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Chapter 3

Vesicles of Complex Topology

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I. INTRODUCTION

Surfaces of complex topology are observable in a variety of multicomponent mixtures such as surfactant-
water-oil systems (bicontinuous or cubic phase), amphiphilic molecules (such as egg lecithin) swelled in
water, or even natural systems such as plastids in plant Golgi apparatus. All these systems are
characterized by a fluid membrane dividing the space into symmetric or asymmetric compartments,
possibly filled with the same kind of solvent. Membranes can also be randomly pierced by holes or
interconnected by passages of a scale ranging from nanometers to micrometers. Holes and passages
appear spontaneously during the swelling stage; they can also be created by artificial means, such as via
strong electric fields or osmotic pressure. These passages can be described as topological defects
whose number and position might fluctuate in time. In all cases, the surface cannot be continuously
transformed into a sphere without tearing and pasting the membrane so that it has a more complex
topology than the sphere. The surface of vesicles can be easily studied by optical microscopy, and this
allows a precise geometrical description on the micrometer scale.

Because there already exist reviews on vesicles of spherical topology, this chapter deals with the
equilibrium shape problem of vesicles of complex topology. It is intended to parallel the more
theoretically oriented chapter by Seifert and Lipowsky (Chapter 2), where recent developments of the
vesicle shape problem are summarized. As reported by other authors in this book, vesicles are closed
bilayers made up of phospholipids. In the fluid state, the vesicles are subject to a set of constraints
(volume, total area, and mean curvature) and their shapes result from the minimization of a curvature or
bending elastic energy. We will hereafter report on vesicles of nonspherical topology equivalent to
spheres with handles; these surfaces being observable by phase contrast microscopy. This includes not
only one-holed tori, symmetric or asymmetric, but also surfaces of more complex topology, such as
two-holed tori or lattices of passages. For g = 1,2 surfaces, our experimental observations are connected
to recent results in differential geometry, since these vesicles are examples of closed surfaces minimizing
absolutely the curvature energy. For g = 1, this is the case of the Clifford torus, and for g = 2, they
correspond to the so-called Lawson surfaces.

* The number of which gives the topological genus g.
This chapter starts with a short theoretical summary. It recalls the different approaches and it sets up the notations. After a short description of the experimental methods, we deal first with the case of partially polymerized toroidal surfaces, since these vesicles have distinctive properties. For unpolymerized vesicles, we address the case of the toroidal genus, genus 2, and even higher genus surfaces, where comparisons with theoretical results can be made. Temperature is used as a control parameter to scan through the phase diagram and, in particular, through the bifurcation lines where the axisymmetry is broken. These observations can also be directly supported by numerical simulations. The experimental shapes are optically digitized to build a triangular mesh, and these digitized shapes can be used as input to Surface Evolver, a computer program written by K. A. Brakke,\textsuperscript{17} to check whether the observed shapes are stable solutions in one of the different curvature models summarized in SL. For toroidal vesicles, one finds good agreement with the theoretical predictions. One observes the different families of equilibrium shapes predicted by the models which are characterized by the shape of their meridian cross section. However, for some of the nonaxisymmetric ones, we show that they must be interpreted as being in metastable states. In temperature-controlled experiments, the axisymmetry for circular tori is broken at the so-called Clifford torus, and large fluctuations can be observed in some cases. These fluctuations are reminiscent of the conformal symmetry which leaves the bending Hamiltonian invariant, and for higher topology the effect can be more spectacular. Indeed, we provide experimental examples of the so-called conformal diffusion effect,\textsuperscript{18,19} where a vesicle undergoes slow shape changes at constant energy and with constant constraints. In the last part, we concentrate on the problem of necks or passages connecting lamellae of membranes. Experimentally, the effect of fluctuations is much more pronounced for these systems. Using an electrostatic analogy, we derive the interaction between necks, which we show to behave as free particles with hard-core repulsion.\textsuperscript{20}

## II. THEORETICAL SUMMARY

In this section we recall the theoretical results on the equilibrium shapes of free vesicles summarized in Chapters 1 and 2. L. Duplantier was the first to recall the Willmore conjecture in the context of the physics of vesicles.\textsuperscript{21} This mathematical conjecture states that the \textit{minimum minimorum} of the curvature energy\textsuperscript{22}

\[
F_0 = \frac{1}{2} \int dS (C_1 + C_2)^2
\]

