

TUMBLING OF LIPID VESICLES, ENCLOSING A VISCOUS FLUID, UNDER A SHEAR FLOW

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We performed an experimental study of the tumbling motion of deflated lipid vesicles, when the viscosity, η_{in} , of the fluid, enclosed by the vesicle membrane, is sufficiently higher than that, η_{out} , of the outer aqueous solution. Vesicles with reduced volumes (the ratio between the vesicle volume and the volume of a sphere, having the same surface area) in the range 0.74 to 0.96 were subjected to a simple shear flow, and the vesicle tumbling motion was characterized for various viscosity contrasts ($r = \eta_{in}/\eta_{out}$ ranging from 9.6 to 28.3). Experiments reveal a strong effect of vesicle deformability (high flexibility of the lipid membrane) on the tumbling motion in a hydrodynamic field: the vesicle shape significantly changes during the rotation, influencing the relationship between rotation speed and angle. For less deformable vesicles (higher reduced volume or lower capillary number), the experimental motion is in good agreement with simpler analytical models for fixed shape ellipsoidal vesicles. We compare the experimental measurements with numerical results, obtained by a boundary integral method.

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1. Introduction

Representing a simplified model of the lipid matrix of a biomembrane, giant unilamellar vesicles can be used in static and dynamic investigations, aimed to study the pure mechanical response of biomembranes in different processes, inspired by real biological systems. An example is the blood rheology, the investigation of which is of major importance in biomedical applications. Although quite simplified, the vesicle model represents a good physical approach to the erythrocyte rheology in the blood circulation. Recently, the dynamics of lipid vesicles in a shear flow has been the object of theoretical [1, 2], numerical [3-5] and experimental [6] investigations. Two types of vesicle motion have been predicted in the literature. When the viscosity contrast between the interior and the exterior fluid is low enough ($r = \frac{\eta_{in}}{\eta_{out}}$, where η_{in} and η_{out} are the internal and the external viscosities, respectively), the vesicle is reported to assume a fixed orientation with respect to the shear flow, with a tank-treading motion of its membrane. Above a critical value of the viscosity ratio, $r > r_{cr}$, which depends on the vesicle reduced volume (the ratio between the vesicle volume and the volume of a sphere having the same surface area), a transition to an unsteady (flipping) motion of the vesicle is expected, when its major axis rotates with respect to the flow direction. Here, we report our experimental and numerical results concerning the tumbling of giant lipid vesicles, filled up with a viscous aqueous solution of a biopolymer and subjected to a linear shear flow.

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2. Experimental details

Giant unilamellar vesicles were prepared from dioleoyl-phosphatidylcholine (DOPC) (Sigma-Aldrich Chem., Germany), using the electroformation method [7]. In order to modify the viscosity of the interior fluid, the vesicles were swollen in aqueous solutions with different concentrations of a mono- or di-saccharide (glucose or sucrose) and a biopolymer (interior solution). The former is necessary for controlling precisely the osmotic pressures inside and outside the vesicles, and the addition of the latter to the solution modifies its viscosity. After preparation, vesicle suspensions were taken out of the electro-formation chamber, and diluted with a hyperosmotic sugar solution (hereafter referred to as the exterior solution), provoking the deflation of the vesicles (due to the non-zero membrane permeability towards water). It is important to note that on the time-scale of our flow experiments (a few dozens of seconds) the membrane can be considered as impermeable. Two types of polymer were used: dextran or sodium carboxymethylcellulose (CMC), both provided by Sigma. Dextran is a biocompatible high molecular weight (in our case $MW \approx 5 \cdot 10^5 \text{ g/mol}$) polymer of glucose. Readily soluble in water, dextran forms clear and stable aqueous solutions, which show Newtonian behavior over a large range of shear rates. To attain the desired viscosity of dextran solutions, it is necessary to use relatively high weight concentrations of dextran. This makes the vesicles heavy compared to the outer solution, and thus promotes their rapid sedimentation during the flow experiment. Thus, approaching the bottom of the shear cell, vesicle tumbling will be affected by the substratum proximity. In order to compensate for the influence of gravity on the vesicle tumbling motion, a series of experiments was performed in ‘microgravity’ conditions (parabolic flights during the spring campaign of C.N.E.S.¹ in 2004 in Bordeaux, France). Another possibility for avoiding the undesired wall effect was to prepare the most viscous solutions with another glucose-based biopolymer, CMC, the solutions of which behave as Newtonian fluids for the concentration and shear rates used in our experiments. The advantage of CMC over dextran is that it allows the preparation of very viscous solutions of low density. Vesicles, swollen in CMC solutions, practically do not fall down over the time scale of a typical shear flow experiment (a few dozens of seconds). The viscosities of all polymer solutions were measured with a rheometer of the cone/plane type. All experiments were done at 25 °C on an Olympus IX71 inverted microscope, working in a phase contrast regime and inclined at 90° with respect to its usual position. The flow was imposed using a syringe pump, connected to a parallelepiped flow chamber. Vesicles were gently injected into the flow chamber, and shear flow was applied immediately after their injection in order to avoid the influence of the substratum on the vesicle motion.

