Impermeability effects in three-dimensional vesicles

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Abstract

We analyse the effects that the impermeability constraint induces on the equilibrium shapes of a three-dimensional vesicle hosting a rigid inclusion. A given alteration of the inclusion and/or vesicle parameters leads to shape modifications of different orders of magnitude, when applied to permeable or impermeable vesicles. Moreover, the enclosed-volume constraint wrecks the uniqueness of stationary equilibrium shapes, and gives rise to pear-shaped or stomatocyte-like vesicles.

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1 Introduction

Lipid proteins embedded in biological membranes strongly influence both the geometric and the elastic properties of the hosting membranes. The rigid inclusions are able to induce vesicle budding \[1, 2, 3\], while the interplay between the protein-membrane interaction and the spontaneous curvature may yield a loss of regular equilibrium configurations \[4\]. Moreover, the elasticity of the hosting vesicle induces a membrane-mediated interaction that has been widely studied both experimentally \[5\] and theoretically, in the cases of planar \[6, 7\], quasi-planar \[8, 9, 10\], and quasi-spherical vesicles \[11\].

In this paper we focus attention on the equilibrium shapes of a three-dimensional vesicle hosting a rigid inclusion, and in particular on the effects of the permeability properties of the vesicle. In fact, a given slight perturbation applied to a quasi-spherical vesicle may induce quite different changes on the resulting equilibrium shape, depending on whether the enclosed volume of the vesicle is constrained or not. More precisely, an \(O(\epsilon)\) relative perturbation of the external parameters induces an equivalent \(O(\epsilon)\) modification in the shape function of a permeable vesicle, but a quite stronger \(O(\sqrt{\epsilon})\) relative perturbation if the enclosed volume is kept fixed. Furthermore, the volume constraint yields a multiplicity of stationary equilibrium shapes, and it leads to abandon the spherical shape towards either pear-shaped or stomatocyte-like vesicles.

The volume enclosed by the vesicle may be constrained or not depending on both the chemical properties of the aqueous solution which surrounds the vesicle, and the time scales in which the (meta)equilibrium shapes are observed \[12, 13\]. When the water is essentially free of molecules that cannot permeate the bilayer membrane, no volume constraint stands. On the contrary, when some of the molecules in the solution are unable to permeate the bilayer, the resulting osmotic pressure gives rise to an enclosed-volume constraint. However, even in this latter case, on long time scales water molecules succeed in permeating the membrane. Eventually, the vesicle reaches its true equilibrium shape, which minimizes the free energy with respect to the enclosed volume.

Throughout our development, we will model proteins as rigid inclusions, and we will assume that the protein-membrane interaction simply fixes the contact angle, i.e. the angle the vesicle normal determines with the inclusion plane \[14, 8, 15\]. However, only slight modifications need to be applied to our results to take into account interactions which determine the contact curvature instead of the contact angle \[16, 17, 18\]. More drastic changes, though substantially the same mathematical setting, requires the weak anchoring case \[15, 19, 4\], in which the inclusion-vesicle interaction provides an additional term in the free-energy functional, instead of a fixed boundary condition.

We describe vesicle elasticity through Helfrich’s (spontaneous curvature) model \[20, 21\]. The free-energy functional is then

\[
\mathcal{F}[\Sigma] := \kappa \int_{\Sigma} (H - \sigma_0)^2 \, da,
\]

where \(\Sigma\) is a closed surface describing the vesicle shape, \(H\) denotes the mean curvature along \(\Sigma\), \(\kappa\) is the bending energy, and \(\sigma_0\) the spontaneous curvature. In the minimizing procedure at fixed area (and possibly fixed volume) we replace (1.1) with the effective free-energy functional

\[
\mathcal{F}_{\text{eff}}[\Sigma] := \kappa \int_{\Sigma} (H - \sigma_0)^2 \, da + \lambda \left( \text{Area}(\Sigma) - A \right) + \left[ \mu \left( \text{Vol}(\Sigma) - V \right) \right] .
\]

The Lagrange multipliers \(\lambda, \mu\) have the physical meaning of surface tension and pressure difference, and the brackets are there to remind that the volume constraint will not be always applied. In \[12\] the area constraint has been inserted as a global, instead of a local constraint. We recall that, in the absence of external forces, both choices are equivalent \[22\].

The Euler-Lagrange equation associated to the functional \[12\] is the shape equation \[22, 23\]:

\[
\kappa \left[ \Delta_s H + 2H \left( H^2 - K \right) + 2\sigma_0 K - 2\sigma_0^2 H \right] - 2\lambda H - \left[ \mu \right] = 0,
\]

where \(\Delta_s\), the tangential divergence of the tangential gradient, is the Laplace-Beltrami operator on \(\Sigma\), and \(K\) denotes the Gaussian curvature along \(\Sigma\).
The plan of the paper is as follows. In the next section, we introduce the surface parameterization and we derive the conditions satisfied by spherical and quasi-spherical shapes. In Section 3 we analyse the vesicle shapes obtained in the presence of the area constraint alone \( (i.e., \) the long-time equilibrium vesicle shapes). Section 4 is devoted to the peculiar role played by the volume constraint. In Section 5 we review and discuss our results.

2 The model

Let us consider a vesicle which embeds an inclusion, that we model as a symmetric conical frustum of negligible height, base radius \( a \), and apex angle \( \psi \). The inclusion-vesicle interaction fixes the angle between the vesicle normal and the inclusion plane at the contact points to be equal to \( \left( \frac{\pi}{2} - \psi \right) \). Figure 1 illustrates the geometric setup of the model. For a more detailed description of the inclusion-vesicle interactions and their modeling we refer the reader to the paper by Biscari and Rosso [4].