(1)

for genus $g = 1$ is obtained for the so-called Clifford torus. This torus is characterized by a ratio of the radii of its generating circles: $r/R = 1/\sqrt{2}$.\textsuperscript{23} Although experiments provide examples of this conjecture (see Section IV), the shape of a vesicle results from a trade-off between a curvature energy and a set of constraints so that a toroidal vesicle is not necessarily the Clifford torus, but is a member of one of the different families of equilibrium shapes (see Figure 1 and Figure 2 for some examples). Up to now, three models have been proposed. We summarize their properties to compare with experimental observations.

1. In the spirit of a Landau functional approach, and following Canham\textsuperscript{24} and Helfrich,\textsuperscript{25} the spontaneous curvature (SC) model states that the shapes are minimal energy solutions of a curvature Hamiltonian with the constraints of a given volume $V$ and surface $A$ lumped together into a dimensionless constant corresponding to the reduced volume constraint $v = 6 \sqrt{\pi} V/A^{1/2}$. A spontaneous curvature term $C_0$ breaking the inside-outside symmetry is generally taken into account such that the bending energy reads

\[
F_{sc} = \frac{1}{2} \kappa \int dS (C_1 + C_2 - C_0)^2
\]

(2)

where $\kappa$ defines the bending modulus, which is typically of the order of $10 k_B T$. Shapes are thus determined by the two parameters $v$ and $c_0 = C_0 R_0$, where $R_0 = (A/4 \pi)^{1/2}$ sets the scale of the equivalent surface sphere.

\* One of the two circles is the meridian cross section, whose center, rotating around the symmetry axis, generates the other one.
2. Originating from the work of Sheetz and Singer,26 Evans,27 and others,28-30 the bilayer coupling (BC) model introduces another constraint on the difference of area $\Delta A$ between the two monolayers making up the bilayer. Because each individual monolayer keeps a constant number of molecules over many hours, and possibly days, it is relevant to consider that $\Delta A$ is constant on short time scales. To leading order in the bilayer thickness $d$, $\Delta A$ can be made dimensionless and is related to the mean curvature:

$$m = \frac{\Delta A}{d R_0} = \frac{1}{R_0} \oint dS(C_1 + C_2)$$  

(3)

In this model, the equilibrium shapes are determined by the two parameters $v$ and $m$.

3. This latter constraint can be relaxed by introducing a second elastic energy term for the area difference between the two leaflets.31-33 An optimal leaflet area difference $m_0$ is introduced and the total energy reads

$$F_{ADE} = \frac{1}{2} \kappa \oint dS(C_1 + C_2 - C_0)^2 + \frac{1}{2} \alpha \kappa (m - m_0)^2$$  

(4)

This is the area difference elasticity model (ADE). According to Reference 33, $\alpha$ is a phenomenological parameter which depends on physicochemical parameters and is of the order of unity for bilayer membranes. When $\alpha$ approaches infinity, one recovers the hard constraint of the BC model, but in the other limit, $\alpha \to 0$, one recovers the spontaneous curvature model case. The ADE model is less restrictive than the previous two in that the shapes are now a function of three parameters ($v$, $c_0$, $m_0$).
When \( c_0 = 0 \) the bending term is invariant under a continuous set of transformations corresponding to the conformal group in three dimensions. This includes not only the rotations, the translations, and a global rescaling, but also inversions with respect to a sphere. The special conformal transformations can be labeled by a vector \( \mathbf{a} = (a_x, a_y, a_z) \) and they transform any point \( \mathbf{r} \) of the surface as

\[
\mathbf{r} \rightarrow \frac{\mathbf{r} + \mathbf{a}}{\mathbf{r}^2 + \mathbf{a}^2}
\]

Minimization of the curvature energy thus leads to a degenerate ground state of different shapes related by an inversion. For low topology surfaces, the constraints of constant reduced volume, of constant reduced mean curvature, or a spontaneous curvature term break this degeneracy. However, for surfaces of higher genus \( (g \geq 2) \), this degeneracy might still persist in some regions of the phase diagram \((v, c_0)\) or \((v, m)\).

