Sedimentation of vesicles: from pear-like shapes to microtether extrusion

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\textbf{Abstract.} We study the sedimentation of buoyant giant lipid vesicles in a quiescent fluid at velocities ranging from 5 to 20 $\mu$m s\textsuperscript{-1}. Floppy vesicles are deformed by the flow. Their bottom (upstream) part remains spherical, while their top (downstream) part narrows down and elongates along the direction of motion, resulting in pear-like shapes or in the reversible formation of a micron-size tube at the vesicle top. The sedimentation velocity of a vesicle is very similar to that of a rigid sphere. Using a thermodynamic approach, we show that the hydrodynamic force acting at the top of a floppy vesicle can exceed the critical force needed to draw a membrane tube. We predict that the tube radius scales as the power $1/3$ of the ratio of the bending energy to the typical hydrodynamic stress, $\eta U/R$, where $\eta$ is the fluid viscosity, $U$ the sedimentation velocity and $R$ the vesicle radius. This result is consistent with the previously reported experimental data. The tensions of vesicles exhibiting a tube and of pear-like shape are deduced from the thermodynamic approach.

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1. Introduction

The behavior of living cells subjected to an external force or a hydrodynamic flow is an important issue both for physiology and for in vitro processing and manipulation. It has been much studied in cells and giant lipid vesicles. The latter are basic soft shells whose behavior is a physical reference for the cell ‘passive’ behavior. For instance, in a shear or an elongational flow, giant vesicles and red blood cells present a variety of motions and deformations whose commonalities and differences have been characterized and understood on the basis of the specificity of the mechanical properties of each particle [1]–[3]. Another example is the behavior of vesicle and cell membranes subjected to an external pulling point force. It is well known that there is a critical force for which a nanotube, called a tether [4]–[9], is drawn from the vesicle/cell and coexists with the spherical body of the particle. In vivo, tether formation is involved in many biological processes, such as intracellular trafficking [10]–[12] and cell migration [13]. In vitro, tethers extruded from the membrane (by pulling a small bead adhering on the membrane) are used as sensors to gain information about vesicle/cell mechanical properties (cortical tension, coupling energy between the membrane and the cytoskeleton) [14]–[17].

Surprisingly, the behavior of vesicles and cells suspended in a fluid and submitted to a gravitational force is not thoroughly documented. One theoretical study [18] predicts that sedimenting vesicles should deform and present either pear-like shapes or bean-like shapes, with no experimental support. However, the question of the movement of deformable objects due to a body force in a viscous fluid arises naturally in different situations, such as centrifugation, magnetophoresis and cell sorting in microfluidics. For instance, recent observations of red blood cells during centrifugation show how the cells resume very peculiar pear-like shapes [19]. In a more general point of view, sedimentation and shape instability of simple viscous drops had also been observed. While a spherical drop remains spherical during sedimentation (the sphere is a steady solution to the Stokes equations [20]), non-initially spherical drops are unstable [21]
and prolate shapes can develop long tails at their rear [22]–[24]. Initially, spherical droplets can also be destabilized when surfactants are present. In this case, the flow gathers the surfactants at the rear of the droplet, drastically reducing the local surface tension and leading to tip streaming [25].

In this paper, we study the deformation of giant unilamellar vesicles (GUVs) sedimenting in a quiescent fluid. We deal with very deformable floppy vesicles. We show that, depending on their sedimentation velocity, the vesicles either present original pear-like shapes or, above a critical hydrodynamic force, develop at a micron-size lipid tube, which coexists with a quasi-spherical shape. A striking point is that the tube extraction does not require the application of a point force. The distributed hydrodynamic stress, which is much weaker than point forces generally used to extract tethers, is, however, sufficient to pull micron-size tubes on floppy vesicles. We show that a free energy analysis describes such a behavior.

We first detail the materials and method before describing the regimes of motion and the deformations of the vesicles. Then we show that vesicle velocities are similar to those of rigid spheres translating in a quiescent fluid. We propose an approach for describing and understanding the formation of membrane tubes. Finally, we estimate and discuss the values of the surface tensions of sedimenting vesicles.

