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Spontaneous curvature-induced pearling instability

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We investigate the instability of a tubular fluid membrane made of a water-soluble surfactant. The tubules are obtained at high brine salinities. The instability is due to introduction within the vesicle multilayer of an alkane. We measure the wavelength of this instability versus the unperturbed radius of the tubules and interpret this selection by using a model that includes not only the surface tension in the elastic energy, but also the effect of the asymmetry induced by the oil as a spontaneous curvature. The linear stability analysis shows that within this model the selection is indeed due to nonzero positive spontaneous curvature.

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I. INTRODUCTION

Vesicles naturally exist in different shapes. The most common shapes are spherical vesicles, and vesicles with oblate shape (potato shape). Toroidal vesicles also exist under some particular conditions [1,2]. Tubular vesicles are also common in nature, but they are not an equilibrium solution for the elastic energy [3]. One of the most fascinating phenomena are the shape changes of cells and the manifold of local instabilities of cell plasma membranes in particular and vesicle membranes in general. Under physiological conditions the red blood cell for example, exhibits the familiar biconcave shape (the discoide form). Removal of some particular proteins leads to the echinocyte form, or to the stomatocyte form [4]. The interest of vesicles as a model system of living cells, for drug delivery or for different industrial usage, is the origin of several fundamental studies over a couple of decades [5]. Local instabilities play an essential role in material transport process between cellular compartments or through the plasma membrane. An important example is the transport of newly synthesized membranes to the cell envelope, and it involves three steps: (a) budding of vesicles, (b) their fission from a parent membrane, (c) their fusion with the target compartment. Tubular vesicles, excited by different techniques (uv radiation, optical tweezers, etc.), exhibit curious behaviors similar to the Rayleigh instability in liquid columns. Such behavior is known as ''pearling'' instability [6–8,10]. After exposure to radiation, tubular vesicles become a succession of spheres, separated by narrow necks. In the case of uv radiation, long time radiation induces a separation of the spheres [8]. When tubular vesciles are excited by optical tweezers, the shape of the spheres depends on the intensity of the laser. A long time exposure of the optical tweezers on the tubules induces a separation of the spheres by narrow necks [6,7]. Also, it was found that vesicles can suffer the same pearling instability when subjected to a change of the water pH between the inside and the outside of the vesicle [9]. It is important to point out that Deuling and Helfrich [11] showed theoretically that pearled shapes can arise in cylindrical vesicles when the tube spontaneous curvature is nonzero. Figures encountered in the literature with such pearls can be found in [12]. This reference showed ''myelin figures,'' which form in aging blood cells. We believe that these figures are experimental evidence of the pearling instability.

In this paper we study an instability of tubular vesicles made of a water-soluble amphiphile. The vesicles are formed in a brine solution (water/NaCl). This instability is similar to the ones cited above at least qualitatively. Contrary to the experiments where pearls form as a consequence to an irradiation, our experiment consists of modifying the interlayer distance, that is, the bilayer distance, by incorporating into the bilayer a solvent of the aliphatic chains which constitute the hydrophobic part of the molecule. In Sec. II we will describe the experiment and in Sec. III we present the theoretical model of the instability observed. Finally, a conclusion is presented.

II. EXPERIMENT

It is known that alkanes are more or less good solvents, depending on their chain length, of the aliphatic chain of the anionic surfactant called AOT (sodium di-2-ethylhexylsulfosuccinate) at the oil-water interface [13,14]. AOT is a water-soluble surfactant that forms micelles when dissolved in water up to a concentration equal to 30 mmol/L. Beyond this ''solubility limit,'' the solution presents two phases: a dense, opaque phase and a lighter and more transparent one. Observation has revealed that AOT forms spherical and multilamellar vesicles [15,16]. Generally, double-tailed surfactants form vesicles [17]. These vesicles are found to be opaque when observed through a microscope and present several layers. We believe they are onionlike vesicles. However, at low salinities and at low AOT concentrations, AOT forms unilamelar sphericals and prolate vesicles as well as dumbell-shaped vesicles. At high salinities (we investigated the range 0.1–0.175 mol/L), we found that AOT in brine forms tubular vesicles [15]. These tubular vesicles can be very long; the length can be several orders of magnitude larger than their diameter. There are two ways of preparing this phase: Either solubilizing AOT in a very short alkane