Because of Equation 3 all three models have the same Euler equations so that the linear stability problem is the same for all cases. In the SC model, \( c_0 \) must be taken into account in the bending energy, but in the latter two cases, \( c_0 \) is a Lagrange multiplier which must be adjusted self-consistently in the ADE model. However, for the same reduced volume \( v \), the total energy depends on the model and the respective phase diagrams are different. This has been extensively studied in the case of the budding phenomenon for \( g = 0 \) surfaces and, more recently, for higher genus vesicles.

As shown by Seifert and Jülicher et al., the general feature of all phase diagrams for toroidal shapes is that there exists a second-order bifurcation line separating axisymmetric toroidal solutions from stable nonaxisymmetric ones. In a schematic way, this line divides the low reduced volume section, where one expects various families of axisymmetric tori with different cross sections, from the high reduced volume section, where the conformally transformed surfaces of axisymmetric solutions are the lowest energy solutions. In the first two of the preceding models, the Clifford torus sits on this line, but in the ADE model, there is a gap between the Clifford torus and nonaxisymmetric tori, such that almost circular tori of reduced volume larger than \( v_{Clifford} = 0.71 \) are in principle observable. Nonaxisymmetric tori, conformal transforms of circular ones, are a particular case of the well-known Dupin cyclides.

Other stable shapes are model dependent. They are characterized by the shape of their meridian cross section. For example, in the SC model, with \( c_0 = 0 \) and no constraint on \( m \), tori with a sickle-shaped meridian cross section (hereafter called spheroidal tori) are minimum energy solutions for \( 0 < v < 0.57 \), but the branch of tori with an almost circular cross section (hereafter called circular tori) is stable for \( 0.57 < v < 0.71 \). For the BC model (i.e., with zero spontaneous curvature and a constraint on \( m \)) there are discoidal, stomatoidal, and spheroidal axisymmetric families of solutions in the \((v, m)\) planes solutions from stable nonaxisymmetric ones. For all of these branches, the hole diameter can shrink to zero. However, in this model, no discoidal or stomatoidal nonaxisymmetric shapes have been calculated up to now. The ADE model predicts also that these three types of solutions are minimal energy shapes in different regions of the \((v, m)\) phase space (at fixed \( c_0 \)).

III. EXPERIMENTAL METHODS

A. PREPARATION OF VESICLES

As described in other chapters, vesicles are made of one or more closed bilayers. Mostly prepared with multicomponent mixtures of natural phospholipids swelled in water using various procedures, they can also be prepared with very pure assays of synthetic surfactant. We used pure synthetic "standard" phospholipids purchased from Avanti Polar Lipids, Inc. (DMPC, DPPC, DOPC) as well as a polymerizable one, \( DC_{10} \).PC.

For the first ones (DMPC, DPPC, DOPC), we used crystallized phospholipids (several milligrams) deposited on a petri dish. A droplet of deionized water (at a temperature \( T > T_m \)) swells the lipids, which are then spread over the bottom of the dish. A further excess amount of heated deionized water (several milliliters) permits the separation of vesicles from the bulk lamellar phase (complete swelling occurs after several hours).
The latter one \((DC_{10,9}PC)\) can be swelled in a similar way. Vesicles undergo a morphological transition toward tubules (diameter: 1 \(\mu m\); length: some 100 \(\mu m\)) when cooled below the melting temperature of the chains \((T_m = 42^\circ C)\). This transition is reversible, which means that one can form vesicles by reheating tubules above \(T_m\). As cooling cannot be avoided between the preparation and the observation stages, tubules can be considered as an intermediate step toward the formation of vesicles. Another, simpler method to prepare tubules has been proposed.\(^{43-45}\)

### B. OBSERVATION AND ANALYSIS

Observation is made at constant temperature using a phase contrast microscope coupled to an image analysis system. Vesicles are best seen when the tangent plane to their contour in the focal plane of the microscope is parallel to the optical axis. Since vesicles are free to rotate under Brownian motion, pictures can be taken from different viewpoints.

