell membrane are much smaller than the resolution of optical microscopes. Model membranes, namely giant unilamellar vesicles (GUVs) composed of unsaturated phospholipids, saturated sphingolipids and cholesterol, have a composition similar to the dominant composition of the cell membrane. On vesicles, phase separation provides a practical pathway for characterizing rafts in biological membranes [2,4]. On GUVs, raft-like lipid domains, usually in the size range of 1–10 μm, are observable via conventional light microscopy [6]. The size and distribution of lipid domains arise owing to competition between different energetic influences such as bending energy and line tension. Line tension is a manifestation of energetic interactions between phases at their interface.

Phase separation on GUVs and supported bilayers has been studied extensively both theoretically and experimentally. A significant part of the relevant literature focuses on studying microdomains from a non-equilibrium perspective, addressing issues such as growth dynamics and the recycling mechanism [7–11]. Numerous studies have also been devoted to understanding equilibrium configurations of multiphase vesicles [6,10,12–23]. For recent reviews, see [24,25]. Most theoretical works in this area involve solving an Euler–Lagrange equation equations (known as the shape equation), which hold in each domain, associated with boundary conditions at the interface between the domains.

Experimental observations on multiphase vesicles in the liquid phase reveal configurations in which the phase-separated domains on vesicles very closely resemble spherical caps, usually in the liquid-ordered phase, surrounded by a...
spherical background, usually in the liquid-disordered phase \cite{6,10,16,17,26,27}. Configurations including both multiple-ordered domains or only one large-ordered domain are commonly observed. Such piecewise spherical configurations are more likely to be observed for vesicles under tension, owing to osmotic pressure. These observations regarding the geometry of multiphase vesicles seem to have convinced more than one researcher \cite{10,27} to confine attention to models in which the domains are taken to be spherical. This assumption obviates the need to solve the shape equation and thus significantly simplifies the calculations. The net potential energy becomes a function of the geometrical parameters, namely the radii of curvatures of the domains and contact angles, and equilibrium configurations result from minimizing with respect to these parameters.

Although the results of Yanagisawa et al. \cite{10} and Semrau et al. \cite{27} compare favourably with experimental observations and, thus, support the applicability of models based on piecewise spherical configurations, more careful attention to the conditions at interfaces remains necessary. In particular, while Yanagisawa et al. \cite{10} and Semrau et al. \cite{27} consider geometrical compatibility at interfaces, they do not require satisfaction of the force balance. Force balance at an interface on a piecewise spherical vesicle relates the surface tensions of the adjacent areas and the line tension of the interface \cite{16,18,28,29}. Notably, the interfacial force balance has been previously used to estimate the line tension from experimental data \cite{16,29,30}—from which it follows that the line tension in such modelling cannot generally be chosen arbitrarily, as it must be compatible with force balance. In addition, piecewise spherical models can overlook potentially important energetic information. This may occur on replacing a thin transition layer with very high curvature by a sharp interface endowed with a (given constant) line tension. Comprehensive discussions of such transition (or boundary) layers appear for example in the works of Fournier & Ben Amar \cite{31}, Allain & Ben Amar \cite{18}, Das & Jenkins \cite{32} and Trejo & Ben Amar \cite{33}. The energy stored in a transition layer consists of two general sources. The first source is the chemical energy owing to very high gradients of lipid species concentrations. The second source is the mechanical energy owing to distortion and misalignment of lipid molecules. For example, the stretching/contraction of different lipid molecules with different hydrophobic tails, splay and tilt of lipid molecules are all mechanical sources of this kind. Then, representing the energy of a transition layer by a sharp interface endowed with line tension is very delicate and requires evaluation of the underlying energies at the boundary layer. Comprehensive studies of the line tension in multiphase lipid membranes have been undertaken \cite{33–37}. In particular, Fournier & Ben Amar \cite{31}, Towles & Dan \cite{37} and Trejo & Ben Amar \cite{33} demonstrate that the line tension may be influenced by the contact angle at the junction between two phases. Questions regarding the suitability of taking the line tension to be constant, therefore, arise.

In a study of the instability of the vesicle owing to protein adsorption, Allain & Ben Amar \cite{28} obtain the equilibrium equations and associated interface conditions for a biphasic vesicle composed of two spherical caps. Working with the same geometrical assumptions, Allain & Ben Amar \cite{18} develop a model for the budding and the fission of biphasic vesicles under tension. In accord with the property of tense vesicles, they implement a capillary solution in which the bending energies of two spherical caps are negligible in comparison with the line and the osmotic-pressure induced energies. They then improve the capillary solution by replacing the sharp interface (across which the slope is discontinuous) with a smooth boundary layer, where the bending energy of the lipid domains is considered. Their analysis leads to an effective line tension that takes the elasticity of the boundary layer into account. Further discussion of their work appears at the end of the section in which we present our numerical results. Trejo & Ben Amar \cite{33} use an asymptotic approach to explore the equilibrium of a biphasic vesicle composed of two spherical domains connected by a boundary layer. They conduct a detailed analysis taking into account various macroscopic and microscopic energetics, including local area-difference elasticity and the thickness change of the membrane. Within the spherical caps, which are referred to as ‘outer layers,’ they account for bending energy. In addition, they take the volume of the vesicle to be fixed. The analysis of Trejo & Ben Amar \cite{33} focuses on understanding the contact angle and effective line tension at a junction separating the phases.

