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Motion of a massive microsphere bound to a spherical vesicle

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Abstract. – We study the motion of a small solid particle (a few micrometers in size) attached to the membrane of a spherical giant lipid vesicle. By means of optical manipulation, the particle is first brought near the top of the vesicle, and released. We determine the friction experienced by the particle moving along the vesicle surface under the action of gravity. From experiments with latex and glass beads, we check that SOPC membranes are fluid at room temperature (static shear modulus $\mu = 0$) and estimate the shear viscosity of SOPC bilayers: $\eta_{\rm m} \approx 3 \cdot 10^{-6}$ surface poise.

In this letter, we study the motion of a small solid particle which is attached to an interface of spherical shape. The particle is spherical too, and a few micrometers in size. The interface may be a surfactant monolayer around a droplet of oil in water, or a vesicle membrane. In the latter case, the membrane is made of two molecular layers and forms a closed spherical shell in water. Experiments to be described in the following are dedicated to so-called "giant vesicles", which are made of phospholipids (the primary constituent of cell membranes), whose sizes are several 10 micrometers in diameter, and whose membranes are in the "fluid" (L_α) state.

Such experiments are a direct method to investigate the film viscosity, or —more generally— viscoelasticity. The basic principle has some in common with that used recently by Petkov *et al.* [1], who studied the motion of particles at the water/air interface under the influence of capillary forces. However, Petkov *et al.*'s technique demands a macroscopic film (Langmuir trough), while ours is applicable to systems of much smaller sizes, namely emulsion droplets or vesicles. In the case of lipid membranes, this distinction is crucial, since such membranes can be produced only up to microscopic scales.

Most of literature data on membrane shear viscosity ($\eta_{\rm m}$) are based on fluorescence techniques, which were used to measure diffusivities ($D_{\rm mol}$) of molecular probes embedded in membranes [2]. Relating $D_{\rm mol}$ to $\eta_{\rm m}$ needs modeling the probe as a macroscopic disk inside a

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continuous film [3], with a well-defined geometry. As well known, this procedure is questionable since the probe is of molecular size. In 1982, Waugh [4] proposed a *macroscopic* method for membrane rheology based on the growth rate of a filament that connects a giant vesicle to an attachment point. Waugh's analysis led to a quantitative determination of η_{m} ($\leq 5 \cdot 10^{-6}$ surface poise for egg phosphatidylcholine), but was based on crucial assumptions about the filament structure and size that cannot be checked independently. Recently, Smeulders *et al.* [5] studied the behavior under shear of colloidal suspensions of small lipid vesicles in the L_α phase. They argued that the suspension shear viscosity $\eta_{\mathrm{susp}}(\omega)$ at high frequencies ($\omega \approx 10^5$ Hz) is influenced by the membrane shear modulus μ and by η_{m}. Data inversion through a —not surprisingly— very complex theory led to $\mu \approx 3$ dyn cm^{-1} and $\eta_{\mathrm{m}} \approx 6 \cdot 10^{-7}$ sp for egg lecithin membranes at 10 °C.

The method presented in this letter is of the macroscopic type too, and offers the advantage of directness, since the experiment amounts to moving a micron-sized mechanical probe across the film. Data inversion has its own difficulties, as we will explain, but does not need special physical assumptions about unknown aspects of the system.

The vesicles in our experiments are made of SOPC (L_α-stearoyl-oleoyl-phosphatidylcholine) at room temperature and grown as clusters using the electroformation method [6]. First, we select a vesicle in the outer region of the cluster. There, one easily finds giant vesicles (50 μm is a typical diameter) which appear as spherical, unilamellar, and bear no internal structure (as far as we can decide from phase contrast views). These vesicles are weakly connected to their neighbours, apparently through "hard sphere" contacts. These connections are useful in our experiments because they prevent the vesicle under study from drifting or rotating as a whole in water.

A very diluted suspension of latex (or glass) particles is injected at some distance (≈ 15 mm) of the vesicles. There, a single particle is picked up by means of a long-working-distance optical trap [7] and then brought in contact with a selected vesicle. Experiments show that the spheres stick on the vesicle surface [8], usually with a finite contact angle α. SOPC membranes are known to be fluid at room temperature, which is directly observable by the fact that such a particle can be moved to any point on the vesicle surface. Conversely, its position perpendicular to the vesicle membrane cannot be varied, in other words α stays constant. In the rheology experiment, the particle is brought near the top of the vesicle surface, and then is released by switching off the laser beams. Then it starts gliding down along the vesicle contour under the action of gravity (fig. 1). The particle path is viewed and video recorded from above. Basically, the recorded trace (see fig. 2a) is the projection of the particle trajectory in a horizontal plane (x, y), near the vesicle equatorial plane. A particle tracking software yields the position of the particle center every 0.2 seconds with a resolution of ± 0.5 μm.