It is also possible to digitize the experimental pictures and build a geometrical model of the observed surface. Using the Surface Evolver computer program of K. A. Brakke,\(^{17}\) it is possible to get the geometrical parameters of the vesicle and to determine by a direct minimization of the bending energy whether the observed shape is a stable, or metastable, solution of one of the curvature models described in Chapter 2.

Finally, in order to test the theoretical predictions concerning the various branches of solutions, we performed temperature change experiments. This permits one to vary the reduced volume of the vesicles, their volume being almost constant when their area changes as

\[
\frac{dA}{dT} = \gamma A
\]

with \(\gamma = 5.010^{-3}\) for most of the phospholipids.

### IV. PARTIALLY POLYMERIZED VESICLES

\(DC_{10,9}PC\) is a polymerizable phospholipid. Partial polymerization can be induced in the gel phase by shining UV light during a time period ranging from several seconds to 10 min. Below \(T_m = 43^\circ C\), the vesicles break up into shards of winding sheets. The helical structure of these ribbons results from an intrinsic bending force in the tilted chiral bilayer.\(^{56,47}\)

The effect of polymerization depends on the time during which the vesicles are exposed to UV radiation. If the irradiation lasts more than 12 min, the rigid structures keep their form when reheated back above \(T_m\). This can be interpreted as a percolation of the polymerized net on the whole surface of the vesicle. For shorter exposure time, however, the needles reverse to vesicles fluctuating with amplitudes which are only slightly reduced with increasing exposure time. Among shapes of spherical topology, one also observes tori with the aspect ratio of the Clifford torus.\(^{48}\) They are axisymmetric with slight fluctuations of the hole around its equilibrium position. These experiments might be interpreted as a partial relaxation of the volume constraint due to leakage of the polymerized vesicle. The vesicle is then free to sit at the absolute minimum of the bending energy. Experimentally, nonaxisymmetric vesicles (Dupin cyclides) are also observed, but less often: as they also correspond to the absolute minimum of the bending energy, they are compatible with this theoretical picture\(^{49}\) (see also Reference 50).

### V. UNPOLYMERIZED MEMBRANES

#### A. EQUILIBRIUM SHAPES IN THE AXISYMMETRIC CASE

Following the experimental procedures introduced earlier, one observes at constant temperature a rich variety of toroidal shapes. These experiments are done with freshly prepared vesicles. The generic toroidal surface has a circular meridian cross section with a reduced volume ranging from 0.3 to 0.71 ± 0.03 (see Figure 1). These circular tori are solutions in all of the above models. For an exact circular meridian cross section, their reduced volume is related to the mean curvature by

\[
\nu_d m_d = 3\pi
\]
In spontaneous curvature model with \( c_0 = 0 \), circular tori are metastable states for \( v \leq 0.57 \). Discoidal toroidal surfaces are also observable. They are always metastable in case 1, but they can be minimal energy shapes in the BC model (case 2). An example is shown in Figure 2. This vesicle has a reduced volume of \( v = 0.51 \) and a mean curvature of \( M/4\pi = 1.38 \). According to Reference 35, such a discoidal vesicle is indeed a minimal energy shape for this set of parameters. However, spheroidal tori predicted in cases 2 and 3 have not been observed, but similar surfaces of a higher genus made of two spheres connected by three passages have been observed (see later Figure 16).

From the numerical point of view, one can test the stability of experimental discoidal tori. The shape is first digitized and then relaxed to a minimum energy surface. If model 1 is used with a zero spontaneous curvature, the shape relaxes to the quasi-circular family. In contrast, if a constraint on \( m \) is taken into account (or with \( c_0 \neq 0 \)), it has been determined that the contour of the meridian cross section remains of the discocyte type (see Figure 3).

**B. BIFURCATION TO THE NONAXISYMMETRIC CASE**

All the theoretical phase diagrams predict a region where the equilibrium shape is nonaxisymmetric. In particular, changing the reduced volume of a vesicle sitting near the transition line allows one to observe this transition. A natural candidate is the Clifford torus with a reduced volume \( v = 0.71 \). This torus is exactly on the transition line in the SC model as well as for the BC case. In case 3, the ADE model, one can in principle observe almost circular tori for \( v \leq 0.77 \) (case \( \alpha = 1 \)).