3. Numerical simulations

The numerical modelling was performed using the “Boundary Integral” method [8]. This formulation addresses the viscous regime (low Reynolds number), and takes advantage of the linearity of the Stokes equation. Indeed, the velocity field reduces to an integration of the force field, with a non-local kernel also called a Green function. Since, in the absence of inertia, the local resulting force applied to an element of fluid cancels out (this is the meaning of the Stokes equation), the only contribution comes from the boundaries (the container and/or the membrane of the vesicle). This formulation thus allows a reduction of the full 3D problem to the treatment of the 2D boundaries only, which is computationally very interesting. We thus discretise the vesicle membrane using a triangular mesh. The boundaries of the container are discretised as well, and we follow the dynamics of the membrane during the shearing experiment. From the knowledge of the vesicular shape at a given time, t , the force field applied by the vesicle to the flow can be computed at each point of the mesh from local geometrical quantities such as the mean curvature and the local Gaussian curvature. The precise expression for the local force will not be reproduced here, but can be found in [9]. We use the description suggested by Helfrich for the curvature Hamiltonian of the membrane. Using the boundary integral formulation, the velocity field can be computed at each point of the mesh, allowing the computation of the new shape at time $t + dt$. A viscosity contrast

¹ CNES – Centre National d’Etudes Spatiales

between the inner and outer fluid can be accounted for as well, by adding an extra contribution to the expression for the force, without changing the overall framework.

4. Results and discussion

Using phase contrast microscopy, we were able to describe quantitatively the vesicle tumbling motion for different viscosity contrasts between the inner and the outer fluid, and to compare our results with the theoretical models for the dynamics of ellipsoidal particles in a shear hydrodynamic field. We investigated experimentally and numerically the effect of the membrane deformability on the vesicle flipping.

For every vesicle studied, its volume, V , and membrane surface area, S , were determined from the vesicle contour at its horizontal position during rotation (assuming axial symmetry). In this

way, each vesicle was characterized by a nominal radius, defined as $R_0 = \sqrt{\frac{S}{4\pi}}$, and its reduced volume $\nu = \frac{3V}{4\pi R_0^3}$. In our experiments, vesicles were deflated with ν between 0.74 and 0.96,

corresponding to prolate spheroidal shapes at equilibrium. The viscosity contrasts between the inner and the outer fluid were between 9.6 and 27.8, i.e. above the threshold of the tank-treading-to-tumbling transition for the range of all reduced volumes studied by us. In their “fluid” state, lipid membranes are characterised by a bending (curvature) modulus, k_c ($\sim 10^{-19}$ J), and a stretching modulus, k_s (~ 0.1 N/m). Thus, two dimensionless “capillary numbers” can be defined:

$$Ca_c = \frac{\eta_{in} \dot{\gamma} R_0^3}{k_c} \text{ and } Ca_s = \frac{\eta_{in} \dot{\gamma} R_0}{k_s}, \text{ where } \dot{\gamma} \text{ is the shear rate.}$$

When subjected to a linear shear flow, far enough from the bottom wall of the experimental cell, deflated vesicles with the viscosity contrasts mentioned above begin a tumbling motion (Fig. 1). The rotation angle is defined as that between the major inertia axis of the vesicle and the shear flow direction.