In this paper, we first discuss the local equilibrium of piecewise spherical vesicles, the junction conditions and different energetics. Next, we consider two different classes of equilibrium configurations, multidomain and ground state configurations. Our analysis of multidomain configurations builds on the works of Yanagisawa et al. \cite{10} and Semrau et al. \cite{27}. Although our analysis accounts only for the bending and line energies, by ensuring that the interfacial force balance is satisfied, we provide a more complete physical description. As a natural consequence of this and consistent with the works of Baumgart et al. \cite{16}, Tian et al. \cite{29} and Hutchison et al. \cite{30}, we view the line tension as a dependent variable that can be obtained by satisfying the interfacial force balance. By contrast, the osmotic pressure is considered as the independent parameter that influences the surface tension in the domains. For multidomain configurations, we assume that the volume of the vesicle is fixed. Although the osmotic pressure does not change the volume of the system (and, hence, has no energetic contribution), we find that it influences the net potential energy of the system through modulation of the line energy. For instance, Akimov et al. \cite{35} discuss how the line tension increases with the surface tension. Importantly, our results show that line tension decreases with the number of domains, despite possible exceptions that may occur for very small numbers of domains. We next focus on ground state configurations, which represent equilibria resulting from the coalescence of multiple domains into a single domain. In contrast to our treatment of multidomain configurations, we allow the volume enclosed by the vesicle in a ground state configuration to change. In so doing, we explore the effect of osmotic pressure and initial excess radius of a vesicle on its equilibrium configuration. We determine critical values of the osmotic pressure and the initial excess radius, and investigate their effect on the final shape of a vesicle in a ground state configuration. Finally, we discuss and compare our approach to ground state configurations with that of Allain and Ben Amar \cite{18}.

2. Formulation

2.1. Local equilibrium of spherical vesicles

Consider a vesicle with superficial energy density $\psi = \psi(H, K)$, where $H$ and $K$ are the mean and Gaussian curvatures,
respectively. The shape equation for such membrane is (see alternative expressions provided, for example, by Steigmann [38] and Steigmann et al. [39])

\[(2 H^2 - K)\psi_{ij} + \frac{1}{2} \Delta \psi_{ij} + 2KH\psi_{ik} - \text{div}_s(L\nabla_s \psi_k) - 2\nabla_s \psi_k \cdot \nabla_s H + 2\Delta(\psi H) - 2\psi_{ij} H - 2H(\psi + \sigma) = p,\]

(2.1)

where \(\psi_{ij}\) and \(\psi_k\) represent the partial derivatives of \(\psi\) with respect to \(H\) and \(K\), \(\nabla_s\) and \(\text{div}_s\) indicate the surface gradient and divergence, and \(L\), \(\sigma\), and \(p\), respectively, denote the curvature tensor, surface tension, and osmotic pressure.

Assume now that the vesicle is spherical with radius \(R\), so that

\[H = -\frac{1}{R}\text{ and } K = \frac{1}{R^2},\]

(2.2)

and the shape equation (2.1) specializes to

\[\frac{1}{R^2} \psi_{ij} - \frac{1}{R^3} \psi_{ik} + \frac{2}{R}(\psi + \sigma) = p.\]

(2.3)

Suppose, further, that \(\psi\) is of the Canham–Helfrich [40,41] form, but with zero spontaneous curvature, so that

\[\psi = \frac{1}{2} H^2 + \kappa K,\]

(2.4)

where \(\kappa\) and \(\kappa\) are the splay and saddle-splay moduli. Substituting (2.4) in (2.3) yields

\[\sigma = \frac{1}{2} \frac{pR}{p},\]

(2.5)

which is the classical Young–Laplace equation. The reduction of the shape equation to the Young–Laplace equation in the absence of the spontaneous curvature is also discussed by Allain & Ben Amar [28], Baumgart et al. [17] and Idema & Storm [23]. A generalization of (2.5) that incorporates spontaneous curvature is provided by Ou-Yang & Helfrich [42,43], Trejo & Ben Amar [33] and Allain et al. [16,17], Allain & Ben Amar [18,28], Tian et al. [29] and Hutchison et al. [30].

Consider now a spherical cap, with arbitrary opening angle \(2\theta\), cut from a spherical vesicle and subject to uniform pressure \(p\), as depicted in figure 1. Suppose that, in addition to the surface tension \(\sigma\), the bilayer supports a transverse shear force \(q\). The force balance in the vertical direction then requires that

\[p(\pi R \sin \theta)^2) = \sigma \sin \theta(2\pi R \sin \theta) + q \cos \theta(2\pi R \sin \theta),\]

(2.6)

which upon using (2.5) yields \(q = 0\). Thus, a spherical bilayer described by the Canham–Helfrich bending energy (in the absence of the spontaneous curvature) under uniform pressure cannot support transverse shear force.

![Figure 1](http://rsif.royalsocietypublishing.org/)

**Figure 1.** A two-dimensional cross section of a spherical cap under pressure \(p\), surface tension \(\sigma\) and transverse shear force \(q\). (Online version in colour.)

2.2. Junction condition

Consider a two-phase vesicle, composed of phases \(\alpha\) and \(\beta\), as depicted in figure 2. Suppose that the domains occupied by phases \(\alpha\) and \(\beta\) are spherical caps, with respective radii of curvature \(R_\alpha\) and \(R_\beta\). Denote the radius of curvature of the junction between the phases by \(\rho\). As a consequence of force balance at the junction in the direction perpendicular to the plane in which the junction resides, it follows that

\[\sigma_\alpha \sin \theta_\alpha = \sigma_\beta \sin \theta_\beta,\]

(2.7)

where \(\sigma_\alpha\) and \(\sigma_\beta\) are the surface tensions in phases \(\alpha\) and \(\beta\), respectively, and the angles \(\theta_\alpha\) and \(\theta_\beta\) as indicated in figure 2. In view of (2.5) and the geometrical relation

\[R_\alpha \sin \theta_\alpha = R_\beta \sin \theta_\beta = \rho,\]

(2.8)

(2.7) is satisfied identically. Further, the balance of force in the plane in which the junction resides yields

\[-(\sigma_\alpha \cos \theta_\alpha + \sigma_\beta \cos \theta_\beta)p = \tau.\]

(2.9)

The surface tensions \(\sigma_\alpha\) and \(\sigma_\beta\) are determined by the Young–Laplace equation (2.5) through

\[\sigma_\alpha = \frac{1}{2} \frac{pR_\alpha}{p} \text{ and } \sigma_\beta = \frac{1}{2} \frac{pR_\beta}{p}.\]

(2.10)

In view of (2.8) and (2.10), (2.9) can be written as

\[\cot \theta_\alpha - \cot \phi = -\frac{2\tau}{p^2},\]

(2.11)

with \(\phi = \pi - \theta_\beta\). Equation (2.11) or equivalent versions of that equation were derived previously by Baumgart et al. [16,17], Allain & Ben Amar [18,28], Tian et al. [29] and Hutchison et al. [30].

