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To cite this version:
François Quéméneur, Catherine Quilliet, Magalie Faivre, Annie Viallat, Brigitte Pépin-Donat.
Gel-phase vesicles buckle into specific shapes. Physical Review Letters, American Physical

HAL Id: hal-00649424
https://hal.archives-ouvertes.fr/hal-00649424
Submitted on 7 Dec 2011

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Gel-phase vesicles buckle into specific shapes

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(Dated: December 7, 2011)

Osmotic deflation of giant vesicles in the rippled gel-phase \( P_{3\beta} \) gives rise to a large variety of novel faceted shapes. These shapes are also found from a numerical approach by using an elastic surface model. A shape diagram is proposed based on the model that accounts for the vesicle size and ratios of three mechanical constants: in-plane shear elasticity and compressibility (usually neglected) and out-of-plane bending of the membrane. The comparison between experimental and simulated vesicle morphologies reveals that they are governed by a typical elasticity length, of the order of one micron, and must be described with a large Poisson’s ratio.

PACS numbers: 46.32.+x ; 87.16.D- ; 87.16.dm

Probing the structural and mechanical properties of soft shells by non-contact techniques is a challenging approach in Soft Matter and in Cell Biology, where concepts may trigger surface and/or cell adhesion and bias results \([1]\). For instance, morphological changes of fluid-phase lipid vesicles under osmotic or temperature variations have been largely studied for the past 30 years. They have shown that vesicle shapes are governed by the bending energy, the spontaneous curvature of the two monolayers of the membrane \([2]\) and by their area difference \([3]\). Surprisingly, very few studies have concerned the shapes of gel-phase vesicles \([4-6]\). In addition to the bending stiffness and the stretching elasticity, the existence in the gel state of a lipid bilayer of a nonzero shear modulus is likely to generate specific deformations and new vesicle shapes. This was indeed observed in the model of coupled bilayer-cytoskeleton proposed in \([7,9]\) for red blood cells, and in the buckling instability that occurs under large local external forces on actin-coated \([10]\) and on gel-phase vesicles \([11]\). Here, we report observations of buckling induced by a non-local constraint on gel-phase Giant Unilamellar Vesicles (GUVs, diameter > 500 nm) upon deflation induced by applying an isotropic osmotic pressure. We propose a simple model that captures the major observed morphologies. The study highlights the relationship between the elastic properties of the lipid membrane and the specific faceted shapes taken by the vesicles.

Deflation experiments were performed on DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) GUVs in the rippled gel phase \( P_{3\beta} \) at 15°C. GUVs were prepared by electroformation \([12]\) above the main acyl chain crystallization temperature \( T_m = 23.6°C \) \([13]\) in a 100 mM sucrose solution, and by slowly decreasing the temperature down to 15°C with a cooling rate of 0.05°C/min. In order to prevent the breaking of the lipid membrane and the volume of vesicles was decreased to adjust to their loss of surface area (\(~ 28\%\) between the \( L_o \) fluid and the \( P_{3\beta} \) rippled phases \([14]\)) by adding a controlled sucrose solution in the external solution. Gel-phase GUVs obtained with this protocol were spherical and presented no observable defects in the membrane. Finally, GUVs sedimented in an iso-osmolar glucose solution were kept at 15°C and osmotically deflated by adding controlled amounts of glucose solution of suitable concentration in the external solution. GUVs were observed by phase contrast microscopy. The obtained shapes displayed in Fig. 1 line (a) show obvious differences with the classical shapes observed on vesicles in the fluid state \([15]\). Subjected to the osmotic shock, gel-phase GUVs shrink and develop a large variety of morphologies, from stomatocytes to concave polyhedra (i.e. sphere paved with depressions). The final faceted state is reached around 40 minutes after the beginning of the deflation (the whole process is limited by diffusion of glucose molecules in the surrounding medium), and, thereafter, no shape modification is observed over several hours, when temperature and osmolarity are kept constant.

