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Vesicles are closed fluid membranes with a size in the range $10 - 100\mu m$. Under flow they exhibit various dynamics such as tank-treading, tumbling, and vacillating-breathing. Part of these dynamics are studied experimentally both on earth and under microgravity conditions. We report on the basic results of vesicles dynamics. Deviations from existing theories are discussed. A small deformation theory is presented that shows notable differences from those imposing a fixed shape: the vesicle shape is shown to undergo a temporal oscillation. This theory allows one to extract the effective viscosity of the suspension.

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I. INTRODUCTION

Phospholipids are amphiphilic molecules that form bilayers in aqueous solutions to prevent contact between hydrophobic aliphatic chains and water. Phospholipid bilayers can form closed membranes known as vesicles or liposomes (Fig.1). Specifically, giant vesicles have a diameter between one and several hundred microns. The mechanical characteristics of usual vesicles are simple. Their internal fluid is generally a viscous liquid which may be more or less complex in order to mimic viscoelastic properties of real cells. The interest of such systems lies in their relative simplicity in comparison to real cells (though they are complex at the absolute level). They are thus thought of as model systems allowing us for the study of transport phenomena of blood elements. The attractive feature of these systems was revealed in the 70's when it became clear that a relatively simple model (based on the curvature energy, very much like the problem of thin plate buckling), due to Helfrich[1], reproduced several shapes which are reminiscent of those met in biology. Of particular interest is the biconcave shape of a red blood cell which has been captured by the Helfrich model, and which has been also reproduced experimentally by vesicles[2].

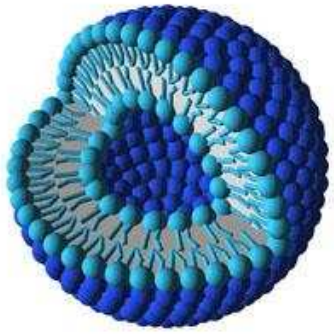

FIG. 1: Schematic view of a vesicle made of a bi-layer of phospholipid molecules

Vesicles under flow have been the subject of intensive work both theoretically[3] and exeprimentally[4, 5]. Let us summarize the main types of dynamics in non confined geometries. So far, three basic types of motion have been reported on: (i) tank-treading, (ii) tumbling, (iii) and vacillating-breathing (VB). Under shear flow and if there is no viscosity contrast between the interior and the exterior, the vesicle makes an angle (smaller than 45°) with the shear direction, while the membrane which is fluid executes a motion which is similar to a tank-treading one. If the vesicle were solid inside, it would just tumble since a shear flow possesses a solid-rotation component. If the inside is fluid, part of the solid-rotation torque due to the applied shear is transferred to the membrane, which in turns transfers it to the enclosed fluid. If the inside viscosity is not too large then an equilibrium position is found with an orientation angle $0 < \theta < 45°$. On increasing the inside viscosity, the transfer of the torque to the enclosed fluid by the tank-treading membrane becomes more and more difficult; dissipation inside the vesicle refrains membrane tank-treading. At a critical value of the viscosity contrast, an equilibrium tank-treading solution ceases to exist, whereby a new solution in the form of tumbling takes place. This occurs as a *true* bifurcation: a saddle-node bifurcation. In the tumbling regime, a new type of motion is found. This motion is called *vacillating-breathing* (VB mode): the vesicle orientation undergoes an oscillation around the flow direction, while the shape executes breathing dynamics. The first two solutions are observed experimentally on vesicle and red blood cells, while the VB has not yet been clearly identified.

The change of the internal viscosity of the vesicle is achieved thanks, for example, to the dextran (see below). This leads inevitably to a density contrast between the inside and outside. Adding additives in the outside solutions would break the osmotic equilibrium, and it is almost unfeasible to obtain an iso-density situation. Therefore, sedimentation is unavoidable. This has several consequences: free and permanent tumbling can not be easily studied, and for a suspension sedimentation leads to a rheolgy that should critically depend on the vesicle-substrate interaction, and makes the analy-

sis quite difficult. For all these reasons several experiments were conducted under a microgravity environment (CNES parabolic flights), and others are scheduled with CNES and ESA, together with a sounding rocket experiment which is scheduled in 2007. These series of studies should help shedding light on the complex phenomena related to dynamics of individual vesicles, and their collective behavior, with both fundamental and potential technological impacts. Here we report on the main results, some of them are obtained under microgravity conditions. We report on tumbling and show the difference with theory, and present theoretical results on dynamics and rheology.