The central experimental information is the friction ζ experienced by the particle along the vesicle contour. Usually, the particle path shows Brownian excursions, superposed to what is perceived as a mean sedimentation path (see fig. 2). In the case of a "large and heavy" particle, Brownian noise is very small. If we neglect this "entropic" contribution, the particle path is the solution of the simple mechanical equation of motion

$$\tilde{m}g \cdot \sin\theta = \zeta \cdot \tilde{R}\dot{\theta}\,. \tag{1}$$

Here $\tilde{m}g$ is the particle weight corrected for buoyancy, θ is the polar angle defined in fig. 1, and $\tilde{R}$ is the distance between the particle and vesicle centers. In eq. (1), the effect of particle inertia is neglected because of the very small velocities and sizes involved in our experimental situation. Equation (1) is easily integrated in spherical coordinates and gives

$$f[\theta(t)] = f[\theta_0] - \frac{\tilde{m}g}{\tilde{R} \cdot \zeta} \cdot t\,. \tag{2}$$

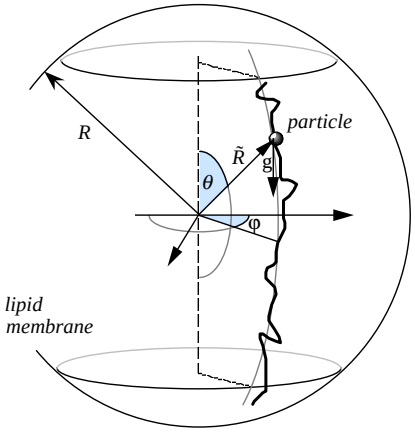

Fig. 1. – Schematic representation of the sedimentation path of a solid particle bound to the surface of a spherical vesicle.

Here $f(\theta) = a\tanh[\cos(\theta)]$ and θ_0 is the particle position at time $t = 0$. Equation (2) can be thought of as the "zero temperature" limit of the motion. Note that the experimental path shown in fig. 2b nicely follows eq. (2), except for θ close to π (see inset of fig. 2b). In fact, the "zero temperature" solution (eq. (2)) fails near the poles of the vesicle. This is not surprising, because in these regions gravity is essentially perpendicular to the membrane, and consequently the external force acting on the particle is very small. Then the motion is mainly Brownian.

The importance of sedimentation relatively to diffusion is measured by the Peclet number, which we define here as $\mathrm{Pe} = \tilde{m}g \cdot \tilde{R}/kT$ (k is the Boltzmann constant and T is the absolute temperature) [9]. To estimate the applicability of eq. (2) quantitatively, we performed numerical Monte-Carlo simulations of particle motion for different values of Pe. We computed $f[\theta(t)]$ and ensemble averages $\langle f[\theta(t)]\rangle$ from many (1000) trajectories. Brownian motion shows up as a noise in $f[\theta(t)]$, which increases when Pe decreases. We find that $\langle f[\theta(t)]\rangle$ follows eq. (2) only in a limited domain around $\theta = \pi/2$, say $[\pi/2 - \theta_\mathrm{m}\,;\pi/2 + \theta_\mathrm{m}]$, and plateaus to a constant value for $\theta > \theta_\mathrm{m}$ (This asymptotic limit is just the equilibrium $\langle f(\theta)\rangle$, which is finite). This explains the behavior shown in fig. 2 b. The width of the above domain, $2\theta_\mathrm{m}$, depends very much on Pe. For "large or heavy" particles, the domain is very large (*e.g.*, $\theta_\mathrm{m} \approx 1.4$ rd for $\mathrm{Pe} \approx 100$). It becomes smaller if Pe is decreased and finally disappears when $\mathrm{Pe} \approx 10$. When $\mathrm{Pe} < 10$ ("small or light" particles), eq. (2) is not at all applicable, whatever θ.

In the following, we will discuss experimental results obtained with "heavy" spheres. The slope of $f[\theta(t)]$ gives $\tilde{m}g/\tilde{R}\cdot\zeta$. The apparent weight $\tilde{m}g$ is measured before bringing the particle in contact to the vesicle from its sedimentation velocity (ν_s) in bulk water: $\tilde{m}g = \zeta_0 \cdot \nu_\mathrm{s}$, where $\zeta_0 = 6\pi \cdot \eta \cdot a$ is the Stokes friction coefficient (η is the water viscosity and a is the particle radius). $\tilde{R}$ is found as the maximum distance between the vesicle and particle centers in the recorded path. Following this procedure, we measure $\bar{\zeta} = \zeta/\zeta_0$, with a reproducibility which is on the order of $\pm 2\%$ for the case shown in fig. 2. In general, the error on $\bar{\zeta}$ depends on Pe, since Brownian excursions lead to fluctuations in the measured quantities.