We restrict our analysis to axisymmetric vesicle shapes, and parameterize them in spherical coordinates centered in a fixed point \( O \), which lies in the inclusion symmetry axis \( z \) (we assume that this is the symmetry axis of the vesicle too, since the inclusion does not upset the cylindrical symmetry):

\[
P(\vartheta, \varphi) - O = r(\vartheta) \sin \vartheta \cos \varphi \mathbf{e}_x + r(\vartheta) \sin \varphi \mathbf{e}_y + r(\vartheta) \cos \varphi \mathbf{e}_z .
\]

The unit vectors \( \{\mathbf{e}_x, \mathbf{e}_y, \mathbf{e}_z\} \) form an orthogonal basis, with \( \mathbf{e}_z \) parallel to the inclusion axis; \( (\vartheta, \varphi) \) are the polar and azimuthal angles, respectively. The area element, the Laplace-Beltrami operator, the mean curvature, and the Gaussian curvature along \( \Sigma \) are given by:

\[
da = \sqrt{g} \, d\vartheta \, d\varphi , \quad \Delta = \frac{1}{\sqrt{g}} \left[ \partial_\vartheta \left( \frac{r \sin \vartheta}{\sqrt{r^2 + r^2}} \partial_\vartheta \right) + \partial_\varphi \left( \frac{\sqrt{r^2 + r^2}}{r \sin \vartheta} \partial_\varphi \right) \right] ,
\]

\[
H = \frac{2r^3 + 3rr'^2 - r^3 \cot \vartheta - r^2 (r' \cot \vartheta + r'')} {2r (r^2 + r'^2)^{3/2}} , \quad \text{and}
\]

\[
K = \frac{(r \sin \vartheta - r' \cos \vartheta) (r^2 + 2r^2 - r r'')} {r \sin \vartheta (r^2 + r'^2)^2} ,
\]

where a prime denotes differentiation with respect to the polar angle, and \( g := (r^2 + r'^2) r \sin^2 \vartheta \).
The spherical parameterization transforms the effective free energy \( \mathcal{F}_{\text{eff}} \) as follows:

\[
\mathcal{F}_{\text{eff}}[r] = 2\pi \int_{\vartheta_{\text{f}}}^{\vartheta_{t}} \left( \kappa (H[r] - \sigma_0)^2 + \lambda \right) r \sqrt{r^2 + r'^2} \sin \vartheta \, d\vartheta + \left[ \frac{2\pi}{3} \mu \int_{\vartheta_{\text{f}}}^{\vartheta_{t}} r^3 \sin \vartheta \, d\vartheta \right].
\]

The integration limit \( \vartheta_{\text{f}} \) depends on the position of the origin \( O \). We choose to fix \( O \) at a distance \( \Delta z = (a \cot \psi) \) above the inclusion, which yields \( \vartheta_{\text{f}} = (\pi - \psi) \). This choice simplifies the attachment condition on the inclusion and the constraint on the direction of the contact normal, which become:

\[
r(\vartheta_{t}) = \frac{a}{\sin \psi} \quad \text{and} \quad r'(\vartheta_{t}) = 0 .
\]

The vesicle is free on its top \( (\vartheta = 0) \). The boundary conditions therein follow from regularity requirements on the vesicle shape:

\[
\lim_{\vartheta \to 0^+} r'(\vartheta) = 0 \quad \text{and} \quad \lim_{\vartheta \to 0^+} r''(\vartheta) = 0 .
\]

### 2.1 Internal actions

Let us consider a subsurface \( \Sigma' \subseteq \Sigma \), and let \( \mathbf{n} \) and \( \mathbf{t} \) respectively denote the normal to \( \Sigma \) at a point \( P \in \partial \Sigma' \), and the tangent to the curve \( \partial \Sigma' \). Furthermore, let \( \mathbf{k} := \mathbf{n} \wedge \mathbf{t} \) denote the direction in the tangent plane at \( P \) pointing outwards with respect to \( \Sigma' \). The internal actions at \( P \) consist in a distributed force \( \mathbf{f} \) and a distributed torque \( \mathbf{m} \), whose densities per unit length of \( \partial \Sigma' \) are

\[
\mathbf{f} = \left[ \kappa (H - \sigma_0)^2 + \lambda \right] \mathbf{k} - \kappa \frac{\partial H}{\partial k} \mathbf{n} \quad \text{and} \quad \mathbf{m} = \kappa (H - \sigma_0) \mathbf{n} .
\]

The above equations generalize to three-dimensional vesicles the internal actions derived in [7] in the two-dimensional case. They show that the surface tension \( \lambda \) may become negative without giving rise to the collapse of the vesicle, provided that \( \mathbf{f} \cdot \mathbf{k} = \left[ \kappa (H - \sigma_0)^2 + \lambda \right] \) remains non-negative all along the vesicle. Furthermore, they provide an alternative way of deriving the free-boundary conditions (2.2). In fact, these conditions are equivalent to the vanishing of the internal force and torque acting on an infinitesimal cap which surrounds the vesicle top.

### 2.2 Spherical shapes

The equilibrium shape of a vesicle is a sphere of radius \( r_o \), centered in \( O \), whenever the base radius \( a_o \), the apex angle \( \psi_o \), and the vesicle area \( A_o \) satisfy

\[
a_o = r_o \sin \psi_o \quad \text{and} \quad A_o = 4\pi r_o^2 \cos^2 \frac{\psi_o}{2} .
\]

If we eliminate \( r_o \) from equations (2.3) \_1 and (2.3) \_2, we obtain:

\[
\pi a_o^2 = A_o \sin^2 \frac{\psi_o}{2} .
\]

If, in addition, the vesicle is impermeable, the enclosed volume \( V_o \) must match

\[
V_o = \frac{4}{3} \pi r_o^2 \cos^2 \frac{\psi_o}{2} + \frac{1}{3} \pi a_o^2 r_o \cos \psi_o = \frac{\pi a_o^3}{6} \cos \frac{\psi_o}{\sin^3 \frac{\psi_o}{2}} (2 - \cos \psi_o) .
\]

In particular, area, enclosed volume, and apex angle must obey:

\[
v_o := \frac{36\pi V_o^2}{A_o^3} = (2 - \cos \psi_o)^2 \cos^2 \frac{\psi_o}{2} .
\]

We remark that for any value in \( v_o \in [1, 2] \) (the end cases corresponding to the cases of a sphere and a half-sphere), there is exactly one value of \( \psi_o \in [0, \frac{\pi}{2}] \) that satisfies (2.4).
2.3 Quasi-spherical shapes