Figure 4 shows three views of this torus at different temperatures. As explained in the experimental section, a decrease in temperature amounts to an increase in the reduced volume. Vesicles are observed at constant temperature after the temperature has been decreased by 10°C. This allows one to determine all the geometrical parameters, such as the reduced volume, i.e., the eccentricity \( e \) of the hole. For nonaxisymmetric shapes, we have used the Dupin cyclides parametrization to get \( v \) from \( e \). For \( T = 50°C \), the torus is axisymmetric. When the temperature decreases, the hole is shifted off the center due to the increase of the reduced volume. Since the minimal temperature accessible for this phospholipid is of the order of 25°C, we have not been able to move the hole off the center such that the shape looks like a sphere with a small handle. (This is because of the small thermal expansion of DC_{8,9}PC, the thermal expansion of DMPC is at least two times larger.) This transition is reversible as predicted in models 1 and 2. In all experiments on circular tori, the bifurcation took place at a reduced volume of \( v = 0.71 \pm 0.03 \). For \( \alpha = 1 \), the ADE model predicts that the transition could also take place for \( v < v_{\text{Clifford}} \) (with \( m_0 > 1 \)) or for \( v > v_{\text{Clifford}} \) (with \( m_0 < 1 \)). None of these cases has been observed.

Varying the temperature can also be done for vesicles which are spontaneously nonaxisymmetric, such as the Dupin cyclides. These tori are characterized by circular lines of curvature but, as seen in Figure 5, the two meridian cross sections have a different radius. In all cases, the effect of decreasing the
temperature is to increase the eccentricity. The maximum reduced volume we have been able to obtain in this case is of the order 0.92.

C. NONAXISYMMETRIC CASE

As in the axisymmetric case, the meridian cross section of a nonaxisymmetric torus is not necessarily circular. Nonaxisymmetric stomatoidal or discoidal tori, obtained by conformal transformation of stable or metastable axisymmetric shapes, are expected. This transformation has been used by Seifert\textsuperscript{31} and by Jülicher et al.\textsuperscript{35} to test the stability of axisymmetric solutions with respect to axisymmetry-breaking perturbations.

Figure 6 shows a nonaxisymmetric stomatoidal torus. This solution is predicted by none of the models. However, using the Surface Evolver program, it is possible to check that it is a metastable solution, with bending energy $F_\gamma/8\pi\kappa = 1.61$, mean curvature $m/4\pi = 1.05$, and reduced volume $\nu = 0.70$. For this set of parameters, one expects in the BC model an axisymmetric stomatoidal torus sitting near the bifurcation line from nonaxisymmetric to axisymmetric shapes. A small spontaneous curvature may explain the deviation of this line towards the low reduced volume tori. In the framework of the ADE model, and for $\alpha = 1$, the situation is more complex. Stomatoidal tori are not minimal solutions near the bifurcation line. For this set of parameters, this torus can only be interpreted as being in a metastable state.

Figure 7 shows another example of a nonaxisymmetric torus. This discoidal shape has a reduced volume of $\nu = 0.52$ with a mean curvature $m/4\pi = 1.11$ as calculated by the Surface Evolver program. As in the preceding example, the phase diagram of Reference 35 for the BC model predicts for this set of parameters that the stable shape should be an axisymmetric discoidal torus.
Figure 6 Nonaxisymmetric stomatoidal torus. (a) Top view. (b) Front view. (c) Side view, showing the nonaxisymmetry. Bar indicates 10 μm.

Figure 7 Nonaxisymmetric discoidal torus. (a) Top view. (b) Front view. (c) Side view, showing the nonaxisymmetry. Bar indicates 10 μm.

To clarify this point, the contour shape of Figure 7 can be numerically digitized and then numerically equilibrated. For the BC model, there is a nonaxisymmetric torus with a discoidal cross section and with the same mean curvature and reduced volume as the experimental one (see Figure 8). The resulting shape has, however, an energy of $F_\gamma/8\pi \kappa = 2.25$, which is larger than the axisymmetric discoidal torus of the same reduced volume. This observation shows that a knowledge of metastable states is crucial to interpretation of experiments on vesicles.