II. EXPERIMENTAL TECHNIQUES

A. Vesicle preparation

Giant vesicles were prepared from dioleoylphosphatidil choline (DOPC)(Sigma) using the electroformation method [6]. At room temperature, DOPC membranes are in a fluid state. Vesicles were swollen in aqueous solution with different concentrations of glucose and a polymer (referred to as interior solution), used in order to modify the inner viscosity of vesicles. Two types of polymer where used : dextran or sodium carboxymethylcellulose (CMC). In the range of concentrations and shear rates we used, rheometer measurements showed that the behaviour of all our solutions was Newtonian. Dextran (D5251 from Sigma) is a biocompatible high molecular weight ($MW \approx 5 \cdot 10^5 g/mol$) polymer of glucose. It is necessary to use relatively high weight concentrations of Dextran to obtain viscosities around 10 times the viscosity of water. This makes vesicles denser than the exterior solution.

We used dextran concentrations up to 11% w/w, providing viscosity ratios $C = \eta_{in}/\eta_{out}$ between 8.7 and 19.9. Longer polymers are also used: carboxy-methylcellulose. With a concentration of 0.1 % w/w of CMC, we expect a viscosity ratio of 23.6 from viscosity measurements.

In addition to polymers, glucose (or sucrose for CMC vesicles) was added in electroformation solutions in order to control the osmotic pressure inside the vesicle. Sugar concentrations were in the range 50–400 mM. After formation, vesicle suspensions were diluted with a hyperosmotic sucrose (glucose for CMC vesicles) solution (hereafter referred to as exterior solution). Due to the permeability of the membrane, this dilution leads to the deflation of initially quasi-spherical vesicles. In addition to their size and the viscosity ratio between interior and exterior solutions, vesicles are therefore characterized by a swelling factor or reduced volume whose definition is given below, and which compares the volume of a vesicle to that of a sphere having the same surface area.

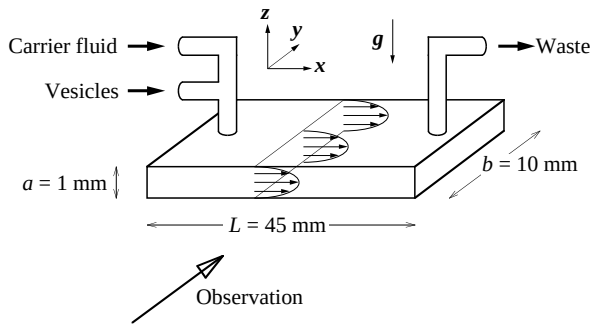

FIG. 2: Schematic view of the flow chamber

B. Experimental setup

Two types of flow chambers were used in this study: a modified quartz Hellma flow chamber, and home made pexiglass chambers with glass optical windows (microscope cover slides). The system is depicted on Fig. 2. Flow chamber dimensions are: $L = 45$ mm along the flow axis $\mathbf{x}$, $b = 10$ mm along the $\mathbf{y}$ axis and $a = 1$ mm along the $\mathbf{z}$ axis. Previously deflated vesicles were gently injected at one end of the channel and mixed with carrier fluid (exterior sucrose solution), and a constant flow rate q (between 5 and 50 ml/h) is imposed by a KDS syringe pump. A nearly 2D Poiseuille flow takes place inside the chamber with a parabolic velocity profile across the thickness a, which is approximately invariant in the $\mathbf{x}$ and $\mathbf{y}$ directions, provided the observed object is at least 1 mm away from optical windows and not too close from the inlet and outlet channels. The flow chamber is mounted on a phase contrast microscope stage The microscope, ordinarily vertical, is rotated by 90 degrees with respect gravity, to allow for a side view.

The local shear rate in the flow is:

$$\dot{\gamma}(z) = \frac{\partial u}{\partial z} = \frac{6q}{a^3 b}(a - 2z) \qquad (1)$$

The maximum shear rate near the wall $\dot{\gamma}(0) = 6q/(a^2 b)$ is therefore approximately between 1 and 10 s^{-1} in our range of flow rates.

However, one consequence of the density difference between vesicles (especially with high dextran concentrations) and carrier fluids, which is only partly reduced by putting glucose in the inner solution and sucrose in the outer one, is a sometimes significant sedimentation of vesicles in the $\mathbf{z}$ direction, resulting in an unsteady shear rate around the vesicle and possible wall effects if it comes too close to the bottom wall. To avoid sedimentation effects for heavier vesicles, some results presented here were obtained in microgravity conditions (CNES parabolic flight campaign, April 2004, Bordeaux, France).

Vesicles are hereafter defined by their nominal radius $R_0 = \sqrt{S/4\pi}$ and reduced volume $\nu = 3V/(4\pi {R_0}^3)$. By definition, $\nu = 1$ corresponds to non-deflated sperical vesicles.