2.3. Geometrical relations

Consider a vesicle consisting of \(N\) identical spherical caps of phase \(\alpha\) surrounded by a background phase \(\beta\) with constant radius of curvature \(R_\beta\). Straightforward calculations show that the net areas \(A_\alpha\) and \(A_\beta\) occupied by the phases \(\alpha\) and \(\beta\) are given by

\[A_\alpha = 2\pi R_\alpha^2(1 + \cos \theta_\alpha)^{-1}\]

and

\[A_\beta = 4\pi R_\beta^2 - 2\pi N p^2(1 + \cos \phi)^{-1}.\]

(2.12)

As the interface separating each domain of phase \(\alpha\) from phase \(\beta\) has length \(2\pi\rho\), the total length \(s\) of the interface...
between the phases is

\[ s = 2\pi N \rho. \]  

Further, the volume enclosed by the vesicle is

\[ V = \frac{4\pi}{3} R^3 + \frac{\pi}{3} N \rho \left[ (1 - \cos \theta_r)^2 (2 + \cos \theta_t) \right] \sin^3 \theta_a. \]  

In view of (2.12), the net area \( A \) of the vesicle is simply

\[ A = A_a + A_b. \]  

(2.15)

Let \( R_a \) and \( R_b \) be defined such that

\[ A = 4\pi R^2 \quad \text{and} \quad V = \frac{4}{3} \pi R^3. \]  

(2.16)

Note that the area inextensibility of the vesicle requires that \( R_a \) be fixed.

As a sphere encloses the maximum volume for a given area, it is evident that \( R_a > R_b \), and it seems reasonable to work with the excess radius

\[ \xi = \frac{R_a - R_b}{R_b}. \]  

(2.17)

introduced by Yanagisawa et al. [10]. Consideration of (2.17) shows that \( \xi \) measures the extent to which the lipid domains of phase \( \alpha \) are allowed to bud outward from phase \( \beta \). An alternative to this measure is the reduced volume \( V / (4\pi R^3) \) used by Seifert et al. [44], Jülicher & Lipowsky [13] and Baumgart et al. [17].

Species conservation requires that the net area occupied by each phase be fixed. On defining the ratio \( \xi = A_a / A \) of the net area of phase \( \alpha \) to the net area of the vesicle, it follows that

\[ \xi = \text{const.} \]  

(2.18)

Further, by (2.12) and (2.16),

\[ \xi = \frac{N \rho^2}{2(1 + \cos \theta_r)}. \]  

(2.19)

where

\[ \tilde{\rho} = \frac{\rho}{R_a} \]  

(2.20)

is a dimensionless version of the radius of curvature of the junction between the phases. Next, using (2.8), (2.12) and (2.19) in (2.15) gives

\[ \frac{2}{\sin^2 \phi} - \frac{N}{1 + \cos \phi} = \frac{2(1 - \xi)}{\tilde{\rho}^2}, \]  

(2.21)

which can be solved for \( \phi \) to yield

\[ \phi = \cos^{-1} \left( \frac{\sqrt{N^2 + 4\alpha (a + N - 2) - N}}{2a} \right), \]  

(2.22)

with

\[ a = \frac{2(1 - \xi)}{\tilde{\rho}^2}. \]  

(2.23)

### 2.4. Energetics

The net potential energy \( \Psi \) of the system can be expressed as

\[ \Psi = \Psi_b + \Psi_l + \Psi_v, \]  

(2.24)

where \( \Psi_b \) and \( \Psi_l \) are bending and line contributions. Assuming that the compositions of phases \( \alpha \) and \( \beta \) are uniform, the bending moduli and the line tension must also be uniform and it follows that

\[ \Psi_b = \frac{\kappa_a A_a + \kappa_b A_b}{2R^2} \]  

(2.25)

and

\[ \Psi_l = \tau \xi. \]  

where \( \kappa_a = \kappa_a + 2\kappa_b \) and \( \kappa_b = \kappa_b + 2\kappa_b \) serve as effective bending moduli. Note that this reduction stems from the assumption that the domains occupied by phases \( \alpha \) and \( \beta \) are spherical.

The volumetric (or pressure) contribution \( \Psi_v \) to (2.24), defined by

\[ \Psi_v = -\int_{V_\alpha} p \, dV, \]  

(2.26)

is relevant only if the volume of the vesicle changes. In (2.26), \( V_\alpha \) and \( V_\beta \) represent the initial and final volumes of the vesicle. Also, the osmotic pressure \( p \) is viewed as a known (that is, experimentally controllable) input.

### 2.5. Scaling

To streamline the presentation and interpretation of results, we work exclusively with dimensionless quantities. Specifically, we introduce

\[ \frac{R_a}{R_b} = \tilde{R}_a, \quad \frac{R_b}{R_a} = \tilde{R}_b, \quad \frac{R_b}{R_a} = \tilde{R}_b, \quad \frac{A_a}{R_b^2} = \tilde{A}_a, \quad \frac{A_b}{R_a^2} = \tilde{A}_b, \quad \frac{V}{R_b^3} = \tilde{V}, \quad \frac{s}{R_b} = \tilde{s}, \quad \frac{\tilde{\Psi}}{\kappa_a} = \tilde{\Psi}, \quad \tilde{\rho} = \frac{\rho R^2}{R_b^3}, \quad \tilde{\tau} = \frac{\tau R_b}{\kappa_a}. \]  

(2.27)

Accordingly, the dimensionless net potential energy \( \tilde{\Psi} \) of the system can be expressed as

\[ \tilde{\Psi} = \tilde{\Psi}_b + \tilde{\Psi}_l + \tilde{\Psi}_v, \]  

(2.28)

where \( \tilde{\Psi}_b, \tilde{\Psi}_l, \) and \( \tilde{\Psi}_v \) are dimensionless counterparts of \( \Psi_b, \Psi_l \) and \( \Psi_v \) given by

\[ \tilde{\Psi}_b = \frac{\tilde{\tilde{A}}_a + \tilde{\tilde{A}}_b}{2R^2}, \quad \tilde{\Psi}_l = \tilde{\tau} \tilde{s}, \quad \tilde{\Psi}_v = -\int_{V_\alpha} \tilde{\rho} \, d\tilde{V}. \]  

with

\[ \tilde{\kappa}_b = \frac{\tilde{\rho} \rho}{R_b}. \]  

(2.29)

being the dimensionless effective bending modulus of phase \( \beta \).