2. Experimental

2.1. Giant unilamellar vesicle (GUV) preparation

GUVs were prepared by the electroformation method [26]. Dioleoyl-phosphatidylcholine (DOPC; Sigma) was dissolved in a chloroform and methanol solution (9 : 1 volume ratio) at 2 mg ml\(^{-1}\) and 10 \(\mu\)l of the solution was spread on the conductive faces of two transparent glass plates coated with a film of indium tin oxide (ITO). After drying (2 h under primary vacuum), about 2 ml of a sucrose solution (with concentration 300 mM) was injected into a chamber formed by ITO plates facing each other, separated by Teflon spacers and connected to an ac generator (frequency 10 Hz). The potential was very slowly increased from 0.2 to 0.8 V and kept constant overnight. The frequency and voltage were finally decreased to 4 Hz and 0.2 V to detach vesicles from the ITO plates.

The suspension of vesicles we obtained was then diluted in a binary sucrose/glucose solution with the following protocol. 100 \(\mu\)l of the initial sucrose suspension of vesicles was diluted in 1 ml of the chosen binary sucrose/glucose solutions for 1 h. Then, 100 \(\mu\)l of this suspension was rediluted in 1 ml of the binary sucrose/glucose solution for an additional hour. Finally, 100 \(\mu\)l of this suspension was diluted again in 450 \(\mu\)l of the binary sucrose/glucose solution and gently introduced into the observation chamber (volume 0.550 ml). Only a few vesicles were present in the observation chamber in order to limit hydrodynamic interactions. The characteristics of the vesicles are listed in tables 1 and 2.

2.2. Method of observation

We used a parallelepiped chamber from Hellma (Mullheim, Germany) that has four optically transparent faces (width \(x\)-direction: 10 mm; length \(y\)-direction: 55 mm; height \(z\)-direction: 1 mm). Sedimenting vesicles and their reflections on the bottom of the chamber were observed in the vertical \(y\)–\(z\) plane, from a microscope (Leica IRB) tipped to the horizontal [27]. We let the vesicles settle at the bottom of the chamber for 20 min. Then, we turned the chamber
upside down and observed the sedimentation of one vesicle using a CCD camera (Cohu 4910, 25 fps). To prevent vesicle adhesion on the chamber wall, we first incubated a casein solution in the chamber before introducing the vesicles. The vesicles were found to behave similarly both in the chamber rinsed in Millipore water and in the chamber treated with casein. We therefore performed most experiments by simply using the chamber rinsed in Millipore water.

Movies were saved using a Sony DSR25 digital videocassette recorder. Vesicle tracking, contour and center of gravity determinations as well as shape analysis were carried out with IDL software. The volume $V$ and the area $A$ of the vesicles were measured at rest from the shapes of the settled vesicles. The effective radius $R$ was deduced as $(3V/4\pi)^{1/3}$.

3. Observations

3.1. Full experiment

Typical experiments are shown in figure 1. Firstly, the vesicle departs from the top of the chamber and progressively elongates and deforms while its velocity increases. Then, a stationary regime is reached: the vesicle, located halfway to the observation chamber, has a constant velocity and a stable shape. Finally, upon approaching the bottom wall, the vesicle velocity decreases and its shape progressively changes (no more vertical elongation). Then, upon landing on the bottom of the chamber, the vesicle flattens down with a typical gravity-induced shape deformation. When a tube is observed at the top of the vesicle, it retracts and it is swallowed by the main body of the vesicle during the landing stage, as shown by the last images of each sequence represented in figure 1.

3.2. Stationary shapes

Vesicles, which do not present large excess area, cannot significantly deform and thus remain spherical during their sedimentation. However, most vesicles are osmotically deflated and are