In order to quantitatively understand these specific shapes, we model the 2D gel-phase membrane by a surface with an in-plane Hooke elasticity \([16]\) determined by two 2D phenomenological constants, the Young modulus \( Y_{2D} \) and the 2D Poisson’s ratio \( \nu_{2D} \), and by an out-of-plane bending elasticity. We describe the bending contribution by the Helfrich model \([2]\) that involves only two constants, the spontaneous curvature \( C_0 \) and the bending modulus \( \kappa \) of the membrane. An initial vesicle is considered as a spherical surface of radius \( R \), enclosing a volume \( V_0 \). As the vesicle remains spherical during the phase transition towards the \( P_{3\beta} \) phase, we consider that the vesicle remains unstrained, which implies \( C_0 = 2/R \). Dimensional analysis reveals that three dimensionless parameters control the shape of the vesicle when its volume decreases from \( V_0 \) to \( V \): the deflation \( \Delta V = \frac{V_0 - V}{V_0} = 1 - \nu_r \) (\( \nu_r \) is the reduced volume), the Föppl-von Kármán num-
Numerical simulations: each shape is characterized by the number of depressions $N$ (see text). $N = 0$: sphere, oblate, untwined chestnut; $N = 1$: stomatocyte; $N = 2$: discocyte, asymmetric discocyte, bean, crisp; $N = 3$: nipple, 3-blades (or knizocyte), twisted 3-blades, bladed nipple; $N = 4$: tetrahedron, 4-blades. $N = 5$: dumbbell with triangular leg; $N = 6$: cube, dumbbell with square leg, bulged cube; $N = 7$: dumbbell with 5-star leg.

This quantitative shape description allows to study numerically the influence of $\gamma$ and $\nu_{2D}$ on $N$. As shown in Fig. 2 for all $\nu_{2D}$ ranging between 0 and 0.98, $N$ gathers on a quasi-linear master curve as a function of $\sqrt{\gamma / I_2 (1 - \nu_{2D}^2)}$. This latter quantity can be considered as a reduced radius $R/d_{eq}$, where $d_{eq} = \sqrt{I_2 (1 - \nu_{2D}^2)} \kappa/Y_{2D}$ is homogeneous to a length. Within the frame of thin shells deformation theory, this scaling law can easily be understood. A thin isotropic shell of thickness $d$ and radius $R$ submitted to a uniform pressure buckles by reversion of a spherical cap of size $L \approx \sqrt{dR}$ [10]. The maximum number of facets that pave the full surface of the initial sphere therefore scales like $N \propto \frac{L}{2} \approx \frac{R}{d_{eq}}$. This relation replaced in a 3D context yields the numerical scaling obtained in Fig. 2 [10]. It is important to note that (i) this scaling law keeps its validity for a range of parameters much larger than those valid for viruses [17] and desiccated pollens [21].

![Diagram](image-url)
a thin shell of an isotropic material (case which reduces to $\nu_{2D} = \nu_{3D} < \frac{1}{2}$ \cite{18} and $\gamma \gg 1$ in the linear approx.\cite{21},

(ii) the non-zero shear energy of the membrane\cite{22} is responsible for the existence of a typical length of deformation, while in systems only governed by the bending energy, the only length scale is the radius of the object\cite{23}. For $\nu_{2D} > \frac{1}{2}$ (maximum value for bulk materials),\cite{24} $d_{eq}$ has no direct 3D equivalent. It is not necessarily thin, but a characteristic elastic length of the membrane, that gives the typical size of the deformations on the sphere: $d_{eq} \sim 1.8 \mu$m.

Making up for the lack of experimental 3D images,\cite{25} experimental values of $N$ were determined by comparing phase contrast microscopy observations to numerical\cite{26} shapes. Fig. 3 shows a plot of $N$ measured in this way\cite{27} as a function of the initial GUV radius for three reduced\cite{28} volumes and more than 1300 vesicles. In all cases, the number of facets on the vesicles had reached its maximum\cite{29} value and remained constant upon further deflation. The variation of $N$ with $R$ is consistent with the numerical line\cite{30} ear dependence obtained previously in Fig. 2 and allows\cite{31} the experimental determination of $d_{eq} \sim 1.8 \mu$m. This value is several orders of magnitude greater than both bi-lamellar thickness (\~{}$5$ nm), and periodic undulations of the rippled phase (amplitude \~{}$1$-$11$ nm and wavelength \~{}$15$-$55$ nm)\cite{32}. Therefore, despite their relatively small\cite{33} thickness, the vesicles in gel phase can not be regarded as \"thin shells\" (\textit{i.e.} \"of an isotropic material\") where $d_{eq}$\cite{34} is the thickness. This typical elastic length can be rewritten $d_{eq} = \sqrt{6(1+\nu_{2D})} \chi/\chi_{2D}$, where $\chi_{2D}$ is the elastic\cite{35} area compressibility (or \"stretching\") modulus. By taking $\kappa \sim 100$ $k_{B}T$ \cite{14}, we find $\chi_{2D} \approx 1 \mu N/m$. This value is very weak compared to that given in \cite{14}, which corresponds to partial unfolding of the ripples and was measured by micropipette aspiration on vesicles weakly tensed, where undulations at a scale larger than ripples were flattened out. Our low value of $\chi_{2D}$ might be linked to fluctuations at a mesoscopic scale, larger than the individual ripples size but smaller than the vesicle radius.