In view of (2.27), the junction condition (2.11) can be expressed alternatively as

\[ \tilde{\tau} = \frac{1}{2} \tilde{\rho} \tilde{\rho}' (\cot \theta_a - \cot \phi). \]  

(2.30)

Baumgart et al. [16], Tian et al. [29] and Hutchison et al. [30] use counterparts of (2.31) to evaluate the line tension using experimental data. If \( \theta_\alpha, \theta_\beta \) and \( \tilde{\rho} \) are held fixed, it is evident from (2.31) that the dimensionless line tension scales linearly with the dimensionless osmotic pressure and, consequently, with the surface tension.

For brevity, the adjective ‘dimensionless’ is suppressed from here on.
2.6. Equilibrium configurations

In this section, we consider two distinct classes of equilibrium configurations. The first of these classes emerges when a uniform multispecies vesicle undergoing phase separation is transformed to a multidomain vesicle (see the transformation from (figure 3a) to (figure 3b)). In the second class of configurations, the lipid domains have coalesced into a single domain (see the transformation from (figure 3b) to (figure 3c)).

2.6.1. Multidomain configurations

Granted that osmotic-pressure driven solvent transport across the vesicle occurs much more slowly than the phase separation process leading to multiple lipid domains, when considering multidomain configurations we assume that the volume enclosed by the vesicle is fixed. In view of (2.14), we must, therefore, satisfy the condition

\[
\frac{4\pi}{3} R_0^3 + \frac{\pi}{3} N \rho \left[ (1 - \cos \theta_o)^2 (2 + \cos \theta_o) \right] = V_0. \tag{2.32}
\]

Additionally, the conservation of lipid species and the areal inextensibility of the vesicle demand satisfaction of (2.19) and (2.21). Solving (2.19), (2.21) and (2.32) for a given number \( N \) of domains determines the unknowns \( \theta_o, \phi \), and \( \rho \). Also, for a given osmotic pressure \( \tilde{p} \), the line tension \( \tilde{\tau} \) can be obtained from (2.11), as is needed to determine the line energy \( \Psi_1 \).

2.6.2. Ground state configurations

After a sufficiently long time, scattered lipid domains may coalesce into a single domain. We refer to such a state as a ‘ground state configuration’. Yanagisawa \textit{et al.} [10] and Semrau \textit{et al.} [27] observe that the net potential energy of a multidomain configuration decreases with the number of domains. Consistent with this, it seems reasonable to expect that ground state configurations should correspond to minima of the net potential energy \( \Psi \).

Lipid domains usually coalesce very slowly. One reason for this is that coalescence can be resisted by an interdomain repulsive forces that become significant when two domains approach one another sufficiently closely [10,21,27]. In view of this fact and because the time required to reach a ground state configuration is likely to be long enough to allow for solvent transport across the membrane owing to osmotic pressure, it seems reasonable to allow the volume of the vesicle in a ground state configuration to vary. This stands in contrast to our treatment of multidomain configurations. In particular, the volume enclosed by the vesicle in a ground state configuration is determined as a consequence of energy minimization.

When the volume enclosed by the vesicle is allowed to vary, the volumetric energy \( \Psi_v \) defined in (2.26) must be considered in addition to the bending and line energies \( \Psi_b \) and \( \Psi_1 \). Moreover, it is important to account for the dependence of the osmotic pressure on the solute concentration. If the osmotic pressure is assumed to be linearly proportional to the solute concentration, we may consider the interior fluid as an ideal solution [45]. For the sake of simplicity, we neglect the ion concentration in the exterior region. Then, because the pressure vanishes in the fluid surrounding the vesicle, the osmotic pressure \( p \) and the pressure in the fluid enclosed by the vesicle coincide. Thus,

\[
pV = p_0 V_o, \tag{2.33}
\]

where \( p_o \) is the initial osmotic pressure—that is, the osmotic pressure in the vesicle before phase separation and also in a multidomain configuration. Using (2.33) in (2.26) yields

\[
\Psi_v = -p_0 V_o \ln \left( \frac{V}{V_o} \right), \tag{2.34}
\]

which, because \( p_o \) and \( V_o \) are both constant, can be additively scaled according to

\[
\Psi_v = -p_0 V_o \ln \tilde{V} . \tag{2.35}
\]

In view of (2.27), the energy \( \tilde{\Psi}_v \), therefore, reads

\[
\tilde{\Psi}_v = -p_0 V_o \ln \tilde{V} . \tag{2.36}
\]

Also using (2.16)n, (2.17) and (2.27), we have

\[
\tilde{V}_o = \frac{4\pi}{3(1 + \xi_0)} , \tag{2.37}
\]

in which

\[
\xi_0 = \left( \frac{R_o - R_v}{R_v} \right) \tag{2.38}
\]

is the initial excess radius of the vesicle. Substituting (2.37) in (2.36) gives

\[
\tilde{\Psi}_v = -\frac{4}{3} \pi \tilde{P}_o \ln \tilde{V} . \tag{2.39}
\]

where

\[
\tilde{P}_o = \frac{\tilde{p}_o}{(1 + \xi_0)^2} \tag{2.40}
\]

denotes the reduced osmotic pressure. Seifert [45] and Allain & Ben Amar [18] perform similar calculations and explore the importance of the volumetric energy with varying pressure.
Using (2.33), the osmotic pressure can be expressed alternatively as
\[
\tilde{p} = \frac{4\pi \Pi_0}{3V}.
\] (2.41)

As for multidomain configurations, the conservation of lipid species and the constraint of areal inextensibility require satisfaction of (2.19) and (2.21). In addition, the line tension depends on the solution through the junction condition (2.11). At equilibrium, the net potential energy \(\tilde{\Psi}\) is minimized. Solving the three equations (2.11), (2.19) and (2.21), while requiring that the net potential energy be minimized delivers the ground state configuration.