### Table 1. Characteristics of vesicles with a tube.

| No. | Veicle radius, $R$ (µm) | Tube radius, $r$ (µm) | Tube length, (µm) | Velocity, $U$ (µm s$^{-1}$) | Viscosity, $\eta$ ($10^3$ Pa s) | Density, $\Delta \rho$ (g l$^{-1}$) | Reduced volume, $\nu$ | Calculated tension from equation (5), $\sigma$ ($10^8$ N m$^{-1}$) |
|-----|------------------------|----------------------|------------------|-----------------------------|-------------------------------|-----------------------------------|----------------|--------------------------------|
| 1.1 | 30.5                   | 2.71                 | 7                | 8.11                        | 1.33                          | 7.4                               | 0.985          | 0.42                          |
| 2.1 | 25                     | 3                    | 11               | 5.48                        | 1.33                          | 7.4                               | 0.96           | 0.35                          |
| 3.1 | 25.8                   | 3.1                  | 16.4             | 6.18                        | 1.33                          | 7.4                               | 0.96           | 0.36                          |
| 4.1 | 29.5                   | 3.6                  | 18.9             | 4.93                        | 1.36                          | 4.4                               | 0.99           | 0.37                          |
| 5.1 | 48.1                   | 3.1                  | 9                | 12.4                        | 1.36                          | 4.4                               | 0.99           | 0.33                          |
| 6.1 | 48.5                   | 3.3                  | 11               | 10.2                        | 1.37                          | 3.2                               | 0.99           | 0.33                          |
| 7.1 | 34                     | 3.7                  | 14.8             | 5.97                        | 1.33                          | 4.2                               | 0.97           | 0.28                          |
| 8.1 | 28.6                   | 2.7                  | 12.6             | 12.4                        | 1.29                          | 11                                | 0.97           | 0.5                           |
| 9.1 | 17.5                   | 3.6                  | 23.5             | 4                           | 1.26                          | 10                                | 0.97           | 0.31                          |
| 10.1| 30.3                   | 3.6                  | 17               | 5.87                        | 1.33                          | 4.2                               | 0.959          | 0.29                          |
| 11.1| 28.5                   | 3.2                  | 19.8             | 7.64                        | 1.29                          | 6.7                               | 0.95           | 0.35                          |
| 12.1| 25.3                   | 3.6                  | 13.5             | 5.3                         | 1.36                          | 4.4                               | 0.95           | 0.3                           |
| 13.1| 16                     | 2.8                  | 15               | 9.5                         | 1.17                          | 18                                | 0.9            | 0.5                           |
Table 2. Characteristics of pear- and egg-like vesicles.

| No. | Bottom radius, $R$ ($\mu$m) | Top radius, $r$ ($\mu$m) | Velocity, $U$ ($\mu$m s$^{-1}$) | Viscosity, $\eta$ (10$^3$ Pa s) | Density, $\Delta \rho$ (g l$^{-1}$) | Calculated tension from equation (12), $\sigma$ (10$^8$ N m$^{-1}$) |
|-----|----------------------------|--------------------------|-------------------------------|-----------------|-----------------|--------------------------|
| 4–1.2 | 25.4                       | 8.5                      | 10.63                         | 1.29            | 11              | 3                       |
| 5–2.2 | 26.4                       | 12.6                     | 4.66                          | 1.33            | 4.2             | 2.7                     |
| 6–3.2 | 27.2                       | 14                       | 4.8                           | 1.33            | 4.2             | 3                       |
| 7–4.2 | 44.8                       | 24.4                     | 20.9                          | 1.3             | 7               | 13.2                    |
| 8–5.2 | 23.5                       | 15.5                     | 4                             | 1.31            | 4               | 4.5                     |
| 2–6.2 | 30                         | 8.9                      | 6.2                           | 1.33            | 4.2             | 1.5                     |
| 10–7.2 | 24.3                       | 22                       | 5.6                           | 1.33            | 7.4             | 33                      |
| 11–8.2 | 48                         | 13                       | 20.27                         | 1.29            | 11              | 4.5                     |
| 13–9.2 | 25.7                       | 18.2                     | 10.7                          | 1.29            | 11              | 15                      |
| 14–10.2 | 21                        | 14.75                    | 6.5                           | 1.29            | 11              | 9                       |
| 18–11.2 | 10.7                       | 2.2                      | 2.9                           | 1.17            | 18              | 0.3                     |
| 19–12.2 | 12.8                       | 4                        | 3.8                           | 1.17            | 18              | 0.9                     |
| 20–13.2 | 13.7                       | 3.8                      | 6                             | 1.17            | 18              | 1.2                     |
| 21–14.2 | 12.9                       | 3.3                      | 7.1                           | 1.17            | 18              | 1.2                     |
| 23–15.2 | 23.8                       | 8                        | 15.6                          | 1.17            | 18              | 4.2                     |
| 0522 V17 16.2 | 15                  | 5                        | 3.2                           | 1.26            | 10              | 1.3                     |
| 0521 10 17.2 | 32.2                   | 19.2                     | 7.91                          | 1.30            | 7               | 6.9                     |
| 070520 18.2 | 15                  | 4                        | 3                             | 1.33            | 7.4             | 0.9                     |