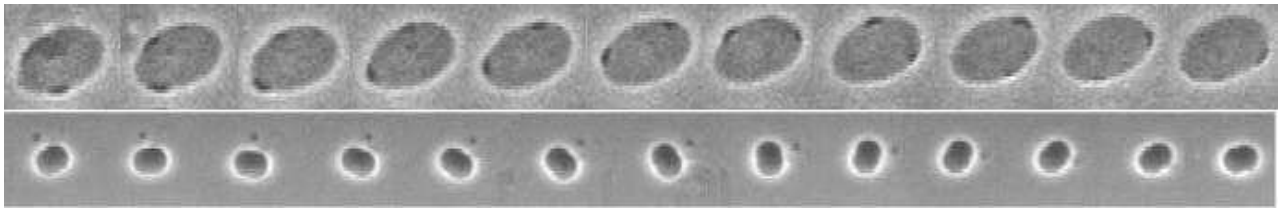

FIG. 3: Top: a snapshot of a tanktreading motion. The dark "defect" on the membrane moves along the membrane from one frame to the next, showing thus the tanktreading motion of the membrane. Bottom: tumbling motion of vesicles filled with dextran. Viscosity ratio $C = 22.5$, reduced volume $\nu = 0.993$, $R_0 = 7.5\mu$m, time interval between pictures = 0.5 s

III. RESULTS AND DISCUSSION

At low viscosity contrast the vesicle is oriented in the flow (Fig.3, top) making a certain angle with the flow direction. At the same time the membrane has a tank-treading motion. The orientation angle decreases upon an increase of the viscosity contrast for a given value of the reduced volume ν. On the other hand, the same tendency is found upon a decrease of ν by keeping the same viscosity contrast. For a given ν, there is a critical value of $\lambda \equiv \eta_{in}/\eta_{out} = \lambda_c$ at which there is a bifurcation from the tank-treading motion towards tumbling. In the tumbling regime the membrane continues to make tank-treading, albeit in a less pronounced fashion. Figure 3 shows a typical spanshot of tumbling. The orientation angle as a function of time is shown on Fig.4. The behavior is nonlinear. It can be shown from a theoretical point of view that if one assumes that the shape of the vesicle remains unchanged, then θ obeys [7] the following equation:

$$\dot{\theta} = A + B\cos 2\theta \tag{2}$$

where $A = -\dot{\gamma}/2$ is the mean rotation velocity, due to the rotational component of the shear flow, and B is a function of the ellipsoid's aspect ratio and viscosity ratio:

$$B = \dot{\gamma}\left[\frac{1}{2} + \frac{C_1}{\left(C_2 - \frac{\eta_{in}}{\eta_{out}}\right)}\right] C_3 \tag{3}$$

C_1, C_2 and C_3 are geometric constants depending on the ratios between the ellipsoid's axes[7, 8].

Physically, the term $B\cos\theta$ in equation 2 is a consequence of the elongational part of the shear flow, which applies a torque on the particle, depending on its orientation.

Equation 2 predicts two types of motion, depending on the ratio $-A/B$. For $-A/B < 1$, there is a stationary solution where the ellipsoid keeps a constant orientation $\theta = \frac{1}{2}\arccos(-A/B)$, and a tank treading motion of the membrane occurs. This motion was well identified and quantified experimentally by several authors [4, 9, 10], with or without a viscosity contrast.

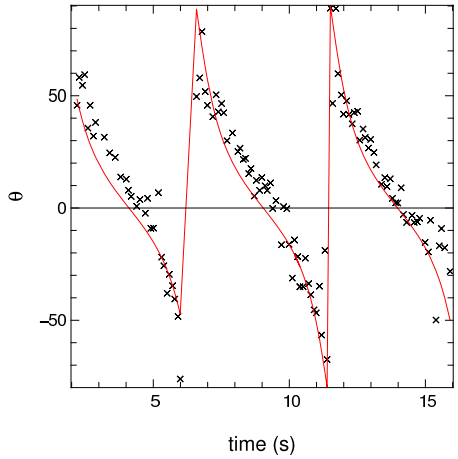

FIG. 4: Orientation θ of a tumbling vesicle with a viscosity ratio $C = 12.4$, a reduced volume $\nu = 0.996$, $R_0 = 13.5\mu$m.