2.6.3. The capillary approximation for the ground state configuration

For sufficiently large values of the osmotic pressure, the net potential energy of a vesicle is dominated by the osmotic pressure effect, rendering the bending energy negligible. Under such conditions, Allain & Ben Amar [18] adopt and study a capillary approximation in which the net potential energy of a mutiphase vesicle includes only the line and volumetric contributions. In terms of the dimensionless quantities defined in (2.29), this approximation leads to
\[
\tilde{\Psi}_{\text{cap}} = \tilde{\Psi}_1 + \tilde{\Psi}_v.
\] (2.42)

On invoking (2.29), (2.31), (2.39) and (2.41), and introducing the purely geometrical quantity
\[
g = \frac{\tilde{p}^2}{V} \left( \cot \theta_0 - \cot \phi \right) + \ln \tilde{V},
\] (2.43)

(2.42) simplifies to
\[
\tilde{\Psi}_{\text{cap}} = \frac{4}{3} \pi \Pi_0 g,
\] (2.44)
showing that \(\tilde{\Psi}_{\text{cap}}\) scales linearly with \(\Pi_0\). Modulo a different non-dimensionalization, (2.44) is equivalent to the expression given by Allain & Ben Amar [18].

3. Numerical results

We next explore various features of multidomain and ground state configurations. Without loss of generality, we take \(\kappa_0/\kappa_\phi = 1.25\). Further, for simplicity, we take the ratio of the effective bending moduli equal to the value of the ratio of the splay moduli reported by Baumgart et al. [16].

3.1. Multidomain configurations

The variations of the net potential energy \(\tilde{\Psi}\), the bending energy \(\tilde{\Psi}_b\) and the line energy \(\tilde{\Psi}_1\) with the number \(N\) of lipid domains for different values of the area ratio \(\xi\) and the osmotic pressure \(\tilde{p}\) are depicted in figure 4. Notably, \(\tilde{\Psi}\) increases with \(N\) and achieves an absolute minimum at \(N = 1\), verifying that ground state configurations correspond to minimizers of the net potential energy. For sufficiently large values of \(N\), \(\tilde{\Psi}\) increases almost linearly with \(N\), consistent with a result owing to Yanagisawa et al. [10]. In addition, \(\tilde{\Psi}\) increases with the osmotic pressure \(\tilde{p}\). The net potential energy \(\tilde{\Psi}\) behaves consistently for different area ratios \(\xi\), except that for smaller values (e.g. \(\xi = 0.2\)) it is less sensitive to the osmotic pressure \(\tilde{p}\). The bending and line energies \(\tilde{\Psi}_b\) and \(\tilde{\Psi}_1\) increase with the number \(N\) of domains. Thus, there is no competition between these contributions to the net potential energy \(\tilde{\Psi}\). Importantly, \(\tilde{\Psi}_b\) is independent of \(\tilde{p}\). This is because the geometry of a multidomain configuration is completely dictated by the geometrical constraints—i.e. by (2.19), (2.21) and (2.32). Also, the ratio \(\tilde{r}/\tilde{p}\) of the line tension to the osmotic pressure is determined from (2.11) and, therefore, is independent of \(\tilde{p}\). However, in view of (2.11), \(\tilde{r}\) and, accordingly, \(\tilde{\Psi}_1\) varies linearly with \(\tilde{p}\). For this reason, \(\tilde{\Psi}_1\), as opposed to \(\tilde{\Psi}_b\), increases with \(\tilde{p}\), and naturally is the source of the dependence of \(\tilde{\Psi}\) on \(\tilde{p}\). As \(\tilde{p}\) increases, the energy difference between configurations with high and low numbers of domains increases, especially for higher values of the area ratio \(\xi\). This potentially favours higher tendency for domain coarsening when \(\tilde{p}\) increases. We believe that this argument is consistent with the experimental observation of Morales-Pennington et al. [25], which reveals the osmotic pressure increase can induce the coalescence of small domains. According to these authors, this process is accompanied by swelling of the vesicles into spheres composed most often of two large domains.

The variations of the characteristic size \(\tilde{\rho}\), the slopes \(\theta_0\) and \(\phi\), and the ratio \(\tilde{r}/\tilde{p}\) of the line tension to the osmotic pressure with the number \(N\) of the domains are depicted in figure 5 for different values of the area ratio \(\xi\) and the excess radius \(\zeta\). For smaller values of the excess radius (e.g. \(\zeta = 0.02\)), the results show that \(\tilde{\rho}\) decreases with \(N\), which is not very surprising because the net area occupied by phase \(\alpha\) is fixed. Also, both \(\theta_0\) and \(\phi\) decrease with \(N\), whereas \(\theta_0 > \phi\) indicates that the domains are dimpled from the vesicle towards the exterior region. In addition, the ratio \(\tilde{r}/\tilde{p}\) decreases with \(N\), meaning that for \(\tilde{p}\) fixed \(\tilde{r}\) decreases with the number \(N\) of domains. As the excess radius increases (from \(\zeta = 0.02\) to \(\zeta = 0.07\)), \(\tilde{p}\) decreases, \(\theta_0\) increases, and \(\phi\) is essentially unchanged. This behaviour seems reasonable because, for larger values of the excess radius, domains have more latitude to bud from the vesicle with higher slope \(\theta_0\) and smaller neck \(\tilde{\rho}\). For smaller area ratios \(\xi\) (e.g. \(\xi = 0.2\)), the influence of \(\zeta\) on the ratio \(\tilde{r}/\tilde{p}\) is more tangible. Moreover, for larger excess radius (e.g. \(\zeta = 0.07\)), the solution experiences a jump between \(N = 1\) and \(N = 2\). This is because the configuration of the vesicle for a constant net
3.2. Ground state configurations

In this section, we explore the consequences of regarding the reduced osmotic pressure $\Pi_c$ as a control parameter. (For brevity, the adjective ‘reduced’ is dropped hereafter in referring to $\Pi_c$.) Under these circumstances, $\theta_o$ and $\phi$ are determined from (2.19) and (2.22) in terms of $\rho$. Accordingly, using (2.14) and (2.27), the volume $V$ is calculated. Next, the osmotic pressure $\rho$ is provided by (2.41). Finally, (2.31) is used to obtain the line tension $\tau$.