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(heptane), and allowing the heptane to evaporate before adding water to the residue, or mixing first AOT and salt in a ratio 1:3 in weight, then pouring gently the appropriate quantity of water to the AOT/salt mixture to have the desired (AOT/salt) concentrations. For the purpose of this work we used the second procedure, so that we could avoid any kind of mixing of the two alkanes. If we prepare a low salinity solution by dissolving the amount of AOT in water and adding brine to bring the solution to the desired salinity, no vesicle is observed. The formation of these vesicles is probably due to an electrostatic effect between the polar heads [17]. In this paper we will not describe the phase observed when changing salinities or amphiphiles concentrations. Instead we will focus on a particular effect of dodecane on tubular vesicles obtained at high salinities. An instability is studied and the wavelength selected at the onset of the instability is measured as a function of the radius of the unperturbed tubules. The instability and the wavelength selection are found to be a result of the fact that the spontaneous curvature deviates from zero because of oil incorporation into the tubular vesicle layers, which makes the bilayer nonsymmetrical. Although spontaneous curvature is a well-known parameter in the study of topological and mechanical properties of membranes, we will explain briefly its origin in Sec. III.

The vesicles are observed through an inverted phase contrast microscope (40× objective, Nikon diaphot 200). The cell where the vesicles are observed is a 1-mm-thick closed glass cell; the gap between these two glass slides is also 1-mm-thick. The AOT-brine solution is left for a couple of days, so that any shape transformation due to macroscopic flow, after transfering the solution from a test tube to the observation cell, disappears. The observation cell is hermetically closed and sealed up in order to avoid any fluid leakage and oil evaporation. The brine salinities used correspond to the minimun of oil-water interfacial tensions and are 0.175 mol/L for dodecane and 0.075 mol/L for decane and an AOT concentration of 7.5 mmol/L and 4 mmol/L, respectively. The critical micellar concentration (CMC) of AOT water solution without salt is around 2.5 mmol/L. (CMC is the amphiphile concentration at which the air-water or oil-water interface is saturated and the first micelles are formed.) It is noteworthy that by adding AOT to water, the surface tension drops continuously and reaches a value, at the CMC, beyond which it (the surface tension) remains constant. At 0.075 mol/L of NaCl, the stable shapes are essentially prolate and spheres; at 0.175 mol/L of NaCl, the shapes are cylinders. This shape transformation from low salinities to high salinities is probably due to an electrostatic effect as AOT is an anionic molecule [17–19]. We observed the effect of dodecane on stable tubular vesicles at 0.175 mol/L (at this salinity the dodecane-brine interface tension is minimum) [13]. The introduction of the oil into the cell does not perturb hydrodynamically the solution and it is a noninvasive way to introduce the drop into the solution. To avoid instabilities such as the Marangoni effect, the oil is introduced through a less than 1-μm-diam crack-type orifice. The drop, with a volume of the order of 0.25 μL, travels through the glass by capillarity before it reaches the solution. The orifice is located at the lateral wall of the cell, that is, between the lower glass slide and the piece separating the two slides. The drop is then directly introduced into the solution. Due to the weak solubility of dodecane in water, and its slow diffusion, it takes several hours for the instability to start. The oil probably travels inside the solution after being incorporated in the swollen micelles. After the oil reaches the tubules situated far enough from the point where the drop was introduced, tubules become unstable, forming pearls similar to the ones observed in Refs. [6–9]. We have not noticed any shape changes when the gap between the horizontal walls of the observation cell is much smaller than 1 mm. (We do not know why this effect depends on the gap between the cell walls where the vesicles are being observed. Oil evaporation may affect the dynamics too.) A sinusoidal instability is initiated, and develops to a peristaltic state, with a reduction in fluctuations. However, large cylinders are stable; this will be clarified in the following model. The structure observed is periodic and typical necks between the pearls are apparent. In the case of thick walled vesicles, the pearls do not disconnect. However, tubules with thin layers are cut into separated spheres at the end of the instability. The time over which the spheres disconnect is still unknown. This kind of state is shown in Fig. 1. Bar-Ziv and Moses [6] suggested, after an experiment where a tubular phospholipid membrane was excited using optical tweezers, that the instability appears because of a change in the surface tension induced by the tweezers with analogy to the Rayleigh instability of a column of liquid [20]. The surface energy should be the source of instability, however as the surface energy increases the linear analysis shows that the instability develops only long-wave modulations without any wavelength selection. Later, and to explain the observations of Bar-Ziv, Tlusty, and Moses [7], Nelson, Powers, and Seifert [21] showed that nonzero wavelength could be selected by the radius of the tubular membrane, because of the fluid viscous motion inside the tube. See also [26]. However, in the case of our experiment this model is not sufficient to select a well-defined periodic spatial modulation of tubular membranes with the right dependence of the wavelength versus the initial size of the cylinder. In fact, in our case an extra length scale becomes important in the problem.