In the absence of a specific theory for the fluctuations of solid membranes, our study, which unambiguously shows a micron-size value for the characteristic length of deformation, provides a clue for a possible entropic origin of the area compressibility modulus.

The diagram of vesicles morphology, determined numerically and characterized by the number of facets, either $N_{\text{transient}}$ or $N$, is represented in Fig. 4 in the plane $(v, R/d_{eq})$ for three values of $\nu_{2D}$. It displays two clearly distinct zones: the $N$-domain where the number of facets has reached its maximum (in which one should find the experimental morphologies of Fig. 1), and the $N_{\text{transient}}$-domain. The coincidence of both experimental and numerical $N$-domains requires that $\nu_{2D}$ is at least equal to $0.8$. Its maximum acceptable limit is $0.95$, for which shapes differ from those displayed in Fig. 1 (e.g. depressions are surrounded by spicules; these poorly compressible surfaces will be treated in a subsequent publication).

This high value of Poisson’s ratio value confirms the fact that gel-phase GUVs cannot simply be regarded as thin shells of isotropic bulk material \cite{19}, where $\nu_{2D} = \nu_{3D} \leq 0.5$. The discrepancy between the lipid membrane thickness and the typical elasticity length may be understood by the anisotropic nature of the constitutive material, \textit{i.e.} the rippled lipid bilayer, that has different properties in its average plane, and in the perpendicular direction. The agreement between experimental and numerical vesicle shapes nevertheless shows the relevancy of this 2D elastic model based on in-plane isotropy, shear modulus and Helfrich curvature energy \cite{23}. Our simulations show a universal sequence of shapes and provide an alphabet to quantitatively interpret deflated morphologies in various experimental systems. More generally, the simulations reveal that the Poisson’s ratio, which generally varies over a narrow range of values and is then often neglected in favor of $\gamma$ in thin shell descriptions, has a crucial role when it approaches 1. Our study explores a wide range of elastic constants suitable to describe many materials, from thin shells of isotropic material ($\nu_{2D} \leq 0.5$) to surfaces with no shear elasticity ($\nu_{2D} \approx 1$), like fluid vesicles. Moderate values of the Föppl-von Kármán constant and small spontaneous curvatures are complementary to that involved in transitions of viral shells, where these two parameters play a different role on the shape \cite{19}. Besides giving quantitative clues on relative elastic features of gel-phase lipid vesicles through mere observations, this study offers interesting insights into the
FIG. 4. Shape diagrams established from Surface Evolver simulations (gray points): number of depressions $N_{\text{transient}}$ or $N$ as a function of $v_r$, and $R/d_{eq}$ for 3 Poisson’s ratios: $\nu_{2D} = 0$ (a), $\nu_{2D} = 0.5$ (b), $\nu_{2D} = 0.8$ (c). Dark and light gray zones correspond to conservation of the spherical symmetry and to deformations without appearance of depressions respectively, and each colored zone to shapes with a given number of concave facets. The red dotted line delimits the zone where the number of depressions has reached its maximum value. In the transient zone, the shape may also evolve with $v_r$ as shown on insert (b): path (2) displays a sphere-discocyte-crisp evolution ($v_r$ indicated under corresponding shapes); while path (1) shows cube becoming bulged cube on path (1). The universal sequence of Fig. 1, recalled and completed in insert (c), may be retrieved by following paths of type (3), within the $N_{\text{transient}} = N$ zone at any $\nu_{2D}$. ❇: experimental points obtained from the set of data at $v_r = 0.6$; for this latter the vesicle radius is averaged for each $N$ and adimensionalized by the $d_{eq}$ obtained in Fig. 3.

structured, reproducible and stable shapes, that can be obtained through the deformation of simple soft objects.


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[23] We consider that mutual sliding of the monolayers at the micron scale is prevented by friction at the ripples edges. Then, contrary to fluid bilayers that may require the ADE model, we model out-of plane deformations of the gel bilayer by a single surface with a Helfrich curvature energy. The spontaneous curvature $C_0$ slightly changes from 15°C to 23.6°C but in no case $C_0$ will exceed the Lobkovski limit $L^{-1/2} \gamma (L)^{1/6}$ ($L \equiv R/\sqrt{N} \approx \sqrt{R d_{eq}}$ is the length of the rims between concave facets), above which $C_0$ could have a significant impact on the vesicle shape [19].