We now assume that some of the control parameters $a$, $\psi$, $A$ (and possibly $V$) are slightly perturbed with respect to their values satisfying (2.3) and (2.4). In this case, we look for solutions of the shape equation (1.3) that represent a perturbation of a sphere:

$$r(\vartheta) = r_0 \left(1 + \epsilon \varrho_1(\vartheta) + o(\epsilon)\right). \tag{2.5}$$

Consistently, we also expand the Lagrange multipliers by perturbing their “spherical” values:

$$\lambda = \frac{\kappa}{r_0^2} (\Lambda_0 + \epsilon \Lambda_1 + o(\epsilon)) \quad \text{and} \quad \mu = \frac{2\kappa}{r_0^3} (\eta_0 + \epsilon \eta_1 + o(\epsilon)). \tag{2.6}$$

In (2.6), the normalizing factors $\kappa$ and $r_0$ have been inserted in order to proceed with the dimensionless quantities $\Lambda_1$ and $\eta_1$. Furthermore, in order to make the whole shape equation dimensionless, we define the reduced spontaneous curvature as

$$\varsigma_0 := \varsigma_0 r_0. \tag{2.7}$$

If we insert (2.5), (2.6) and (2.7) in (1.3), we derive at $O(1)$ the condition

$$\Lambda_0 + \eta_0 = \varsigma_0 (1 - \varsigma_0), \tag{2.8}$$

linking the spherical values of the Lagrange multipliers.

When we push the expansion to $O(\epsilon)$, we obtain a fourth-order linear differential equation for $\varrho_1$. If we further introduce the variable $s := \cos \vartheta$, and perform the substitution $\varrho_1(\vartheta) = \omega_1(\cos \vartheta)$, the so-obtained differential equation reads as:

$$(1 - s^2)^2 \omega^{(4)} - 8s (1 - s^2) \omega^{(3)} + \left[12s^2 - 4 + g_1 (1 - s^2)\right] \omega^{(2)} - 2g_1 s \omega^{(1)} + g_0 \omega = -4(\Lambda_1 + \eta_1), \tag{2.9}$$

where

$$g_0 = 2g_1 := 4(\varsigma_0 (2 - \varsigma_0) - \Lambda_0),$$

and the superscripts denote differentiation with respect to $s$. Equation (2.9) is an inhomogeneous fourth-order Legendre differential equation. Its general solution can be expressed in terms of Legendre functions of the first and second kind as:

$$\omega(s) = -\frac{4(\Lambda_1 + \eta_1)}{g_0} + C_1 P_{\nu_+}(s) + C_2 Q_{\nu_+}(s) + C_3 P_{\nu_-}(s) + C_4 Q_{\nu_-}(s),$$

where the orders of the Legendre functions are given by

$$\nu_{\pm} = -\frac{1}{2} + \frac{1}{2} \sqrt{5 + 2g_1 \pm 2\sqrt{(g_1 + 2)^2 - 4g_0}}. \tag{2.10}$$

If we replace $g_0 = 2g_1$ in (2.10), we obtain

$$\nu_{\pm} = -\frac{1}{2} + \frac{1}{2} \sqrt{5 + 2g_1 \pm 2|g_1 - 2|},$$

so that

$$\{\nu_+, \nu_-\} = \left\{1, \frac{1}{2} \left(\sqrt{1 + 4g_1} - 1\right)\right\}.$$
Some further investigation will turn out to be necessary in the particular cases \( \nu = 0 \) (i.e. \( g_1 = 0 \)) and \( \nu = 1 \) (i.e. \( g_1 = 2 \)). We postpone the analysis of these cases to the sections below.

In the following, we will analyse and compare the perturbed equilibrium vesicle shapes of permeable and impermeable vesicles. The derivation below works when any or even all of the parameters \( a, \psi, A, \) or \( V \) are varied with respect to their spherical values. However, and only in order to shorten our presentation, we will henceforth restrict our development to the case in which only the area, and possibly the enclosed volume, are varied with respect to the values satisfying (2.3) and (2.4), while the inclusion parameters are kept unchanged. These are the easiest perturbations to be implemented experimentally: for example, an area variation in a biological membrane may be simply induced by adding extra lipid molecules to the membrane bilayer.

## 3 Permeable vesicles

When the vesicle is inextensible but permeable, no volume constraint stands. For all practical purposes, this is equivalent to assume that the Lagrange multiplier \( \mu \) vanishes identically. When this is the case, condition (2.8) reads as

\[
\Lambda_0 = \psi_0 (1 - \psi_0)
\]

which implies \( g_0 = 2g_1 = 4\psi_0 \), and

\[
\nu = \frac{1}{2} (\sqrt{1 + 8\psi_0} - 1) \quad (3.1)
\]

The linear perturbation of the spherical shape becomes:

\[
\varrho_1(\vartheta) = \frac{\Lambda_1}{\psi_0} + C_1 \cos \vartheta + C_2 Q_1(\cos \vartheta) + C_3 P_3(\cos \vartheta) + C_4 Q_4(\cos \vartheta).
\]

(3.2)

All Legendre functions of the second kind \( Q_{\nu}(s) \) are singular when \( s \to 1^- \). Thus, the free-boundary conditions (2.2) require \( C_2 = C_4 = 0 \). The remaining parameters \( \Lambda_1, C_1, \) and \( C_3 \) can be determined with the aid of the contact conditions (2.1) and the area constraint:

\[
\varrho_1(\vartheta) = 0, \quad \varrho_1'(\vartheta) = 0, \quad \text{and} \quad 4\pi r_0^2 \int_0^{\theta_1} \varrho_1(\vartheta) \sin \vartheta d\vartheta = \Delta A,
\]