VI. FLUCTUATIONS AROUND STABLE SHAPES

Surfaces of complex topology are more sensitive to fluctuations than those of spherical topology. This can already be seen in the case of genus 1, where the Clifford torus plays a special role. Because of its symmetry, this torus is almost conformally invariant with respect to a shift of the hole. At constant reduced volume, a second-order stability analysis shows that the energy increases as

$$\delta \left( \frac{F_\gamma}{2\pi \kappa} \right) = \frac{1}{4} \sum_{n=2} a_n^2 \left( 2 - 3n^2 + n^4 \right)$$

for $c_0 = 0.52$. In Equation 8, $(a_n)_{n\geq 2}$ labels the hierarchy of axisymmetry-breaking perturbations, where $n$ corresponds to the broken symmetry: $n = 1$ moves the hole off the center, $n = 2$ is an elliptical deformation, and $n = 3$ produces a star.\(^{52,53}\) The conformal mode corresponds to $n = 1$, and this shows that the energy increases in proportion to $e^4$, instead of the usual $e^2$ potential, when the hole moves off the center. Therefore,

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* The complete hierarchy of modes can be calculated from a stereographic projection from the three-dimensional sphere on the Euclidean space. In Reference 52 the set of modes was incomplete; this has been corrected in Reference 53.
Figure 8  Numerically equilibrated surface, starting from a digitized surface obtained from Figure 7. \( F_0/8\pi x = 2.25 \), as calculated by the Surface Evolver program.

Figure 9  Fluctuations of a Clifford torus. Bar indicates 10 \( \mu \text{m} \).

\[ v = v_{\text{Cliff}} = 0.71 \]

one expects large fluctuations. For some of the Clifford tori, this is actually the case. Figure 9 shows cut views of the same torus at different times (the membrane is in the fluid state and there is no partial polymerization). The typical amplitude for these fluctuations changes the aspect ratio by 20\%, and this effect is much more pronounced than for common vesicles, where thermal fluctuations affect the aspect ratio by
Figure 10  Some examples of genus 2 surfaces which minimize $F_0$ absolutely. They are conformal transformed one from the others.

a factor of the order of 5 to 10%. When the same stability analysis is performed on nonaxisymmetric Dupin cyclides, one can show that the energy increases in proportion to $e^2$. This shows that the Clifford torus is the only $g = 1$ surface for which the conformal symmetry gives an observable effect.

VII. VESICLES OF TOPOLOGICAL GENUS $G = 2$

Because a circular torus is highly symmetric,\textsuperscript{52,54} there is only one branch of nontrivial special conformal transformations. This branch is characterized by the distance of the inversion center with respect to the symmetry axis. Other positions of the inversion center amount to rescaling, rotations, or translations. Surfaces of higher topology cannot be axisymmetric, so placing the inversion center along the $x$, $y$, or $z$ axis yields three specific branches of shapes with the same bending energy. Mathematicians have already provided examples of surfaces which extremize the bare bending energy of Equation 1. Figure 10 shows examples of genus 2 Lawson surfaces which are all conformal images of one another. According to Kusner et al.,\textsuperscript{55,56} these surfaces play a role analogous to that of the Clifford torus for $g = 1$ tori. They are absolute minima of the bending energy for genus 2 surfaces.

As for $g = 1$ surfaces, the set of genus 2 surfaces can be characterized by a two-parameter phase diagram, $(\nu,c_0)$ or $(\nu,m)$. All shapes of Figure 10 are actually observable. Figure 11 shows a type B Lawson surface. Figure 12 provides an example of type LS with a threefold symmetry axis (a genus $g > 1$ surface can have at most a $g + 1$ symmetry axis). Figure 13 is an example of a type BL surface.

The specificity of high genus surfaces is that the ground state can be degenerate even with the constraints of constant reduced volume and constant mean curvature (the same remark holds in all models).\textsuperscript{18} Because the conformal group in three dimensions is indexed by three parameters, two of them are enough to satisfy the constraints and the last one generates a whole family. The conformal diffusion of Jülicher et al.\textsuperscript{35} takes place in a low symmetry region of the phase diagram, and it is characterized by entropic diffusion of the shape along a branch of degenerate solutions with a time scale set by the viscosity of the solution.