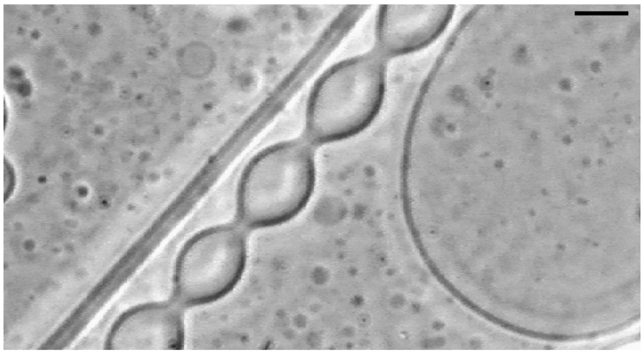

FIG. 1. A peristaltic state, in a thick cylinder. The bar represents 10 μm.

Figure 2 shows the dependence of the dimensionless number $(q_c R_0)$ as a function of the initial radius of the tubule R_0. The wavelength at the onset, q_c, is measured whenever a tubular vesicle starts to suffer the instability. The dispersion in the data is probably due to the fact that the wavelength is not measured exactly at the onset with an error of

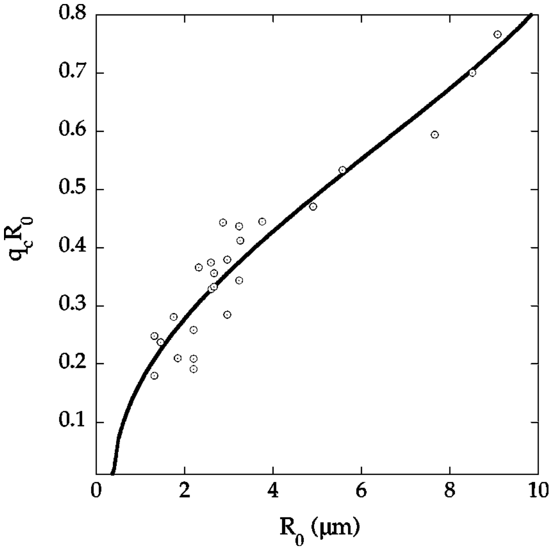

FIG. 2. The product (q_cR_0) as a function of the unperturbed radius (R_0, in μm). The line represents a fit with the theory developed below.

the order of 2 sec after the onset of the peristaltic state. The oil molecules reach the tubular vesicles in a random way, so the instability onset is also random. The finer tubules start to suffer the instability earlier than the larger ones; this fact explains also the dispersion in the data of Fig. 2.

The characteristic slope of the experimental data of Fig. 2 represents an extra length (of the order of 2 μm) in the problem, as we will show later. The inverse of this length may be interpreted as a spontaneous curvature c_0, measuring a symmetry breaking between the two sides of the membrane by incorporating a different amount of oil molecules (in some sense this is the analog of chirality in liquid crystals). In the next section we will introduce a simple model which explains the physical meaning of this length scale.