therefore floppy and deformable. We now describe their stationary shape. The vesicles exhibit a spherical upstream (bottom) region and a vertically elongated downstream (top) part with a radius of curvature, $r$, smaller than the spherical part. Weakly deformable vesicles present an egg shape. More deformable vesicles look like pears with, at their top, a spherical cap of radius $r < R$ and an elongated region of length $L \leq r$ (figure 2). Some vesicles present a spherical part, which coexists with a tube. The tube radius, $r \ll R$, ranges from <1 to 2–3 $\mu$m. It is slightly pinched at its basis and, as seen in figure 1(b) and in figure 2, a catenoid shape is observed at the junction between the spherical part and the tube. Its length $L$ is longer than $r$. Most of these vesicles present large membrane undulations (figure 1(a)), disclosing that the tension is very low. Some vesicles, which are characterized by the highest sedimentation velocities, exhibit several tens of microns long pearling tubes of submicron radius (figure 1(d)).

When vesicles are subjected to two or three successive experiments of sedimentation, similar-shape deformations and sedimenting velocities are retrieved.

Finally, it is worth noting that there is no lipid flow on the membrane surface as attested to by small lipid aggregates bound to the membrane, which do not move relatively to the center of mass of the vesicle. Indeed, the driving force of the motion being vertical, the vesicles present a vertical axis of symmetry. This symmetry prevents the appearance of a lipid recirculation on its surface. Moreover, in contrast to a fluid drop, the two-dimensional (2D) confinement of lipid molecules and the symmetry of the experiment prevent any momentum transfer to the inner fluid, and therefore no flow recirculation is observed.
Figure 1. Vesicle sedimentation. (a) Vesicle (2.2), pear shape, full process from departing to landing, $t = 0, 0:57, 2:22, 4:46, 5:08$ and $6:28$ s, shapes are fluctuating. (b) Vesicle (12.1), short tube, full process, $t = 0, 0:24, 1:04, 1:49, 2:51, 4:38$ and $6:14$ s. (c) Vesicle radius $6 \mu m$, landing process from stationary shape to settled vesicle, $t = 4:40, 5:32, 7:04, 8:30, 9:12$ and $12:00$ s. (d) Departing of a vesicle (radius $49 \mu m$) and tube growth (time between images = 1 s). (e) Landing of the vesicle with tube retraction (60, 76, 84, 90, 92 and 93 s).

4. Velocities

We show in this section that the velocity field around a sedimenting vesicle is given by the flow produced around a rigid sphere with a radius equal to the effective radius of the vesicle and shows that at the leading order, the deformation of the vesicle does not modify the velocity field of the equivalent sphere.

The evolution of the vertical velocity of the center of mass of the vesicles as a function of time is illustrated in figure 3a) for one pear-like shape vesicle. The existence of the stationary regime is clearly observed (figure 3(a)). The two transient regimes correspond to the departing and the landing of the vesicle and the associated velocities are represented in figure 3(b) as a
Figure 2. Typical stationary vesicle shapes. Top, pear-like shapes: (1.2) radius 26 µm, (14.2) radius 13 µm and (6.2) radius 32 µm; bottom, vesicles with tubes: (11.1) radius 30 µm and (13.1) radius 16 µm.

Figure 3. (a) Velocity versus time: the steady regime is reached in the middle of the chamber. (b) Velocity during (▲) departing of the vesicle from the top substrate, (●) landing of the vesicle on the bottom substrate and (—) equation (1); h is the distance between the vesicle membrane and the top wall (during departing) or between the vesicle membrane and the bottom wall (during landing).