We carried out several experiments with latex spheres whose radii were in the range $2 \div 10$ μm and some with glass spheres ($0.5 \leq a \leq 2$ μm). Vesicle radii were between 10 μm and 50 μm. The contact angle (α) takes on a particular value in each experiment, which depends on the initial tension of the vesicle and on the nature of the particle surface [8]. In the different

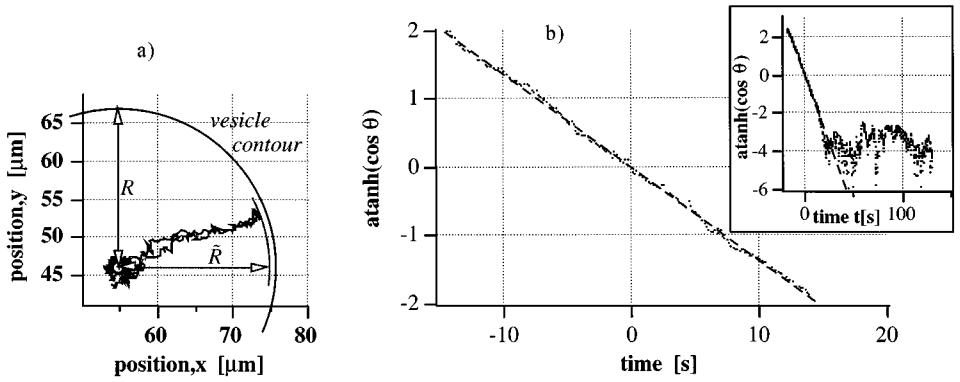

Fig. 2. – a) Example of an experimental record, with a glass sphere ($a = 1.21$ μm). b) The same trajectory, in the $a\tanh(\cos\theta)$ *vs.* t representation. The inset shows the long-time part of the record.

experiments that we performed, various contact angles were realized between 0° and 180°.

All experiments gave well-identified sedimentation paths, with friction coefficients ($\bar{\zeta}$) ranging from 1.2 up to about 3. This, together with the fact that each particle could be moved everywhere on the vesicle surface by means of optical manipulation, proves that the membrane is really in the fluid state, *i.e.* $\mu = 0$, since no static shear elasticity is detected. In principle, the parameter $\bar{\zeta}$ contains the information on the membrane shear viscosity $\eta_{\rm m}$. However, inverting data rigorously needs an exact theory that depicts the movement of the particle bound to a finite size vesicle. This theory is not available yet. For a preliminary estimate, we propose to adapt the theory worked out by Danov *et al.* [10] (hereafter referred to as DADL) for a spherical particle across a film at the *water/air* interface, supposed flat and infinite, as depicted in fig. 3. The latter condition will limit our data to small particles for which $a \ll R$.

Here we will use data obtained with latex particles large enough to be sedimenting (*i.e.* "large or heavy" in the sense defined above), that would definitely intercept the vesicle contour (*i.e.* α far from 0° and 180°) and which we will suppose to be small enough to feel the membrane as flat. An experimental example meeting all these conditions is:

$$a = 2\ \mu\text{m}; \quad R = 29\ \mu\text{m}; \quad \alpha = 137°; \quad \text{Pe} = 122; \quad \bar{\zeta} = 1.35\,.$$

We adapt DADL theory in the following way: The membrane, of thickness h, separates two regions, water and water (W/W) in our situation, or water and air (W/A) in DADL theory (see fig. 3). Essentially, we write ζ as the superposition of two terms, $\zeta = \zeta^0 + \zeta^{\rm ex}$, where ζ^0 is the sphere friction in the limit of a non-viscous film ($\zeta^0_{\rm WW} = 6\pi\cdot\eta\cdot a$ in the W/W case), and $\zeta^{\rm ex}$ is the excess friction due to the film. $\zeta^{\rm ex}$ depends on two variables, which we may choose as the radius of the disk that the particle intercepts in the film, $a^* = a\cdot\sin\alpha$, and the contact angle, α. We define the functions $\Lambda_{\rm WA}$ and $\Lambda_{\rm WW}$ such that

$$\zeta^{\rm ex}_{\rm WA} = 4\pi\cdot\eta\cdot a^*\cdot\Lambda_{\rm WA}(x_{\rm WA},\alpha)\,, \tag{3a}$$

$$\zeta^{\rm ex}_{\rm WW} = 8\pi\cdot\eta\cdot a^*\cdot\Lambda_{\rm WW}(x_{\rm WW},\alpha)\,. \tag{3b}$$