The variations of the net potential energy $\Psi$, the slopes $\theta_o$ and $\phi$, and the line tension $\tau$ with the domain size $\rho$ are depicted in figure 6 for the area ratio $\xi = 0.5$ along with two values $\Pi_c = 1$ and $\Pi_c = 10$ of the osmotic pressure. For the smaller value of $\Pi_c$, the results show that $\Psi$ decreases monotonically with $\rho$, achieving an absolute minimum at $\rho = 1$. This corresponds to a ground state configuration in which the vesicle is composed of two spherical caps (in this case, two hemispheres) of phases $\alpha$ and $\beta$ with identical radii (see also figure 6b), which indicates that $\theta_o = \phi = \pi/2$ at $\rho = 1$. For the higher value of $\Pi_c$, the results show that $\Psi$ possesses a local minimum at $\rho = 0$ and, much like what arises at lower osmotic pressures, an absolute minimum at $\rho = 1$. The size $\rho = 0$ corresponds to a ground state configuration, wherein the domain occupied phase $\alpha$ is about to pinch off from the vesicle (figure 6b, which shows that $\theta_o = \pi$ and $\phi = 0$ at $\rho = 0$). The other ground state configuration occurs at $\rho = 1$, corresponding to the complete sphere configuration.

In the figure 6c, we see how the line tension $\tau$ changes with the domain size $\rho$. Importantly, at the ground state configurations arising for $\rho = 0$ and $\rho = 1$, the model predicts that the line tension vanishes. This result is consistent with the observation that, by (2.11), $\tau$ vanishes for $\rho = 0$ and $\rho = 1$. As it might seem unreasonable to allow $\tau$ to vanish, we will return to this issue shortly.

On the basis of figure 6, we note that the structure of the net potential-energy curve is strongly influenced by the value of the osmotic pressure $\Pi_c$. For $\Pi_c = 1$, $\Psi$ has an absolute minimum at $\rho = 1$. However, for $\Pi_c = 10$, in addition to an absolute minimum at $\rho = 1$, $\Psi$ has a local minimum at $\rho = 0$. Note that $\rho = 1$ is the locus of the absolute minimum only for $\xi = 0.5$. Regardless of the value of the area ratio $\xi$, numerical results verify that the location of the absolute minimum of $\Psi$ corresponds to a configuration in which the vesicle resembles a complete sphere composed of two spherical caps of the phases $\alpha$ and $\beta$ with the same radii of curvature and identical slopes (i.e. $\theta_o = \phi$) at their interface.

Under these conditions, the geometrical relations (2.19) and (2.21) simply imply that the size $\rho$ corresponding to a completely spherical ground state configuration is

$$\rho = 2\sqrt{\xi(1-\xi)}. \tag{3.1}$$

Importantly, the transition from the case where $\Psi$ exhibits a single absolute minimum at $\rho = 2\sqrt{\xi(1-\xi)}$ to that wherein $\Psi$ exhibits two minima (specifically, one local minimum at $\rho = 0$ and one absolute minimum at $\rho = 2\sqrt{\xi(1-\xi)}$) occurs for $\Pi_c$ in excess of a critical value $\Pi_c$. Figure 7 shows the variation of $\Pi_c$ with $\xi$. The circles in the figure indicate the distinct values of $\Pi_c$ determined by numerical trial and error for various choices of $\xi$. The graph of $\Pi_c$ versus $\xi$ seems to be almost symmetric about the line $\xi \approx 0.5$. The critical value $\Pi_c$ is very large for $\xi$ close to either zero or unity, and achieves a minimum at approximately $\xi = 0.5$,
for which $\Pi_0 \approx 1.8$. For $\Pi_0 < \Pi_c$, the only ground state configuration is a complete sphere. For $\Pi_0 > \Pi_c$, two ground state configurations, one a complete sphere and the other pinched-off, exist. Although the pinched-off configuration can be considered as metastable relative to the complete sphere configuration, it qualifies as an equilibrium configuration as long as $\Psi$ does not pass the energy barrier between the two configurations.

We propose that the initial excess radius $z_o$ is a key parameter that determines which configuration, the pinched-off or the complete sphere, is likely to be selected as the ground state. Figure 8 shows the variations of the net potential energy $\tilde{\Psi}$ and the excess radius $z$ with the size $\tilde{\rho}$ of the domain for $\Pi_0 = 10 > \Pi_c$ and two choices $\xi = 0.5$ and $\xi = 0.2$ of $\xi$. Notably, $\tilde{\Psi}$ has an absolute maximum at a critical size $\tilde{\rho}_c$. For this value of $\tilde{\rho}$, the excess radius is $z_c$, and $\Psi$ is the critical excess radius. We propose that the ground state configuration is pinched-off for initial excess radii greater than $z_c$. Otherwise, for $\xi < z_c$, the ground state configuration is a complete sphere. In fact, $\xi_c$ serves as an initial value which, depending on its value compared with the critical excess radius, leads to different ground state configurations.

The variations of the critical excess radius $z_c$, and the critical domain size $\tilde{\rho}_c$ with the initial osmotic pressure $\tilde{\rho}_o$ are depicted in figure 9 for two choices $\xi = 0.5$ and $\xi = 0.2$ of the area ratio $\xi$. Obviously, $\tilde{\rho}_c$ exceeds the critical value exhibited in figure 7. The critical excess radius $z_c$ attains a maximum at the critical pressure (equal to 2.5 and 4.4 for $\xi = 0.5$ and $\xi = 0.2$, respectively). It also decreases with $\tilde{\rho}_c$. In addition, schematic representations of the corresponding ground state configurations are shown above and below the critical excess radius line.