Function of the distance h to the departing or the landing wall of the chamber. In comparison, we also plot the sedimentation velocity U of the equivalent sphere given by the following law,

\[ U = \frac{2g}{9} \frac{\Delta \rho R^2}{(1 + (R/h))}, \]

where \( \Delta \rho \) is the difference in density between the inner fluid of the sphere and the outer suspending fluid and g is the gravitational acceleration. U is derived from the approximated expression for the drag force of a rigid sphere moving close to a wall perpendicular to the direction of the sphere motion [28]. One can clearly see that departing and landing velocities
Figure 4. Variation of the translation velocity observed for different vesicles in the steady state versus $\Delta \rho g R^2/\eta$; square: velocity of an agarose rigid bead ($R = 36 \mu m$, $\Delta \rho = 1.0466$).

are pretty similar and are well described by (1). This result shows that the deviation of the vesicle shape from a sphere does not affect the velocity field around the particle.

To confirm this result, we also plot the stationary velocity $U$ observed far from the walls for all of the studied vesicles, whatever their shapes, and for a spherical bead of agarose ($R = 36 \mu m$) as a function of $(g/9)/(\Delta \rho R^2/\eta)$. All data lie on a single line as shown in figure 4. This result confirms that the vesicle behave like rigid spheres in a Stokes flow while their surface is an incompressible 2D fluid. Because the lipids cannot leave the interface, the divergence of the velocity field is exactly zero. This strong constraint does not exist for a liquid drop, where the molecules can leave the interface and transfer momentum to the volume even in an axisymmetric case. A liquid drop therefore has a lower friction force than the fluid vesicle.

As a consequence, we will consider in a first approximation that the drag force that is applied on a sedimenting vesicle far from the walls is equal to the Stokes drag acting on a rigid sphere of equivalent radius: $6\pi \eta RU$.

5. Shape deformation: detailed description

The sedimentation of a vesicle generates an excess pressure upstream and a lower pressure downstream, at its top. This difference in pressure is at the origin of the pear-shaped deformation. The viscous forces exerted by the fluid on the vesicle membrane redistribute a part of the membrane excess area toward the top part of the vesicle and under some conditions are able to draw a membrane tube. Using the pressure field around a rigid translating sphere [29], we can express the variation in the pressure $p$ on the surface of the vesicle at a distance $R$ from the center of mass,

$$p = p_\infty - \frac{3}{2} \eta \frac{\vec{U} \cdot \vec{n}}{R},$$

where $p_\infty$ is the pressure in the fluid far from the vesicle and $\vec{n}$ is the normal to the membrane. The difference in pressure between the top and the bottom part of the vesicle is therefore $3\eta U/R$, which is typically of the order of $10^{-3}$ Pa in our study.

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5.1. Hydrodynamic tube extrusion

We consider the tube observed on the top of the vesicles in the stationary regime, i.e. when \( r \ll R \) and \( L > r \). We write the free energy of the tube membrane by using the approach developed by Waugh et al \([7, 30, 31]\), Evans and Yeung \([32]\), Derényi et al \([33]\), Hochmuth et al \([16]\), Brochard-Wyart et al \([8]\) and Fournier \([34]\). It is based on the Helfrich Hamiltonian, which describes the membrane as a fluid, incompressible surface with a homogeneous surface tension \( \sigma \) and a bending rigidity \( \kappa \). For a fixed tension and a fixed pressure, the tube free energy \( F \) of radius \( r \) and length \( L \) can be written as

\[
F = \left( \frac{\kappa}{2r^2} + \sigma \right) 2\pi r L - \Delta p \pi r^2 L - f L, \tag{3}
\]

where \( \Delta p \) is the pressure difference across the tube membrane and \( f \) is the pulling force, which elongates the tube on a length \( L \).

We consider that the thickness of the membrane is constant. Then, the area difference between the lipid inner monolayer and the outer monolayer of the membrane changes when a tether is pulled out. The change in area difference creates a spontaneous curvature of the membrane calculated in \([35]\). The energetic cost for this curvature was estimated in \([36]\) to be \( \approx \kappa L^2 / R^2 \), where \( R \) is the vesicle radius. This contribution is significant only for tube lengths of more than 100 \( \mu \)m, so that we omit it in the free energy.