Here $x_{\rm WW} = 2\eta\cdot a^*/\eta_{\rm m} = 2x_{\rm WA}$. The function Λ_{WA} can be computed using DADL data (DADL gives $\zeta^0_{\rm WA}$ and $\zeta_{\rm WA}$) . The problem is to find $\Lambda_{\rm WW}$. In our analysis, we suppose

$$\Lambda_{\rm WA}(x,\alpha) = \Lambda_{\rm WW}(a,\alpha)\,, \tag{4}$$

whatever x and α. A simple superposition argument (2 half spheres across a monolayer) shows

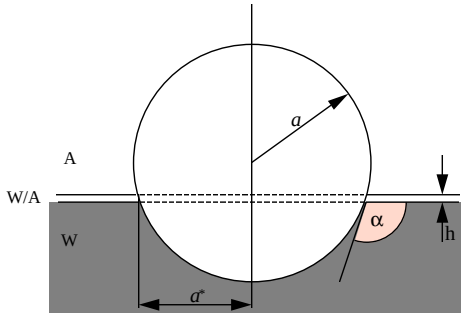

Fig. 3. – Spherical particle across a film at the water/air interface. In our adaptation of DADL theory [10], the upper phase is made of water too.

that eq. (4) is exact when $\alpha = 90°$. We observed that the explicit dependence of Λ_{WA} on α is weak, and that $\Lambda_{\mathrm{WA}}(x, \alpha) \cong \Lambda_{\mathrm{WA}}(x, 180° - \alpha)$ [11]. This makes the above superposition argument (2 truncated spheres, one with α, the other one with $180° - \alpha$) acceptable in general and eq. (4) approximately correct when $\alpha \neq 90°$.

Equation (4) allows us to find x_{WW} from the experimental ζ. With the above given example, we find $x_{\mathrm{WW}} = 0.56$, which gives $\eta_{\mathrm{m}} \approx 5 \cdot 10^{-6}$ sp for the shear viscosity of a SOPC membrane at room temperature.

A few other examples lead to a bunch of values ranging from about 3 to $8 \cdot 10^{-6}$ sp. This range is compatible with Waugh's result for egg-lecithin membranes [4], whose viscosity is probably comparable to that of SOPC at room temperature.

The reason of the dispersion of η_{m} values is unclear to us. The precision in η_{m} is limited by: i) the error on α; ii) the reliability of our approximation (eq. (4)); iii) the error on ζ. In the above example, we estimate the contribution of i) and ii) together to be at worst $\pm 9\%$. The main cause of error is that on ζ, which is about $\pm 6\%$ in the same example, and leads to $\pm 24\%$ in η_{m}. The total error on η_{m} is about $\pm 33\%$, which is large but definitely less than the dispersion.

Following Waugh's analysis [4], one might infer that different vesicles correspond to different values of η_{m} because their membranes may have different structures, in terms of defects or number of lamellae. In this view, the lowest value in the measured range should be taken as the most representative of the shear viscosity of a single defect-free bilayer. However, there is no indication in the particle paths of inhomogeneities in the membranes. On the other hand, if we suppose that many of the vesicles could have been multilayered, this would have resulted in a quantification of η_{m} values, which is not observed too.

In summary, we have analyzed the motion under the action of gravity of a small spherical particle bound to a fluid spherical surface. We have defined a criterion to distinguish "sedimenting" and "diffusing" particles, and set out an approximate method to find the membrane viscosity from experimentally measured friction coefficients. The error in ζ can be reduced by using large (non Brownian) particles, but then the relative importance of the membrane in the particle friction decreases, and the fact that the particle is not small compared to the vesicle makes the above approximate treatment (flat infinite membrane) inapplicable. Consequently, inverting data from large particles demands much accuracy in the interpretation, which means elaborating an exact theory for the particle movement on a sphere. Conversely, very small spheres are mostly sensitive to membrane viscosity and feel the interface as flat, which makes the above treatment acceptable. The friction of diffusing ($\mathrm{Pe} \ll 50$) spheres can be found

within a few percent from the analysis of their Brownian excursions [12], but measuring their contact angles is difficult. Efforts in the above two directions, theory and experimental procedure, are now in progress.

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[11] We checked this property numerically for $40^\circ \leq \alpha \leq 140^\circ$. With $x_{\mathrm{WW}} = 0.56$ (example in the text), we find from DADL that $\Lambda_{\mathrm{WA}}(0.56, 140^\circ)$ and $\Lambda_{\mathrm{WA}}(0.56, 40^\circ)$ differ by less than 10%.

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