Returning to the consideration of figure 6, the present model predicts zero line tension for the two possible ground state configurations corresponding to $\tilde{\rho} = 0$ and $\tilde{\rho} = 2\sqrt{\xi(1 - \xi)}$. In practice, however, it seems likely that, consistent with the results of Towles & Dan [37] and Trejo & Ben Amar [33], the line tension should have a positive non-zero lower bound. To rule out the vanishing of the line tension without recourse to any fundamental analysis of its nature, we may simply stipulate that it be greater than some chosen value. To illustrate the outcome of this strategy, we take the lower bound to be $\tilde{\tau}/\Pi_0 = 0.1$. Accordingly, only values of $\tilde{\rho}$ for which $\tilde{\tau}/\Pi_0$ exceeds 0.1 must be considered. Instead of adopting pinched-off or complete sphere configurations, the ground states then consist of a budded configuration (in which two spherical phases are connected by a very narrow neck) and a very slightly budded spherical domain of phase $\alpha$ on an otherwise spherical vesicle, as shown in figure 10. Both of these configurations are commonly observed in experiments [16,17,26].

It is important to investigate the extent to which including the bending energy in the net potential energy influences the equilibrium configurations of a two-phase vesicle. With this aim, we next compare the net potential energy given in (2.28) with the energy, given in (2.42), arising in the capillary limit. The variations of $\Psi/\Pi_0$ and $\Psi_{cap}/\Pi_0$ with $\tilde{\rho}$ are depicted in figure 11 for different values of the osmotic pressure $\Pi_0$ and two different choices $\xi = 0.5$ and $\xi = 0.2$ of the area ratio $\xi$. To allow comparisons of the results for different values of the osmotic pressure in the same plot, the net potential energies have been divided by $\Pi_0$. Note from (2.44) that $\Psi_{cap}/\Pi_0 = -4/3\pi\delta$, implying that $\Psi_{cap}/\Pi_0$ is independent of $\Pi_0$. Comparing the curves associated with each osmotic pressure value with the curve corresponding to the capillary approximation indicates that the bending energy is significant for the lower values of the osmotic pressure. Including the bending energy also changes the structure of the net potential-energy function. From figure 11, the capillary approximation to the net potential-energy function has a fixed local maximum and a global minimum at $\tilde{\rho} = 2\sqrt{\xi(1 - \xi)}$. However, including the bending energy alters the structure of the net potential-energy function. It is noteworthy that the critical osmotic pressure $\Pi_0$ arises only in the presence of the bending energy. Finally, as figure 11 reveals (in agreement with an observation of Allain & Ben Amar [18]), for sufficiently large values of the osmotic pressure the bending energy has a negligible contribution and the capillary approximation is acceptable.

As part of their study, Allain & Ben Amar [18] apply a methodology similar to that presented in §2 to consider the capillary limit of equilibrium of a two-phase vesicle composed of two spherical caps. By neglecting the bending energy of the domains, and taking the osmotic pressure to be constant while varying the volume enclosed by the vesicle, they arrive at a net potential energy that, apart from the geometrical parameters, depends only on the dimensionless quantity $\tau/\rho R_c^2$. If this quantity is taken to be a control parameter, their equations deliver two solutions for the equilibrium configurations. Allain & Ben Amar [18] present a plot similar to those in figure 6c or that in figure 10 (with horizontal and vertical axes interchanged). For any given $\tau/\rho R_c^2$, only the lowest energy solution is kept, while ruling out the remaining solution as unstable. The importance of a critical value of $\tau/\rho R_c^2$ corresponding to the maximum value of $\tilde{\tau}$ in figure 6 or $\tilde{\tau}/\Pi_0$ in figure 10, is addressed as well. For this critical value, the neck size $\tilde{\rho}$ is finite (cf., figure 10, where $\tilde{\rho} \approx 0.5$). Allain & Ben Amar [18] mention that if $\tau/\rho R_c^2$ exceeds this critical value, then no solution exists for the equilibrium configuration, but two disconnected spheres of each phase can provide a solution. However, a more detailed analysis would be needed to explain such a discontinuous transition. Indeed, Allain & Ben Amar [18, pp. 413–414] state that the transition is a dynamical process and has similarities with the breaking of a soap film between two rings (the catenoid instability) [41]. Nevertheless, complete fission requires a
microscopic reorganization, such as hemifission [42] which occurs at small scales and is out of reach of the present treatment. A detailed analysis may be found in [43] for example. It is why we cannot predict if the two daughter vesicles remain connected by a small filament of lipids or prefer to separate.

After discussing the capillary solution, Allain & Ben Amar [18] replace the sharp interface between the domains with a smooth boundary layer in which the bending energy of the boundary layer is incorporated.

A subtle difference between our approach to ground state configurations and the approach of Allain & Ben Amar [18] should be clarified. Because we consider the bending energy of the domains, our solution cannot be characterized by the single ratio \( \gamma / \rho R_c^2 \). Indeed, we consider \( \Pi_c \) as a control parameter. As the volume of the vesicle is allowed to change, we allow the system to take on different values for \( \rho \) between 0 ≤ \( \rho \) ≤ 2\( \sqrt{\xi (1 - \xi)} \), spanning from the pinched-off to the complete sphere configurations. Next, for each value of \( \Pi_c \) and each set of \( \rho, \theta_u \) and \( \phi \), the line tension \( \tau \) is calculated from (2.31). As our numerical results show, \( \tau = 2 \sqrt{\xi (1 - \xi)} \)

Figure 8. Variation of the net potential energy (a) \( \bar{\Psi} \) and the excess radius (b) \( \bar{z} \) with the size of the domain \( \bar{\rho} \) for \( \Pi_c = 10 \) and two values (i) \( \xi = 0.5 \) and (ii) \( \xi = 0.2 \) of the area ratio \( \xi \). (Online version in colour.)