(3.3)

where \( \Delta A \) is the area excess with respect to the spherical value \( A_0 \). With the aid of (A1.2)-(A1.4) conditions (3.3) yield

\[
\Lambda_1 = \psi_0 C_3 \csc^2 \psi_0 \left[ \nu \cos \psi_o P_{\nu-1}(-\cos \psi_o) + (\sin^2 \psi_o + \nu \cos^2 \psi_o) P_\nu(-\cos \psi_o) \right],
\]

\[
C_1 = -\nu C_3 \csc^2 \psi_0 \left[ P_{\nu-1}(-\cos \psi_o) + \cos \psi_o P_\nu\nu(-\cos \psi_o) \right], \quad \text{and}
\]

\[
C_3 = \left( \frac{4(P_{\nu-1}(-\cos \psi_o) - P_\nu\nu(-\cos \psi_o))}{1 + \cos \psi_o} \right) \left( \frac{\nu + 1)(1 - \cos \psi_o)}{(\nu - 1)(\nu \cos \psi_o + 2)P_\nu(-\cos \psi_o) - (\nu^2 + \nu + 2)P_{\nu-1}(-\cos \psi_o)} \right) = \frac{\Delta A}{A_0}.
\]

We have already announced that the differential equation (2.9) admits (3.2) as its general solution only when \( \nu \not\in \{0, 1\} \), that is when the spontaneous curvature is neither null nor equal to the inverse of the unperturbed radius \( r_0 \). We will now solve (2.9) in these cases.

When \( \psi_0 = 0 \), the general solution of the homogeneous differential equation associated to (2.9) is still as in (3.2), with \( \nu = 0 \). However, and since \( P_0(s) = 1 \), the particular solution of the equation is not a constant. By using the method of variation of parameters, and requiring also the free-boundary conditions (2.2), we find:

\[
\varrho_1^{(\nu=0)}(\vartheta) = C_1 \cos \vartheta + C_3 + \Lambda_1 \left( 3 + 2 \log(1 + \cos \vartheta) \right).
\]
In addition, conditions (3.3) yield:

\[ \Lambda_1 = -C_1 \sin^2 \frac{\psi_0}{2}, \quad C_3 = C_1 \left[ 1 + \left( 1 + 2 \ln \left( \frac{\sin^2 \frac{\psi_0}{2}}{2} \right) \right) \sin^2 \frac{\psi_0}{2} \right], \]

and

\[ 2 \left( 1 + \sin^2 \frac{\psi_0}{2} + 2 \tan^2 \frac{\psi_0}{2} \ln \sin^2 \frac{\psi_0}{2} \right) C_1 = \frac{\Delta A}{A_o}. \]

When \( \varsigma_0 = 1 \), the particular solution of the differential equation (2.9) is again a constant, but the solution of the homogeneous equation is not of the form (3.2). If we solve (2.9) explicitly and use the free-boundary conditions (2.2), we arrive at:

\[ \varrho_1^{(\varsigma_0=1)}(\vartheta) = C_1 \cos \vartheta + C_3 \left( \cos \vartheta \ln(1 + \cos \vartheta) - 1 \right) - \Lambda_1. \]

Finally, conditions (3.3) now require:

\[ C_1 = -C_3 \left( \ln \left( 1 - \cos \psi_o \right) - \frac{\cos \psi_o}{1 - \cos \psi_o} \right), \quad \Lambda_1 = -C_3 \left( 1 + \frac{\cos^2 \psi_o}{1 - \cos \psi_o} \right), \quad \text{and} \]

\[ \cos^2 \frac{\psi_o}{2} \cot^2 \frac{\psi_o}{2} C_3 = \frac{\Delta A}{A_o}. \]

Figure 2 shows how the perturbed vesicle shape depends on the spontaneous curvature for a prescribed area increase (5% with respect to the spherical value), while Figure 3 shows the volume variation induced by \( \Delta A \).

- Since the inclusion is placed at \( \vartheta = \vartheta_f \), Figure 2 shows that in vesicles characterized by greater spontaneous curvatures the shape modifications gather away from the inclusion.

- An area increase induces a shape perturbation \( \varrho_1 \) that does not change sign all along the vesicle (we will find below that this is not the case when also the volume is constrained).

- Figure 3 shows that the volume increase induced by \( \Delta A \) increases monotonically with \( \Delta A \). However, the remarkable increase in \( \Delta V \) that shows up when \( \varsigma_0 \simeq 3 \) is to be linked with the spontaneous-curvature driven budding transition [24] that is close to occur. A detailed analysis of the inclusion’s influence on this transition can be performed only by studying the nonlinear shape equation (1.3), and will be reported elsewhere [25].

### 3.1 Small inclusions

By using the asymptotic expansion (A1.5), it is possible to show that in the small inclusion limit \( a \ll \sqrt{A_o} \), which implies \( \psi_o \ll 1 \) by virtue of (2.6), the perturbed vesicle shape becomes independent of \( \varsigma_0 \):

\[ r(\vartheta) = r_o \left( 1 + \frac{\Delta A}{A_o} \frac{1 + \cos \vartheta}{2} \right) + O \left( \psi_o^2 \ln \psi_o, \left( \frac{\Delta A}{A_o} \right)^2 \right). \]  

Figure 2 shows that when the spontaneous curvature is small, the asymptotic expression (3.4) is more rapidly approached. In fact, if we compute the volume variation associated with the perturbed shape (3.4) we obtain:

\[ \frac{\Delta V}{V_o} = \frac{3}{2} \frac{\Delta A}{A_o} + O \left( \psi_o^2 \ln \psi_o, \left( \frac{\Delta A}{A_o} \right)^2 \right). \]

Figure 3 confirms that the relative volume variation is closer to the small limit prediction \( \frac{3}{2} \Delta A/A_o \) when \( \varsigma_0 \) is small.
Figure 2: Perturbed shapes of a vesicle hosting an inclusion, when the area is slightly greater than the area corresponding to a spherical equilibrium solution. The plots correspond to $\psi_0 = 0.1\pi$, $\Delta A = 0.05 A_0$, and $\varsigma_0 = 0, 1, 2, 3$ (the arrow points towards increasing values of $\varsigma_0$).