III. THEORY

One may model this by considering a membrane layer as composed of two different layers separated by a distance h and with different values of surface tension and surface elements, one with σ_+ and dS_+ and the other with σ_- and dS_-. Therefore, one has an energy of the form $\sigma_+\int dS_+ + \sigma_-\int dS_-$. This energy would include all the elastic contributions (curvature and surface tension of the whole layer) [17]. Expanding now both surface elements $dS_\pm$ in powers of h, with $dS_\pm = dl^1_\pm dl^2_\pm = dl^1 dl^2[1\pm h/(2R_1)][1\pm h/(2R_2)]$, one gets [we leave out the $\int dS/(R_1R_2)$ term because it is a topological invariant which does not contribute to the equations of equilibrium]

$$\int dS\left[(\sigma_+ + \sigma_-) + \frac{(\sigma_+ - \sigma_-)h}{2}\left(\frac{1}{R_1}+\frac{1}{R_2}\right)\right]dS.$$

Here $1/R_1 + 1/R_2$ is the mean curvature and $dS = dl^1 dl^2$ is a geometrical neutral surface element between the two layers. Finally, adding a ''bending'' energy of a tube, the free energy is [22]

$$F = \frac{\kappa}{2}\int dS\left(\frac{1}{R_1}+\frac{1}{R_2}-c_0\right)^2 + \sigma\int dS, \tag{1}$$

where κ is the rigidity, $\sigma = (\sigma_+ + \sigma_-) - \kappa c_0^2/2$ is the ''effective'' surface tension of the bilayer, and $c_0 = (\sigma_+ - \sigma_-)h/\kappa$ is the spontaneous curvature of the layer and is zero when the bilayer is symmetric, or when the neutral surface of the layer is in the middle of the layer [23]. Here the spontaneous curvature is not introduced as a parameter, but is considered as a measure of the asymmetry in the layer and a shift of the neutral surface from the middle of the layer. Also the surface tension is expressed as a combination of the different mechanical parameters of the membrane and has a different meaning than the surface tension of a monolayer or a liquid-liquid interface. For an axisymmetric surface characterized by a local radius $R(z)$, the mean curvature is

$$\frac{1}{R_1}+\frac{1}{R_2} = \frac{1}{R\sqrt{1+R'^2}} - \frac{R''}{(1+R'^2)^{3/2}}, \tag{2}$$

on the other hand the surface element is $dS = 2\pi R\sqrt{1+R'^2}\,dz$. Here $R' = dR/dz$ and z is the cylinder axis.

Let R_0 be the radius of a nonperturbed tube; as in [20], we set

$$R(z) = R_0\left[\sqrt{1-|u_q|^2} + \left(\frac{u_q}{\sqrt{2}}e^{iqz} + \text{c.c.}\right)\right] \tag{3}$$

into the free energy getting a power expansion in u_q (the square root appears because of conservation of the volume of the tube; note also that $u_{q=0}$ cannot hold a value diffrent from zero):

$$F = F_0 + \alpha(q)|u_q|^2 + \frac{\beta(q)}{2}|u_q|^4, \tag{4}$$

where

$$F_0 = \frac{\pi L\kappa}{R_0}[(1-c_0R_0)^2 + 2\mathcal{S}],$$

$\mathcal{S} \equiv \sigma/\kappa c_0^2$ is a dimensionless number, and the coefficient of the quadratic term is

$$\begin{aligned}\alpha(q) = \pi L\kappa/2R_0\{&-(c_0R_0)^2 - (qR_0)^2 - 4(c_0R_0)(qR_0)^2\\ &+(c_0R_0)^2(qR_0)^2 + 2(qR_0)^4\\ &-2\mathcal{S}(c_0R_0)^2[1-(qR_0)^2]\}.\end{aligned} \tag{5}$$

The linear instability appears for $\alpha(q) < 0$. Geometrically, the transition $\alpha(q) = 0$ allows us to express the control parameter $\mathcal{S}$ as a function of the perturbation wave number qR_0. For $0 < c_0R_0 < 1/2$, $\mathcal{S}$ has a minima at $q = 0$. The cylinder is stable for low values of $\mathcal{S}$, however as one increases $\mathcal{S}$ up to a critical value $[\mathcal{S}(q=0)]$, the tube suffers a long wavelength instability, without wave-number selection. For $1/2 < c_0R_0 < 1$, $\mathcal{S}$ has a minima for $q = q_c$. A short wavelength instability appears as soon as $\mathcal{S} > \mathcal{S}_c$. This is shown as local minima in Fig. 3. From Eq. (5) one gets explicitly the threshold of the instability, hence the wave vector of the