The tube radius and the pulling force are calculated at equilibrium by taking \( \partial F / \partial r = 0 \) and \( \partial F / \partial L = 0 \). The first derivative gives the equilibrium Laplace law for a cylinder,

\[
\frac{\sigma}{r} - \frac{\kappa}{2r^3} = \Delta p. \tag{4}
\]

We estimate the pressure difference across the tube membrane by considering that the hydrostatic pressure out of the tube is roughly equal to the pressure upstream at the vesicle top: \( \Delta p = p_{in} - (p_{\infty} - 3\eta U / 2R) \), where \( p_{in} \) is the pressure within the vesicle. Here, the vesicles are initially tensionless since they have been deflated. In this case, the difference in pressure, \( p_{in} - p_{\infty} \approx 0 \ll 3\eta U / 2R \). Therefore, (4) can be rewritten as

\[
\frac{\sigma}{r} - \frac{\kappa}{2r^3} = \frac{3\eta U}{2R}. \tag{5}
\]

The surface tension of the vesicle is induced by the flow and is therefore of the order of the ‘hydrodynamic’ tension \( \sigma \approx \eta U \approx 10^{-8} \text{ N m}^{-1} \). The pressure term in the right-hand term of (5) is one order of magnitude smaller \( (\approx 10^{-3}) \) than the tension and the bending term \( (\approx 10^{-2}) \) since the tube radii are much smaller \( (r \approx 1 \mu \text{m}) \) than \( R \). The right-hand side term for pressure in (5) therefore plays only the role of a small corrective factor.

If the vesicle is initially tense \( (\sigma \gg \eta U) \), the pressure terms in (5) are not dominant. One retrieves the classical frame \([8, 33, 34]\) \( r = (\kappa / 2\sigma)^{1/2} \), which yields the typical size for \( r \) of the order of 40–50 nm for a standard value of \( \sigma \approx 10^{-5} \text{ N m}^{-1} \).

The second derivative \( \partial F / \partial L = 0 \) gives the classical value of the equilibrium pulling force exerted on the tube of radius \( r \),

\[
f_c = \frac{3\pi \kappa}{2r} + \pi r \sigma. \tag{6}
\]

The combination of the two equations, (5) and (6), sets the equilibrium radius \( r_0 \) of the tube coexisting with a quasi-spherical vesicle and the associated equilibrium pulling force \( f_0 \).
In the case of a sedimenting vesicle, the force exerted on the vesicle surface is the drag force induced by the flow.

The pulling force, which initiates the tube, is exerted on the top of a spherical cap of area \( S \),

\[
\vec{f}_H = -\frac{3\eta U}{2R} S. \tag{7}
\]

When this hydrodynamic force is equal to the extrusion force \( f_0 \), it is possible to draw a tube. The condition \( f_H = f_0 \) is

\[
\frac{3\eta U}{2R} S = \frac{3\pi \kappa}{2r_0} + \pi r_0 \sigma. \tag{8}
\]

Noteworthy is that equation (8) compares the critical force of extraction at a tension and for hydrodynamic conditions that are not exactly those of the stationary regime. However, since we see a tube emerging for a value of the velocity close to 90\% of the stationary regime, we believe that our approximation is valid.

We assume that the radius of this spherical cap is of the order of magnitude of the tube radius plus the crossover region that relates the quasi-spherical vesicle to the tube (red line in figure 5). As pointed out in [37], the crossover region is an exponentially damped sinusoid with a decay length of the order of \( r_0 \). We therefore write \( S \approx \pi (\alpha r_0)^2 \), with \( \alpha \) being the only adjustable parameter, close to 2. By using (5) and (8) to eliminate \( \sigma \), one obtains a relation for the equilibrium tube radius

\[
r^3 (\alpha^2 - 1) = \frac{4\kappa R}{3\eta U}. \tag{9}
\]

Figure 6 shows a plot of the parameter \( r^3 \) as a function of \((4R/3\eta U)\) measured for 13 vesicles of different sizes \( R \) (from 16 to 48.5 \( \mu \text{m} \)) and velocity \( U \) ranging from 4 to 12.5 \( \mu \text{m s}^{-1} \). Data are fitted using (9) the slope, \( \kappa/(\alpha^2 - 1) \), equal to \( 8.11 \times 10^{-21} \) J. By setting that the bending rigidity \( \kappa \approx 10 \text{kT} = 4 \times 10^{-20} \) J, which is a value currently accepted for DOPC membranes, we find that \( \alpha = 2.45 \), which is in good agreement with the prediction for the crossover region given in [37].
Figure 6. Variation of a parameter scaling as the cube of the tube radius versus the reduced variable $4R/3\eta U$. The straight line is the theoretical prediction from (9) with a slope equal to $8.11 \times 10^{-21}$ J.