Figure 3: Volume variation induced by a given area variation in a permeable vesicle hosting an inclusion, as a function of the reduced spontaneous curvature $\varsigma_0$. As in Figure 2 $\psi_0 = 0.1\pi$ and $\Delta A = 0.05 A_0$. 
4 Impermeable vesicles

We now focus on the solutions of the differential equation (2.9) that satisfy both area and volume constraints, when these geometrical quantities are close to satisfy the spherical condition (2.11). Leaving aside for the moment the cases \( \nu = 0 \) and \( \nu = 1 \) (that turn out to be meaningless in the impermeable case), the solution of (2.9) is of the form (2.12) with the following parameters to be determined: \( C_1, C_2, C_3, C_4, A_0 \) (i.e. \( \nu \), by virtue of (2.11)), and the combination \((\Lambda_1 + \eta_1)\).

4.1 Singular perturbations

The boundary conditions (2.1) and (2.2) may determine the four parameters \( C_1-C_4 \). A problem arises when we try to determine \( \nu \) by using the area and volume constraints. In fact, the \( O(\epsilon) \) of the above constraints reads as:

\[
\int_0^{\vartheta_1} \varrho_1(\vartheta) \sin \vartheta \, d\vartheta = \frac{\Delta A}{4\pi r_o^2}, \quad \int_0^{\vartheta_1} \varrho_1(\vartheta) \sin \vartheta \, d\vartheta = \frac{\Delta V}{2\pi r_o^3}.
\]

(4.1)

It is clearly impossible to satisfy both constraints if

\[ \Delta A \neq \frac{2}{r_o} \Delta V. \]

From the analytical point of view, the degeneracy of the constraints (4.1) stems from the fact that the first variations of area and volume of a surface are linearly dependent when computed in a spherical shape. In fact, if we perturb any surface with constant mean curvature \( H \) (the so-called Delaunay surfaces [26, 27]), the area and volume of the resulting surface satisfy [23]

\[ \Delta A = 2H \Delta V. \]

The area and volume constraints become linearly independent only when the second variations come into play. Thus, if we are willing to perturb the assigned area and volume to an arbitrary \( O(\epsilon) \), we have to perturb the shape function \( r(\vartheta) \) to \( O(\sqrt{\epsilon}) \), as we shall show next.

We begin by replacing (2.9) by

\[ r(\vartheta) = r_o \left( 1 + \sqrt{\epsilon} \varrho_+^o(\vartheta) + \epsilon \varrho_1(\vartheta) + o(\epsilon) \right). \]

(4.2)

The singular perturbation \( \varrho_+^o \) is of the form (2.12), with \( \Lambda_1 \) and \( \eta_1 \) replaced by their half-order counterparts \( \Lambda_+^\frac{1}{2} \) and \( \eta_+^\frac{1}{2} \). Correspondingly, the area and enclosed volume of the resulting vesicle shape are given by:

\[
A_o + \epsilon \Delta A = 2\pi \int_0^{\vartheta_1} r \sqrt{r^2 + r_o^2} \sin \vartheta \, d\vartheta = A_o + 4\pi r_o^2 \sqrt{\epsilon} \int_0^{\vartheta_1} \varrho_+^o \sin \vartheta \, d\vartheta + \pi r_o^2 \epsilon \int_0^{\vartheta_1} \left( 2\varrho_+^o + \varrho_+^2 + 4\varrho_1 \right) \sin \vartheta \, d\vartheta + o(\epsilon); \quad (4.3)
\]

\[
V_o + \epsilon \Delta V = \frac{2\pi}{3} \int_0^{\vartheta_1} r^3(\vartheta) \sin \vartheta \, d\vartheta = V_o + 2\pi r_o^3 \sqrt{\epsilon} \int_0^{\vartheta_1} \varrho_+^o \sin \vartheta \, d\vartheta + \pi r_o^3 \epsilon \int_0^{\vartheta_1} \left( 2\varrho_+^o + 2\varrho_1 \right) \sin \vartheta \, d\vartheta + o(\epsilon). \quad (4.4)
\]

Both (4.3) and (4.4) can now be satisfied, provided the functions \( \varrho_+^o \) and \( \varrho_1 \) are such that:

\[
\int_0^{\vartheta_1} \varrho_+^o(\vartheta) \sin \vartheta \, d\vartheta = 0, \quad (4.5)
\]

\[
\int_0^{\vartheta_1} \left[ \varrho_+^2(\vartheta) - 2\varrho_+^o(\vartheta) \right] \sin \vartheta \, d\vartheta = \frac{r_o \Delta A - 2\Delta V}{\pi r_o^3}, \quad \text{and} \quad (4.6)
\]

\[
\int_0^{\vartheta_1} \varrho_1(\vartheta) \sin \vartheta \, d\vartheta = \frac{\Delta V}{2\pi r_o^3} - \int_0^{\vartheta_1} \varrho_+^o(\vartheta) \sin \vartheta \, d\vartheta. \quad (4.7)
\]
Equations (4.5) and (4.6) fix $\varrho_2^1$, as we will show below; then, equation (4.7) allows to determine also the now next-order correction $\varrho_1$.

Before entering in the detailed analysis of equations (4.5) and (4.6), the above result deserves some remarks.

- Equation (4.2) underlines the most striking effect of the impermeability constraint on the vesicle: an $O(\epsilon)$ perturbation of the assigned geometrical values induces an $O(\sqrt{\epsilon})$ perturbation in the vesicle shape.
- Equation (4.5) is an eigenvalue equation. We have to look for non-trivial (i.e., non-vanishing) perturbations $\varrho_2^1$ that satisfy it. It will prove to admit a countable infinity of independent solutions.
- Equation (4.6) is quadratic in $\varrho_2^1$. We will thus find two, rather than one, possible perturbed shapes for any non-trivial solution of (4.5). An energy argument will be needed to identify the preferred perturbation among the double-infinity of possible choices at our disposal. Both perturbed shapes arising from (4.6) will deserve notice, since they display two qualitatively different vesicle reactions to the perturbation.
- The quadratic expression in the left-hand side of (4.6) forces the right-hand side combination $(r_2 A - 2\Delta V)$ to assume only non-negative values. This property is not peculiar of vesicle theory: it reflects a classical isoperimetric inequality. In fact, for any closed surface, the ratio $A^2/V^2$ is bounded from below by the value $36\pi$, which is attained only by a sphere (see, e.g., [28], p. 8). Thus, for example, it does not exist a closed surface with the same enclosed volume of a sphere and smaller area.