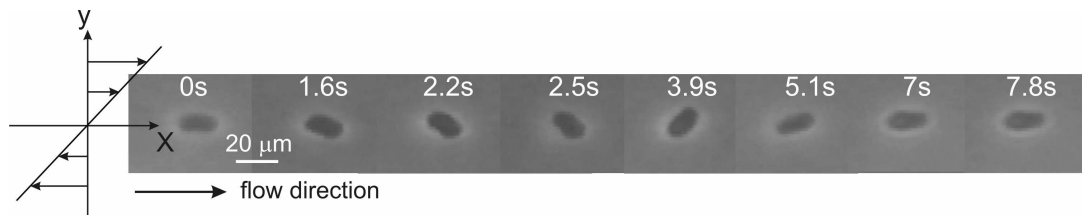


Fig. 1. A tumbling DOPC vesicle in a simple shear flow, as observed by phase contrast microscopy (int. solution: 7 wt% of Dextran and 0.05 mol/l of glucose; ext. solution: 0.065 mol/l of sucrose; $\nu = 0.8$; $R_0 = 7.5 \mu\text{m}$; $\dot{\gamma} = 0.52 \text{ s}^{-1}$). The experiment was carried out under microgravity conditions.

Our experiments revealed a strong effect of the vesicle deformability (high flexibility of the lipid membrane) on the tumbling motion. The vesicle shape changed significantly during the rotation (as in Fig. 1), influencing the relationship between rotation speed and angle. For vesicles with high reduced volumes or lower capillary numbers, the experimental motion is well described by simpler analytical models for fixed-shape ellipsoidal vesicles (Fig. 2a). Even for less deflated vesicles (Fig. 2b), the vesicle shape changes during tumbling and the principle axis lengths vary with time. The experimental observations are in good agreement with numerical simulations using the boundary integral method (Fig. 3). The deformation of the shape during tumbling is visualised in Fig. 3a (experimental results) and Fig. 3b (numerical simulation). Shadows correspond to the mean curvature, dark regions indicate a positive curvature while brighter areas are negative.

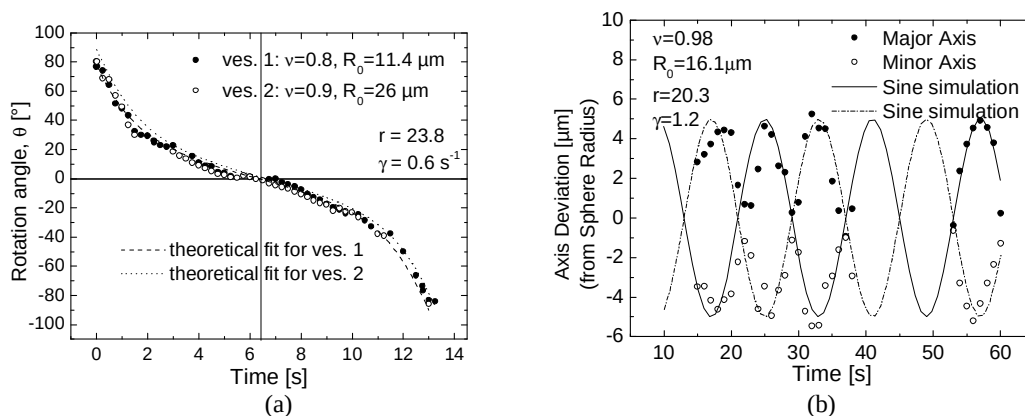


Fig. 2. (a) The rotation angles of two tumbling vesicles with different reduced volumes as a function of time (int. solution: 0.1 wt% of CMC and 0.025 mol/l of sucrose; ext. solution: 0.03 mol/l of glucose). The theoretical fit is performed according to [1]; (b) Vesicle deformation during tumbling (int. solution: 11 wt% of Dextran and 0.38 mol/l of glucose; ext. solution: 0.4 mol/l of sucrose)



Fig. 3. Deformation of a tumbling vesicle: (a) experimental observation in phase contrast light microscopy; $\nu = 0.96$; $r = 13.6$; (b) numerical simulation for $\nu = 0.9$; $r = 10$ (shadow code: dark represents a positive curvature, bright corresponds to a negative one);

Our experimental and numerical results reveal the strong influence of the membrane flexibility on the morphology of vesicles, tumbling in a hydrodynamic field. The relationship between the rotation speed of the vesicle and the rotation angle is also affected by the membrane deformability. The reported results are a first step towards the description of the mechanical response of biological cells to external hydrodynamic stress, which is an open question of major importance in many biological processes.

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