For a given shape, if the viscosity ratio η_{in}/η_{out} becomes sufficiently large, we get $-A/B > 1$ and the solution for $\theta(t)$ is a periodic tumbling motion:

$$\theta(t) = arctan\left(\frac{A+B}{\sqrt{A^2-B^2}} tan\left(\sqrt{A^2-B^2}\,(t-t_0)\right)\right) \tag{4}$$

An example of this motion can be seen in fig. 4. As far as we know, quantitative data on the tumbling motion of vesicles has never been reported before. Recently, an analytical treatment was conducted in the small deformation theory[11]. The new feature of this study was to relax the constraint of a fixed shape. During tumbling there is ample experimental evidence that the shape evolves in time. The result of this study was that the dynamics are described by two coupled equations: one for θ, and the other for the evolution of the shape. These equations have the form

$$\epsilon\partial_t R = h\left[1 - 4\frac{R^2}{\Delta}\right]\sin(2\theta) \tag{5}$$

$$\epsilon\partial_t \theta = -\frac{1}{2} + \frac{h}{2R}\cos(2\theta) \tag{6}$$

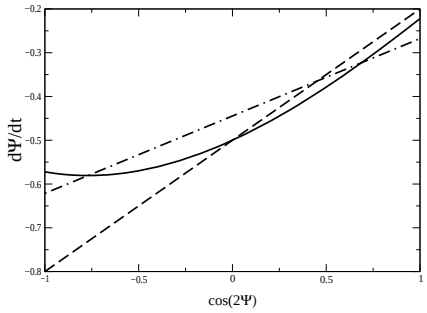

FIG. 5: Full line: the full calculation. The dashed line: the KS theory. The dashed-dotted line: a linear fit from the full calculation. $h = 0.3$, $\Delta = 1$

$R = 0$ corresponds to a sphere. R represents the amplitude of the second spherical harmonics. Δ is the membrane excess area defined by $A = 4\pi+\Delta$, and is related to ν by $\Delta = 4\pi[\nu^{-2/3} - 1]$, and $h = 60\sqrt{2\pi/15}/(32 + 23\lambda)$, and ϵ is a small parameter measuring the strength of deviation from a sphere. The additional feature here is that R is an oscillating function of time which is in qualificative agreement with the observation. At first sight we found that θ has the same qualitative feature as that shown on Fig.4. However, a comparison of the KS theory to the present analysis reveals significant differences. $d\theta/dt$ is plotted as a function of $\cos(2\theta)$ (Fig.5). This leads, for the KS theory, to a straight line (as can be seen from eq.(6). Taking into account the deformability of the vesicle, a marked difference is found as shown on Fig.5 (full line). A linear fit, dictated by the KS theory, produces the dashed-dotted line in that figure. This fit conveys the impression that the effective rotation frequency (represented by A) is smaller (in absolute value) than the KS one. The same holds for B (representing the slope). The present state is that we have not yet made a systematic comparison between theory and experiments (especially that A and B are significantly smaller than those obtained from the KS theory, and that this becomes more dramatic upon an increase of the shear rate)[8]. When allowance is made for deformability one finds from the small deformation theory smaller values than the KS ones (as seen experimentally). However the small deformation theory does not contain the information about the evolution of A and B as a function of the shear rate. This requires pushing the analytical theory to the next order in the deformation expansion in order to capture this effect. This is why we have not attempted here to make a quantitative comparison with the experimental study, and this is currently under investigation. Nevertheless, for the set of parameters explored so far in the small deformation theory provides us with values of A and B which are smaller than those which follow from the KS theory.

Following Einstein[12] we could derive the expression of the effective viscosity of a dilute suspension of vesicles. The result is given by

$$\eta_{eff} = \eta \left[1 + \frac{5}{2}\phi - \phi\frac{\Delta(23\lambda + 32)}{16\pi}\right] \qquad (7)$$

For $\Delta = 0$, we recover the Einstein result, $\eta_{eff} = \eta[1 + \frac{5}{2}\phi]$. For $\Delta = 0.5$ (corresponding to only 4% in relative excess area where a perturbative scheme is expected to make a sense), we find for $\lambda = 1$ and 2, $\eta_{eff} \simeq \eta[1 + 2\phi]$ and $\eta_{eff} = \eta[1+1.5\phi]$, respectively. These are significant shifts. Note that the above relation was derived in the tank-treading regime. In the tumbling regime and the VB one we do not know how would this be altered. In addition, our study allows one to derive a rheological constitutive law, which is currently under preparation.

The overall rheology will be made in connection with the rheological measurement which will be performed soon on the ground (in a cone-plate rheometer), and under microgravity conditions (sounding rocket in 2007), as well as in parabolic flights shortly before the sounding rocket experiments. We hope then to report on extensive results of rheology under gravity and its comparison under microgravity conditions.

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