### 4.2 Multiplicity of stationary perturbed shapes

The free boundary conditions (2.12) require that the coefficients of the singular Legendre functions of the second kind in (2.12) must be null: $C_2 = C_4 = 0$. Afterwards, the contact conditions (2.11), which are linear in the shape function, supply two relations that connect $C_1$, $C_3$, and $(\Lambda_1^1 + \eta_1^1)$ in (2.12). As a result, the leading perturbation $\varrho_2^1$ in (4.2) can be given the form:

$$\varrho_2^1(\vartheta) = \left[C_3 \left[\alpha(\nu, \psi_0) + \beta(\nu, \psi_0) \cos \vartheta + P_\nu \cos \vartheta\right]\right], \quad \text{with} \quad (4.8)$$

$$\alpha(\nu, \psi_0) := -\csc^2 \psi_0 \nu \cos \psi_0 P_{\nu-1}(-\cos \psi_0) + (\sin^2 \psi_0 + \nu \cos^2 \psi_0)P_\nu(-\cos \psi_0)$$

$$\beta(\nu, \psi_0) := -\nu \csc^2 \psi_0 \nu \cos \psi_0 P_{\nu-1}(-\cos \psi_0) + \cos \psi_0 \cos \psi_0 P_\nu(-\cos \psi_0).$$

In order to determine completely the function $\varrho_2^1$, we have to use (4.5) and (4.6) to derive $\nu$ and $C_3$. Any non-trivial solution of (4.6) possesses $C_3 \neq 0$. Thus, we can drop $C_3$ out from it, to obtain an eigenvalue equation in $\nu$, depending only on $\psi_0$. Figure 4 illustrates the numerical solutions of (4.6). These solutions exhibit the following properties:

- The spontaneous curvature does not enter in (4.6). Thus, the stationary shape modifications of an impermeable vesicle do not depend on $\varrho_0$, as they did in the permeable case (see (3.1) and Figure 2).
- For any $\psi_0 \in \left[0, \frac{\pi}{2}\right]$, there is a countable infinity of values $\nu_k(\psi_0)$ satisfying (4.6).
- For any $\psi_0 \in \left[0, \frac{\pi}{2}\right]$, the solutions $\nu_k(\psi_0)$ are symmetric with respect to $\nu = -\frac{1}{2}$. However, since the symmetric solutions are identical (see (A1.1)), we can restrict our attention to solutions with $\nu \geq -\frac{1}{2}$.
- When $\psi_0 \ll 1$ (small protein limit), the use of (A1.5) allows to prove that $\nu_k$ tends to an integer value for any $k$:

$$\nu_k(\psi_0) = (k + 1) + \frac{k - 1}{4} \psi_0^2 + o(\psi_0^2) \quad \text{for any} \quad k \in \mathbb{N}. \quad (4.9)$$
The shape function thus approaches a linear combination of Legendre functions of integer order, that is, Legendre polynomials. Furthermore, both (4.9) and Figure 4 show that only Legendre polynomials of order equal to or greater than 2 come into play, while only low-order Legendre polynomials \((P_0 \text{ and } P_1)\) entered the small-protein limit of permeable vesicles (see (3.4)). This yields more drastic shape modifications in the incompressible case, since Legendre polynomials are more and more oscillating as their order increases. The expansion of the shape function (in the absence of inclusions) in terms of Legendre polynomials was first used in [24].

- For any \(k \in \mathbb{N}\), the functions \(\nu_k(\psi)\) increase monotonically with \(\psi\), and do not intersect.
- It is possible to prove by direct inspection that \(\nu = 0\) and \(\nu = 1\) do not solve (4.5) for any value of \(\psi\).

\[
\begin{align*}
\psi_o & \quad \nu_k \\
\frac{\pi}{6} & \quad 5 \\
\frac{\pi}{4} & \quad 4 \\
\frac{\pi}{3} & \quad 3 \\
\frac{\pi}{2} & \quad 2 \\
\pi & \quad 1 \\
\end{align*}
\]

Figure 4: Order \(\nu\) of the Legendre functions entering in the perturbation of an impermeable vesicle shape, as a function of the inclusion apex angle \(\psi_o\). The graphs display the smallest five numerical solutions \(\{\nu_k, k = 1, \ldots, 5\}\) of the equation (4.5).

Once we have identified the \(\nu\)-values that satisfy (4.5), we can insert (4.8) in (4.6) to determine \(C_3\), the only remaining free parameter in the singular perturbation \(\varrho_{1/2}\). We stress again that, being \(\varrho_{1/2}\) linear in \(C_3\), this latter parameter enters quadratically in (4.6). This fact, on the one hand fixes a sign for the geometrical quantity \((r_o \Delta A - 2 \Delta V)\), and on the other hand implies that, for any \(k \in \mathbb{N}\) with \(\nu = \nu_k(\psi_o)\), there are exactly two values of \(C_3\) (one the opposite of the other) that satisfy (4.6). Thus, for any \(k \in \mathbb{N}\), there are two possible perturbed shapes:

\[
r_{k \pm}(\vartheta) = r_o \left(1 \pm \sqrt{\varrho_{1/2,k}(\vartheta)} + O(\epsilon)\right).
\]

Only an energy estimate can help us in determining, for any \(\psi_o \in [0, \pi/2]\), both the value of \(\nu_k\) and the sign of \(C_3\) that minimize the elastic energy. This will be the aim of the remaining part of this section.