From the experimental measurements of the equilibrium tube radius and of the sedimenting velocity, we have estimated the tension of the 13 vesicles during their sedimentation from equations (5) and (8). These are very low (table 2), as expected for floppy vesicles. Noteworthy is that the force $f_0$ required for drawing a tube from a floppy vesicle is quite small, of the order of $10^{-13}$ N.

For tensed vesicles, where the pressure term in equation (4) is neglectable, the same power law is retrieved, $r = (\kappa R/6\eta U)^{1/3}$, and the tension scales as $\sigma = (2/3)(6\eta U/R)^{2/3}$. The velocity required for drawing a tube from a vesicle of tension $\sigma$ and bending energy $\kappa$ is $U = (R(2\sigma)^{3/2}/6\eta\kappa)^{1/2}$.

In figure 7, we show the theoretical curves of variation of the ratio of the tube radius to the vesicle radius and of the adimensioned tension, $\sigma R^2/\kappa$, as a function of the adimensioned capillary number $\eta U R^2/\kappa$ for a vesicle drawing a tube.

To get an idea of the evolution of the vesicle tension during sedimentation, we measure its apparent surface area. When the tension increases, the apparent vesicle area $A$ also increases, unfolding the submicron thermal fluctuations of the membrane. We plotted in figure 8 the parameter $R_{\text{Area}} = (A/4\pi)^{1/2}$ as a function of time. While decelerating just before landing, the vesicle reduces its area, which reaches a minimum, revealing a minimal surface tension. At this stage, the vesicle flattens and swallows the tube. Then, the area increases again when the vesicle settles onto the substrate and reaches a value similar to that observed during the steady sedimentation stage. Vesicle tensions falling steadily in a fluid or settled onto a substrate are
Figure 7. Theoretical curves (small dots) of the variation of the ratio of the tube radius to the vesicle radius and of the adimensioned tension, $\sigma R^2/\kappa$ as a function of the adimensioned capillary number $\eta UR^2/\kappa$ for a vesicle drawing a tube. Filled circles correspond to pear-like vesicles (table 2), where the tension is calculated from equation (12), while squares correspond to vesicles drawing a tube (table 1), where the tension is calculated from equation (5). The bending energy is set to $4 \times 10^{-20}$ J.

similar. This is not surprising since the vesicle is subjected in both cases to the same gravitational force. In one case, the reaction is the drag force, whereas in the other case, it is the reaction of the substrate. The vesicle area and volume are similar in both cases but the repartition of the force is different, yielding different shapes.

The tube comes from the change of the repartition of the volume and the surface area of the vesicle with respect to the shape of the vesicle settled at the bottom of the chamber. It is possible since the vesicle presents an excess of area. It is characterized by the reduced volume,

$$\nu = \frac{3V}{4\pi (A/4\pi)^{3/2}}$$

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Figure 8. Variation of \((A/4\pi)^{1/2}\) (□) and velocity (●) versus time. Top: pictures of the vesicle at various times during the sedimentation process.

defined as the ratio of the volume of the vesicle to the volume of the sphere with the same surface area \((\nu \leq 1, \nu = 1\) for a sphere), where \(V\) is the vesicle volume. It is deduced from azimuthal integration over the contour of the vesicle settled on the substrate by applying the Papus–Guldin theorem (assuming an axisymmetric shape) [38]. The length of the tube \(L\) can then be estimated by expressing the overall volume of the spherical part + the tube part as \(V = 4\pi R^3/3 + \pi r^2 L\), and the surface area as \(A = 4\pi R^2 + 2\pi r L\) and by replacing \(V\) and \(A\) in the expression for the reduced volume. This yields

\[
\nu = \left( R^3 + \frac{3}{4} r^2 L \right) \left( 1 + \frac{r L}{2R^2} \right)^{-3/2} R^{-3}.
\] (10)

After linearization, we obtain the expression for the tube length,

\[
L = \frac{4 R^3 (1 - \nu)}{3 r (\nu R - r)}.
\] (11)

Figure 9 shows the plot of \(L\) measured as a function of the reduced parameter \(\frac{4 R^3 (1 - \nu)}{3 r (\nu R - r)}\) for the 13 vesicles, which exhibit micron-size tubes. The agreement with the theoretical curve, without any adjustable parameter, is quite good.