Figure 14 shows this phenomenon for a LS vesicle. Different views are taken at intervals of 30 s such that the focal plane corresponds to the equatorial plane. The three piles parallel to the principal axis undergo a scale change on a slow time scale characteristic of the conformal diffusion but remain circular. Figure 15 provides an example similar to the one published in Reference 35. It is a BS-type vesicle for which, in the first view, the focal plane does not correspond to a symmetry plane. However, after 20 s, the shape becomes symmetric with respect to a plane passing through the principal axis.
Figure 11 Button vesicle. Its numerically calculated geometrical parameters are $\nu = 0.52$, $m/4\pi = 1.17$. Bar indicates 10 $\mu$m.

Figure 12 LS-type vesicle. Three views of the same vesicle can be compared to a numerical approximation of the surface computed by the Surface Evolver program. Bar indicates 10 $\mu$m.

Other genus $g = 2$ surfaces are observable. In agreement with the theoretical predictions, they do not all exhibit the conformal diffusion phenomenon. A paradigmatic surface of this type is presented in Figure 16. It can be viewed as two concentric spheres connected by three passages. By enlarging one of these necks, this shape is transformed into a torus with two holes. Its genus is therefore 2. Fluctuations around equilibrium shapes will be studied in the next section. It suffices to notice that these passages fluctuate strongly, their distance varying by a factor 2.

VIII. HIGHER GENUS SURFACES AND GIANT FLUCTUATIONS

Higher genus surfaces similar to the one of Figure 16 are also observable. They are of the same type but with a larger number of passages connecting the two concentric spheres. It is an experimental fact that all these low reduced volume surfaces (the volume corresponds to the region included between the two spheres) fluctuate strongly on a 1 s time scale. This time scale contrasts with the longer one necessary for the conformal diffusion to take place, thereby indicating that there is a restoring force. A model surface to study this effect has been described in Reference 20. A simple geometry corresponds to two lamellae connected by a periodic network of passages. Thus the system is divided into elementary cells with one or more holes per cell. We impose periodic boundary conditions on each cell and, for simplicity, we take the geometry of a square.
Figure 13  BL-type vesicle. Three views of the same vesicle can be compared to a numerical approximation of the surface computed by the Surface Evolver program. Bar indicates 10 μm.

Figure 14  LS-type vesicle exhibiting conformal diffusion. Snapshots are some 20 seconds apart and correspond to different geometrical shapes. No Brownian motion takes place during this observation. Bar indicates 10 μm.

Figure 15  BS-type vesicle exhibiting conformal diffusion. Snapshots are some 20 seconds apart and correspond to different geometrical shapes, as can be easily seen: view (a) shows a symmetry plane, but none exists in view (b) or (c), which correspond to different geometrical shapes. Bar indicates 10 μm.

If the radius $a$ of each hole is much smaller than the lattice spacing $2L$, a one-holed elementary cell can be decomposed into a core domain and a slightly bent surface $z(x,y)$, where the mean curvature can be approximate by $H \approx Dz$. This approximation fails in the core region, $r = \sqrt{x^2 + y^2} \approx a$, where the two radii of curvature are large but are of opposite signs. This surface divides the space into two
Figure 16  Fluctuating vesicles of genus 2. Snapshots are separated by some seconds. Arrows indicate the positions of necks connecting the two quasi-spherical parts of the vesicle. Bar indicates 10 μm.

Figure 17  Elementary cell of a periodic network of holes.

compartments and, because there is no minimal surface \( (H = 0) \) of this type, the hole diameter must be kept to a finite value by imposing a pressure difference between the two sides. A more convenient way to consider this problem is to impose the value of \( a \) as a boundary condition. To leading order in \( a \), both ensemble give the same result.\(^{20}\)

In the latter ensemble, there are analogies between an electrostatic problem and the curvature problem, where the electrostatic potential corresponds to the height variable \( z(x,y) \). Since the total electrical charge must be zero the charge \(-a/2\) borne by each hole must be compensated for by a positive background. For a square lattice, the equilibrium shape problem is cast into the Poisson equation

\[
\Delta z = -2\pi \frac{a}{L^2}
\]  

(9)

and, as a result, the curvature energy of each cell scales as \( a/L^3 \).