### 4.3 Energy estimates

#### 4.3.1 Ground state energy

In order to identify which is energetically preferred among the stationary perturbed shapes determined above, we will now compute their elastic energy. When we insert (4.2) in the free-energy
functional (1.1), and we make use of (4.5)-(4.7), we obtain:

\[
F_{k\pm} = 2\pi \kappa \int_0^{\vartheta_l} (H_{k\pm} - \sigma_0)^2 r_{k\pm} \sqrt{r_{k\pm}^2 + r_{k\pm}'^2} \sin \vartheta \, d\vartheta = \\
= \kappa (\vartheta_0 - 1)^2 \frac{A_0}{\varrho^2} + \kappa \vartheta_0 (\vartheta_0 - 1) \frac{\Delta A}{\varrho^2} - \kappa \vartheta_0 \frac{r_0 \Delta A - 2 \Delta V}{r_0^3} + F^{(2)}_k + o(\Delta A, \Delta V),
\]

where \(F_{k\pm}\) denotes the free energy of the \(k\)-th solution of equation (4.5), with the positive or negative sign for \(C_3\), and

\[
F^{(2)}_k := \frac{\pi}{2} \kappa \int_0^{\vartheta_l} \left[ \vartheta_2^{(2)} \vartheta, \kappa(\vartheta) \cos^2 \vartheta \varrho_2^{(2)} \vartheta, \kappa(\vartheta) \right] \sin \vartheta \, d\vartheta
\]

is the leading order that may determine \(k\) and the sign of \(C_3\). However, \(F^{(2)}_k\) depends quadratically on \(\varrho_2^{(2)} \vartheta, \kappa\), so that the sign of \(C_3\) cancels out from it. Thus, the second order expansion turns out to be able to identify only the preferred value of \(\nu_k\). A further term in the free-energy expansion will be needed to complete the determination of the free-energy absolute minimizer.

Figure 5 shows how \(F^{(2)}_k\) depends on \(k\) and \(\psi_0\): the free energy increases when either of these increase. In particular, Figure 5 proves that the stable perturbed shape for a quasi-spherical impermeable vesicle corresponds to the solution with the smallest possible order of the Legendre functions. This is not surprising from the physical point of view, since Legendre functions wrinkle when their order increases, and these oscillations increase the elastic energy.

![Figure 5: Results of the numerical computation of the integral in (4.10), when \(\varrho_2^{(2)} \vartheta, \kappa\) is given in (4.8), and \(\nu\) is the \(k\)-th solution of equation (4.5). The graphs display the results for \(k = 1, \ldots, 5\).](image)

### 4.3.2 Pear-shaped or stomatocytes?

We still have to choose the preferred sign for the parameter \(C_3\). Figure 6 shows the deep, qualitative, differences between permeable (a), and impermeable ((b) and (c)) stationary shapes that arise from the same parameter changes: \(\Delta A = 10^{-4} A_0\) for all the shapes; \(\Delta V\) is left free in (a), while it is kept null in (b) and (c). Permeable vesicles modify their enclosed volume in order to keep an almost-spherical shape. On the contrary, impermeable vesicles move towards pear-shaped or stomatocyte-like equilibrium shapes [29], depending on the sign of \(C_3\).

In order to compare the free energies of pear-shaped and stomatocyte-like vesicles, we need to push further our free energy expansion. The next order depends on the third power of \(\varrho_2^{(2)} \vartheta, \kappa\) and its
Figure 6: Perturbed shapes for a vesicle embedding an inclusion of negligible size ($\psi_0 \ll 1$) when a 10% increase in the vesicle area is imposed (with respect to the value leading to a spherical shape). Picture (a) shows the equilibrium shape of a permeable vesicle, which is allowed to adapt its enclosed volume. Pictures (b) and (c) refer to an impermeable vesicle when the positive or negative sign for the parameter $C_3$ is chosen when solving equation (4.6). The inclusion (not visible in the pictures) sits in the bottom end of the vesicle.

Derivatives: it is $O(r_0 \Delta A, \Delta V)^{3/2}$. By making use of the constraint requirements (4.5)-(4.7), it is possible to obtain:

$$
F_{(ps, st)} = \kappa_0 \left( \frac{A_0}{r_0^2} \right) \Delta A - \kappa_0 \left( \frac{A_0}{r_0^2} \right) \Delta V + \mathcal{F}^{(2)} + \mathcal{F}^{(3)}_{(ps, st)} + o(r_0 \Delta A, \Delta V)^{3/2},
$$

where all the terms up to $\mathcal{F}^{(2)}$ do not depend on the pear shaped vs. stomatocyte choice, and

$$
\mathcal{F}^{(3)}_{(ps, st)} = \pm \left( \frac{r_0 \Delta A - 2 \Delta V}{r_0^3} \right)^{2/3} \left( f(\psi_0) + \varsigma_0 g(\psi_0) \right).
$$

(4.11)

In (4.11), the plus sign corresponds to the pear-shaped vesicle, the minus sign describes a stomatocyte, and $\varsigma_0$ denotes as usual the reduced spontaneous curvature. Plots of the functions $f$ and $g$ (whose explicit expressions can be found in Appendix A2, equations (A2.2)-(A2.3)) are shown in Figure 7. Both $f$ and $g$ are negative for all values of $\psi_0$. Thus, for any positive value of the spontaneous curvature, the pear-shaped vesicle is the absolute minimizer of the free-energy, whereas the stomatocyte represents only a relative minimum. A transition between the two stationary shapes can be observed only when negative spontaneous curvatures are induced; more precisely, the stomatocyte-like phase is preferred if

$$
\varsigma_0 < -\frac{f(\psi_0)}{g(\psi_0)} =: \varsigma_{0, cr}(\psi_0).
$$

(4.12)

Figure 7 also shows how the critical value of the reduced spontaneous curvature depends on the apex angle. In particular, $-\frac{6}{5} < \varsigma_{0, cr}(\psi_0) \leq -\frac{3}{5}$ for all values of $\psi_0$.

### 5 Concluding remarks

In this paper we have analysed how the impermeability constraint may induce singular stationary vesicle shapes, and how the embedding of an inclusion may vary the vesicle topology, promoting pear-shaped geometries that anticipate critical phenomena such as budding or vesiculation [30, 31].

Our analysis has been based on the linearization of the shape equation close to a spherical shape. This approximation does not allow to approach the aforementioned transitions, but in turn yields analytical results which prove that the vesicle reaction to a variation of the external
Figure 7: Plots of the functions $f$ (dashed), $g$ (dotted line), introduced in \[4.11\], and the critical spontaneous curvature $\varsigma_{0,cr}$ (continuous line), defined in \[4.12\], all as functions of the apex angle.

parameters may not be analytical. In Section 4 we have shown that an $O(\epsilon)$ relative variation in the vesicle area may induce either an $O(\epsilon)$ or an $O(\sqrt{\epsilon})$ relative variation in the shape function, depending on the permeability properties of the vesicle and the aqueous solution that surrounds it.