Figure 9. Variations of the critical excess radius (a) \( \xi_c \) and the critical domain size (b) \( \bar{\rho}_c \) with the initial osmotic pressure \( \bar{\rho}_o \) for two values (i) \( \xi = 0.5 \) and (ii) \( \xi = 0.2 \) of the area ratio \( \xi \). (Online version in colour.)

Figure 10. Variation of the line tension (scaled by \( \Pi_c \)) by the domain size \( \bar{\rho} \) for the area ratio equal to \( \xi = 0.2 \) when a lower bound is considered. The shapes of the ground state configurations are also depicted schematically. (Online version in colour.)

Figure 11. Variation of \( \bar{\Psi} / \Pi_c \) with \( \bar{\rho} \) for different values of the osmotic pressure \( \Pi_o \), and two choices (a) \( \xi = 0.5 \) and (b) \( \xi = 0.2 \) of the area ratio \( \xi \). The first five lines from the top correspond to the net potential energy (with bending energy taken into consideration) and the first line from the bottom corresponds to the value of \( \bar{\Psi} / \Pi_c \) under the capillary approximation—namely, \( \bar{\Psi}_{\text{cap}} / \Pi_c \). The triangles show the local maxima of the curves. (Online version in colour.)

correspond to equilibrium configurations for \( \Pi_c < \Pi_c \). For \( \Pi_c > \Pi_c \) we have two equilibrium configurations corresponding to \( \bar{\rho} = 0 \) and \( \bar{\rho} = 2 \sqrt{\xi (1 - \xi)} \). In this case, we do not rule out the configuration with larger net potential energy. Rather, because there is an energy barrier between the two configurations, and as long as the energetic fluctuations are not large enough when compared with the height of this barrier, we believe that both configurations are possible; consistent with this, we regard \( \bar{\rho} = 2 \sqrt{\xi (1 - \xi)} \) as stable and \( \bar{\rho} = 0 \) as metastable. However, as mentioned previously, in view of other studies indicating a positive non-zero lower bound for the line tension [33,37], by setting a lower bound for \( \bar{\rho} / \Pi_c \), we may exclude the extreme cases corresponding to \( \bar{\rho} = 0 \) and \( \bar{\rho} = 2 \sqrt{\xi (1 - \xi)} \) in which \( \bar{\rho} = 0 \).

Also, as shown, the initial excess radius \( \xi_c \) of the vesicle has a strong influence on the nature of the final configuration adopted in any ground state.
4. Summary

A simple model was used to study the equilibrium of the multidomain configurations. Multidomain and ground state configurations were considered. In a multidomain configuration, the vesicle is covered by identical partially budded lipid domains. In a ground state configuration, the vesicle is composed of two coexisting lipid domains. Inspired by experimental observations in which lipid domains appear to be closely approximated by spherical caps, we assumed that all domains have constant radii and, for multidomain configurations, the host phase is assumed to have constant radius.

For multidomain configurations, we studied the net potential energy, the shape of the domains as characterized by their size and slope at the junction, and the line tension at the junctions for various sets of input parameters. In contrast to Yanagisawa et al. [10] and Semrau et al. [27], we ensured that force is balanced at the domain junctions. In this sense, our work is consistent with studies of fully phase-separated configurations conducted by Baumgart et al. [16, 17], Allain & Ben Amar [18] and Tian et al. [29]. As the area of the each phase and the volume of the vesicle are fixed, the geometry of the vesicle is independent of the osmotic pressure. But, as a result of the force balance at the domain junctions, the line tension depends linearly on the osmotic pressure. Thus, although the osmotic pressure increase does not energetically contribute by itself (because the volume is fixed), it increases the line tension and consequently the net potential energy of the system. Consistent with observations of Morales-Penningston et al. [29], this result suggests a preference for domain coalescence at sufficiently high osmotic pressures. Also, the numerical results showed that the line tension decreases by increase of number of domains. However, while this observation is valid for more than three domains, it might not hold in the transition from a pair of domains to a single domain.

A vesicle in a ground state configuration was assumed to be formed by coalescence of distributed domains of a vesicle in the multidomain configuration. As the characteristic time needed to attain such a configuration may be comparable to or greater than that associated with solvent transport across the membrane, we allowed the volume enclosed the vesicle to be non-constant. This stands in contrast to our study of multidomain configurations, which generally form on a time-scale short compared with that associated with solvent transport across the membrane. Allowing for variations of the volume enclosed by the vesicle requires that a volumetric contribution involving the osmotic pressure be included in the net potential energy. In this context, we considered the consequences of the van’t Hoff [46] relation, wherein the osmotic pressure is linearly proportional to the solute concentration. Treating the osmotic pressure as a control parameter, the dependence of net potential energy as a function of the size of domain was shown to depend on whether the osmotic pressure $\Pi_c$ is below or above a critical value $\Pi_c$.

For osmotic pressures below $\Pi_c$, the net potential energy has an absolute minimum at a configuration in which the vesicle resembles a complete sphere divided into two domains of identical radius. For osmotic pressures above $\Pi_c$, aside from a complete sphere configuration like that arising for osmotic pressures below $\Pi_c$, the net potential energy possesses a local minimum, corresponding to two pinched-off spheres of pure phase. These two configurations are separated by an energy barrier that allows both configurations be possible, at least in the absence of energy fluctuations sufficiently large to induce transitions from well to well. We proposed a criterion for determining which of these ground state configurations will prevail based on comparing the value of the excess radius of the vesicle prior to phase separation (or in a multidomain configuration) with a critical excess radius. We also showed that this critical excess radius decreases by increase of the osmotic pressure $\Pi_c$. Motivated by works of Towles & Dan [37] and Trejo & Ben Amar [33], we considered a positive non-zero lower bound for the line tension, leading to slight modifications of two possible ground state configurations. Finally, we studied the effect of the bending energy of the phases. The results revealed that, for sufficiently small values of the osmotic pressure, the bending energy can significantly influence the ground state configuration of a two-phase vesicle.

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References