5.2. Egg- and pear-like shape vesicles

When the sedimenting velocity of a vesicle is too small, the hydrodynamic force is smaller than the critical force required for extruding a tube. This happens for the couples \((\sigma, \eta U)\) located above the curves displayed in figure 7. The vesicle deforms but no tube is extruded at its rear. Because the external pressures at the bottom and at the top part of the vesicle are different,
the curvature radii at the top and at the bottom of the vesicles are different, generating egg- or pear-like shapes.

We propose to calculate the tension of little extended objects like egg- and pear-like vesicles. With a good approximation, we can describe the bottom and the top of the vesicle by two spherical caps of radius $R$ and $r_{\text{top}}$, respectively. In this case, we can roughly estimate the vesicle surface tension by writing the Laplace law on both the spherical parts,

$$p_{\text{in}} - p_{\infty} - \frac{3\eta U}{2R} = 2\sigma_{\text{bot}}/R \quad \text{and} \quad p_{\text{in}} - p_{\infty} + \frac{3\eta U}{2R} = 2\sigma_{\text{top}}/r_{\text{top}}.$$

The flow imposes a pressure gradient inside the membrane surface, which compensates for the applied viscous force per unit area. The tension difference between the bottom and top endcaps is equal to $3\eta U$; that is, $\sigma_{\text{bot}} - \sigma_{\text{top}} = 3\eta U$, leading to the following relation between the velocity, the radii and the tension,

$$\sigma_{\text{top}} = \frac{9\eta U r_{\text{top}}}{2(R - r_{\text{top}}^2)}.$$  \hfill (12)

We measured the values of $R$ and $r_{\text{top}}$ on 18 egg- and pear-like vesicles, and estimated the membrane surface tension of the vesicles, which are reported in table 2.

In figure 7(b), we plot the reduced tension of these vesicles as a function of the capillary number $\eta U R^2/\kappa$ to obtain a phase diagram. The domain of coexistence at equilibrium of a tube and a spherical vesicle is on the black curve. The pear-like regime lies in the uppermost part of the diagram, above the curve of tube–vesicle coexistence. Below the coexistence curve, the vesicles are initially floppy and are not in equilibrium: a tube is initiated, which grows and increases the vesicle tension. The length of the tube stops when the tension reaches the

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**Figure 9.** Experimental tube length versus a reduced parameter (see text). The straight line of slope 1 is the theoretical prediction.
equilibrium coexistence tension. In our case, most vesicles exhibit large excess area but are slightly tensed due to the gravity.

6. Conclusion

We have shown that the drag force is able to draw a tube from the top of a vesicle sedimenting in a quiescent fluid, while it is not a point force as is usually described in the literature for membrane tethers extrusion. In our study, we used vesicles that were osmotically deflated before the experiments and characterized by very small (vanishing) surface tensions. In this case, the critical force to extrude a tube is very small, of the order of $10^{-13}$ N, and a vesicle moving at a few $\mu m s^{-1}$ can develop tubes with micron-size radii. Noteworthy is that no force barrier seems to exist for the formation of tubes, in contrast to the case of tube formation by the application of a point force, where the force barrier grows linearly with the size of the area on which the force is exerted [39].

With a classic thermodynamic approach, we proposed a basic model, which predicts that the tube radius varies as $r = (\kappa R/6\eta U)^{1/3}$ consistent with reported experimental data. For large tensions, we predict that the velocity required for drawing a tube increases with the power 1.5 of the surface tension.

These results may be of importance when GUVs are subjected to centrifugation, as is the case for the recent method called double emulsion, used to prepare vesicles. In this case, the vesicle velocity can reach several hundreds of $\mu m s^{-1}$ and can generate the formation of membrane tubes.

Finally, we hope that our work will stimulate numerical, more detailed theoretical descriptions able to predict all of the observed shapes of vesicles, from the existence of tubes to pear-like shapes, within a single frame.

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