The present analytical study is being currently completed by a numerical study \[25\], which detects how the presence of an embedded inclusion modifies the phase diagrams describing the vesicle topology, and under which conditions it anticipates budding phenomena.

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Appendix A1: Properties of Legendre functions

The Legendre functions of the first and second kind (respectively denoted as $P_\nu(s)$ and $Q_\nu(s)$) are the solutions of the linear differential equation

\[
(1 - s^2) y''(s) - 2s y'(s) + \nu(\nu + 1) y(s) = 0 .
\]

The Legendre functions of the first kind are regular when $s \to 1^-$ (with $P_\nu(1) = 1$), while the Legendre functions of the second kind are singular close to both $s = \pm 1$. The properties we use in this paper are the following (see \[32\], §8.2, and §8.5)

For any $\nu \in \mathbb{R}$ and $s \in [-1, 1]$,

\[
P_{\frac{1}{2} - \nu}(s) = P_{\frac{1}{2} + \nu}(s) , \tag{A1.1}
\]

\[
P_{\nu+1}(s) = \frac{2\nu + 1}{\nu + 1} s P_\nu(s) - \frac{\nu}{\nu + 1} P_{\nu-1}(s) \quad (\nu \neq -1) , \tag{A1.2}
\]

\[
(1 - s^2) P'_\nu(s) = \nu P_{\nu-1}(s) - \nu s P_\nu(s) . \tag{A1.3}
\]

Equations (A1.2) and (A1.3) imply

\[
\int P_\nu(\cos \vartheta) \sin \vartheta d\vartheta = -\int P_\nu(s) ds = \frac{P_{\nu-1}(\cos \vartheta) - \cos \vartheta P_\nu(\cos \vartheta)}{\nu + 1} \quad (\nu \neq -1) . \tag{A1.4}
\]
For any \( \nu \in \mathbb{R}^+ \setminus \mathbb{N} \), the Legendre functions of the first kind admit the following asymptotic expansion:

\[
P_{\nu}(-1 + \epsilon) = -\frac{\log(\epsilon/2) + 2\gamma + \Psi(-\nu) + \Psi(\nu + 1)}{\Gamma(-\nu) \Gamma(\nu + 1)} + O(\epsilon \log \epsilon) \quad \text{as} \quad \epsilon \to 0^+ ,
\]

where \( \Gamma, \Psi \), and \( \gamma \) respectively denote the Euler gamma function, the digamma function, and Euler’s constant.

### Appendix A2: Third order expansion of the free energy

The derivation of the third-order term in the free-energy expansion requires a third-order expansion of the shape function. Thus, (4.2) has to be replaced by:

\[
r(\theta) = r_o \left( 1 + \sqrt{\epsilon} \, \varrho_{\frac{1}{2}}(\theta) + \epsilon \, \varrho_{\frac{1}{2}}(\theta) + \epsilon^2 \, \varrho_{\frac{3}{2}}(\theta) + o(\epsilon^3) \right).
\]

However, the higher-order terms \( \varrho_{\frac{1}{2}} \) and \( \varrho_{\frac{3}{2}} \) turn out to enter in the free-energy expansion only through combinations that can be related to integrals of \( \varrho_{\frac{1}{2}} \) and its derivatives by making use of the area and volume constraints, as it already happens in the second-order expansion (see (4.7)).

More precisely, the third-order expansion of the constraints yields

\[
\int_0^{\vartheta^1} \varrho_{\frac{3}{2}}(\theta) \sin \theta d\theta + 2 \int_0^{\vartheta^1} \varrho_{\frac{1}{2}}(\theta) \varrho_{\frac{3}{2}}(\theta) \sin \theta d\theta = -\frac{1}{3} \int_0^{\vartheta^1} \varrho_{\frac{3}{2}}(\theta) \sin \theta d\theta .
\]

By using (A2.1) it is long but straightforward to prove that

\[
F(\psi_o) = \kappa(\delta_0 - 1) \, \frac{A_o}{r_o^3} - \kappa(\delta_0 - 1) \, \frac{\Delta A}{r_o^3} - \kappa(\delta_0 - 1) \, \frac{2 \Delta V}{r_o^3} + \mathcal{F}^{(2)} + \mathcal{F}^{(3)} + o(\Delta A, \Delta V)^{3/2} ,
\]

with

\[
\mathcal{F}^{(3)}_{(ps, st)} = F(\psi_o) + \kappa(\delta_0 \, G(\psi_o)) ,
\]

where

\[
F(\psi_o) = 2\pi \int_0^{\vartheta^1} \left[ \frac{\nu_k(1 + \nu_k)}{3} \varrho_{\frac{3}{2}}^3 - \frac{\cos 2\theta}{1 - \cos 2\theta} \varrho_{\frac{1}{2}} \varrho_{\frac{3}{2}}^2 + \frac{\cot \theta}{6} \varrho_{\frac{3}{2}}^3 - \frac{1}{2} \varrho_{\frac{1}{2}}^0 \varrho_{\frac{3}{2}}^2 \right] \sin \theta d\theta
\]

\[
G(\psi_o) = 2\pi \int_0^{\vartheta^1} \left[ \varrho_{\frac{1}{2}}^0 \varrho_{\frac{3}{2}}^2 - \frac{2}{3} \varrho_{\frac{1}{2}}^3 + \frac{1}{3} \varrho_{\frac{3}{2}}^3 \cot \theta \right] \sin \theta d\theta
\]

The functions \( f, g \) introduced in (A.11) are related to \( F, G \) through

\[
f(\psi_o) = F(\psi_o) \, \text{sgn}(C_3) \left( \frac{r_o \Delta A - 2 \Delta V}{r_o^3} \right)^{-\frac{3}{2}} ,
\]

\[
g(\psi_o) = G(\psi_o) \, \text{sgn}(C_3) \left( \frac{r_o \Delta A - 2 \Delta V}{r_o^3} \right)^{-\frac{3}{2}} .
\]

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