This analogy can be pushed further for more complex surfaces where the holes do not have the same "electrical charge" or for multilamellar membranes dividing the space into different compartments. Here it suffices to note that the holes have an inner core which matches the external constant curvature region. The radius of this matching domain sets the scale where two holes interact with a hard-core repulsion. A matching analysis gives an effective core radius at which this effect takes place which scales as \( a \) \( \log (a/L) \), \( L \) being the periodicity of the lattice.\(^{39}\)

IX. CONCLUSION

Although the shape problem for phospholipidic vesicles had been tackled 20 years ago, recent developments, both theoretical and experimental, have shown new facets. Among them, vesicles of complex topology have been observed recently, and these studies have exemplified new phenomena related to curvature energy and to their symmetries.

Fluid but partially polymerized vesicles provide first an illustration of the well-known Willmore conjecture on minimal surfaces. When relaxing the constraint of a constant reduced volume, the only
torus which can be observed corresponds to the absolute minimum of the bending energy. In principle, because of the conformal symmetry, this minimum is degenerate, but the constraints break this degeneracy for genus \( g = 1 \) surfaces. Large experimentally observed fluctuations show, however, the importance of this symmetry for dynamic phenomena.

As predicted in all models, experimental observations of vesicles of complex topology show a variety of different families. Even in the simplest case of toroidal shapes, we have provided examples not only of almost circular tori, but also, although more rarely, of tori with a discoidal or stomatoidal cross section. These observations have also shown that nonaxisymmetric families, where the hole is shifted off the center, are also observable for values of the reduced volume where axisymmetric solutions have been calculated. Not all of these surfaces are conformal transforms of axisymmetric ones. However, when the experimental observation is analyzed by a numerical optimization of an approximate triangular mesh, these shapes converge to nearby solutions whose energy is slightly larger than the axisymmetric ones. At the very least, this shows that metastable states are important and that not all families of equilibrium shapes are conformal transforms of axisymmetric ones. It also raises the interesting and open question of denumbering the number of metastable states as a function of the constraints \((v,c_0)\) or \((v,m)\). A bifurcation from an axisymmetric to a nonaxisymmetric shape is evidenced when temperature is varied. By varying the reduced volume, the bifurcation takes place in the neighborhood of the Clifford torus. This is consistent with all the models, though the ADE model predicts that this transition might take place at larger reduced volumes. For a reduced volume larger than 0.71, one scans reversibly through the Dupin cyclides shapes, conformal transforms of the Clifford torus.

The experimental study of the phase diagram requires the knowledge of geometric parameters such as area, volume, and average mean curvature. These are in principle accessible by imaging techniques, and one usually assumes that they are characteristic of a vesicle and that they are time independent. Although it is known that flip-flop of phospholipid molecules is a very slow process compared with the lateral diffusion time scale, it can play a role in the equilibrium shape problem. This discussion is consistent with the generic observation of circular tori for newly, but not freshly, prepared solutions of vesicles. All these observations are in agreement with the three models for the shape problem if metastable states are taken into account. However, one should distinguish between the short time scale observations, where the bilayer couple model may be relevant, and the longer time scale situations, where the spontaneous curvature or the ADE model might apply.

Mathematicians have provided examples of surfaces of higher topology which are experimentally accessible. One of the most spectacular effects for genus \( g \geq 2 \) surfaces is the possible degeneracy of whole minimal families even in the presence of constraints. In this review we have provided examples of vesicles exhibiting the so-called conformal diffusion process, confirming thereby one more time the importance of the curvature energy. Finally, low reduced volume vesicles are very sensitive to thermal fluctuations. Holes or passages fluctuate violently. This effect is best illustrated by an electrostatic analogy where the necks bear an electrical charge. Total neutrality is related to curvature so that one can compute the equilibrium shapes. Dynamic fluctuations can also be understood in this approach, since the interaction between holes is controlled by the overlap domain between their inner core and an outer domain with an almost constant curvature. This point of view may be seen as an intermediate step towards surfaces of more complex topology such as the sponge phases.

REFERENCES