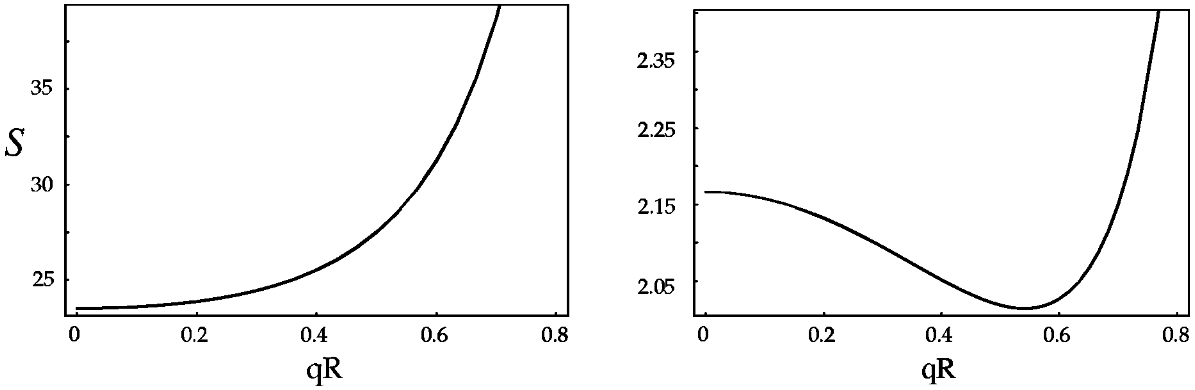

FIG. 3. The order parameter $\mathcal{S}$ as a function of the product qR_0; the figure on the left is obtained for a zero spontaneous curvature and the one on the right is for $c_0 \neq 0$.

most unstable mode (q_c), and the value of the control parameter $(\mathcal{S})$ at the onset of the instability.

In Fig. 4, one can see that the control parameter vanishes as soon as the radius of the tube is equal to the spontaneous curvature, i.e., when the tubule layers are symmetrical. From Eq. (5) and imposing $\alpha(q)=d\mathcal{S}/dq=0$ one gets the critical wave number and the threshold, that is,

$$q_cR_0=\sqrt{1\pm\sqrt{2}\sqrt{1-c_0R_0}}. \tag{6}$$

Note that there are two different solutions: The solution with the + sign represents a situation where $q_cR_0>1$ and this instability is possible only if $\mathcal{S}$ takes negative values (note that this negative surface energy allows the instability to occur with a well-defined wavelength even for $c_0=0$), however the selected wave number in this case has a negative ‘‘mean slope,’’ that is, a line essentially perpendicular to the experimental line given in Fig. 2. The critical surface tension for the case $q_cR_0<1$ is

$$\mathcal{S}_c=\frac{-3+2^{5/2}\sqrt{1-c_0R_0}+4c_0R_0-(c_0R_0)^2}{2(c_0R_0)^2}. \tag{7}$$

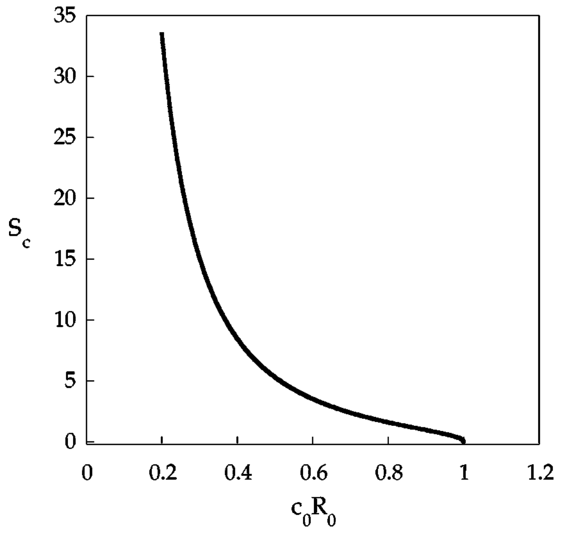

FIG. 4. The critical value $\mathcal{S}_c$ vs the product c_0R_0. As the radius of the tube approaches spontaneous curvature, the tension vanishes, as it should be.

The fit represented in Fig. 2 was performed using the expression (6), i.e., we tried $q_cR_0 \sim \sqrt{1-K\sqrt{1-c_0R_0}}$. The fit gives $K\approx 1.2$ (instead of 1.4) and $c_0=0.16\ \mu\mathrm{m}^{-1}$. This value is in the range of values measured for vesicles. In Fig. 4 the value of $\mathcal{S}_c$ at the q_c is of the order of 2.

At the critical point q_c, the sign of the coefficient $\beta(q_c, \mathcal{S}_c)$ of the fourth-power term $|u_q|^4$ is positive for all values of c_0R_0 such that $1/2<c_0R_0<1$. Therefore, this peristaltic instability is supercritical (a second-order transition). It is interesting to note that for large tubular radius there is no instability, as we can see in expression (6), where q_c becomes a complex number. This characteristic is indeed observed in experiments [15]. On the other hand, for small tubule radius, the instability grows for $q_c=0$, but this is not possible because of volume conservation, in other words a homogeneous growth of u_q is forbidden. In conclusion, in both cases (i) for small tubular radius $R_0<1/c_0$ (experimentally we get $R_0<1\ \mu$ m) and (ii) for large tubular radius $R_0>1/c_0$ (experimentally $R_0>10\ \mu$m) the tubules are stable.

IV. CONCLUSION

The oil, when incorporated in the vesicle membrane, is distributed asymmetrically between the different layers composing the membrane, i.e., between the outer layer and the inner layer, giving rise to a nonzero spontaneous curvature. A possible scenario would be that when the swollen micelles reach the tubular vesicles, they load their amphiphiles at the outer surface and a symetry breaking occurs between the outer and the inner layers. One can wonder, how is it possible to vary the spontaneous curvature without changing the length of the surfactant molecule? As for AOT monolayers at the planar interface between the oil and brine phases, this can be done by changing the brine salinity [24], but in the range of the salinity that one can probe, the Debye length changes from 0.456 nm to 0.608 nm, which is not sufficient to change the spontaneous curvature of the tubular vesicle radius in the range from 5 to 20 μm [27]. This could be perfomed, perhaps, using optical tweezers to bring proteins to a particular region of the membrane.

In conclusion, tubular vesicles can be excited in different ways, from uv radiation to laser tweezers; the latter represents a powerful tool to control the onset of the instability and the size of the pearls that nucleate in the vesicles [6]. It is found that with the incorporation of another molecule, in this case an alkane, in the bilayer, a more or less good solvent for the aliphatic chain of the surfactant, the tubules also suffer an instability similar to the Rayleigh instability. The nonzero spontaneous curvature is created by the incorporation of oil molecules (dodecane) into the membrane of the vesicles, inducing a short-wavelength instability to appear. In our model surface tension of the whole layer is necessary but not sufficient to describe the peristaltic state observed in such fluid tubular membranes. It is then important to introduce an effective surface tension (not to be mistaken for surface tension of monolayers and liquid interfaces), which is probably created by the incorporation of oil molecules between the aliphatic tails of the surfactant, composed of the bilayer rigidity, where the thickness and the surface tension of the outer and inner layers suffer elongation and compression, respectively, and vice versa. A microscopic description of the distribution of molecules, in terms of the volume fraction of oil introduced into the alipahtic part of the layer, is necessary to understand this effect on the spontaneous curvature of the tubules [25]. It is also of importance to notice that myelin figures observed in aged blood cells can be a result of a migration of molecules in the bilayer, which causes the bilayer to be nonsymmetrical [11,12]. This can be checked using the same experimental procedures of Bar-Ziv, Tlusty, and Moses [7].

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