Oblate-Prolate Transition of Ellipsoidal Giant Magnetoliposomes: Experiments Showing an Anisotropic Spontaneous Curvature (Chapter 11)

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Oblate-Prolate Transition of Ellipsoidal Giant Magnetoliposomes: Experiments showing an Anisotropic Spontaneous Curvature

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1. Introduction

The shape of fluid lipid vesicles is governed by the bending elasticity of their membrane characterized by a bending modulus K_c and a spontaneous curvature c_0 [1]. The latter exists only for bilayers separating non symmetric aqueous media. Recently Döbereiner et al described an experimental method based on the thermal fluctuations of giant vesicles to measure c_0 precisely and use it as a control parameter in phase diagrams [2]. We use here particular non symmetric giant vesicles called 'magnetoliposomes' as a tool for the observation of new shape transitions. Magnetoliposomes are giant liposomes filled up with a colloidal dispersion of magnetic particles. As usual liposomes, magnetoliposomes exhibit thermal fluctuations. The effect of a magnetic field is to flatten the fluctuations and to induce a shape deformation even at low field intensity. Two types of deformations are reported here depending on the characteristics of the encapsulated magnetic solution. They are explained by a modification of c_0 induced by the application of the magnetic field.

2. Liposome preparation

Magnetoliposomes are obtained by filling usual giant liposomes with an aqueous magnetic fluid (ferrofluid). They are suspended in a non-magnetic aqueous phase. The ferrofluid is a colloidal suspension of magnetic nanoparticles (iron oxide grains γ–Fe_2O_3) [3]. Particle diameter is about 10 nm and the iron oxide surface is coated by tri-sodium citrate species in order to have negative surface charges at pH 7, which ensure the colloidal stability even in physiological media [4]. There is an equilibrium between the citrate species adsorbed on the grains and the non-adsorbed tri-sodium citrate in the bulk. This electrolyte has a concentration C_s which is monitored by dialysis and controlled by measuring the conductivity of the ferrofluid. The phospholipid constituting the membrane of the magnetoliposomes is 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC from Avanti Polar Lipids, USA, used as purchased).

For the preparation of giant liposomes, we use the spontaneous swelling of a dense phospholipidic film because it enables to encapsulate a ferrofluid inside giant vesicles with a high efficiency. The ferrofluid is firstly prepared and its ionic strength C_s is adjusted by dialysis. A small amount of lipid powder (1 mg) is mixed with 10 µL of this aqueous ferrofluid (pH=7, volume fraction in magnetic particles is 0.05) and sheared on a glass support to obtain a dense lamellar phase. Then tridistilled water is added in excess (4 mL) to allow the spontaneous swelling of the liposomes during one hour incubation at 40°C. Most of the liposomes prepared by this way are spherical with diameter ranging from 10 to 100 µm (Fig. 1). They are red colored and they exhibit thermal fluctuations.
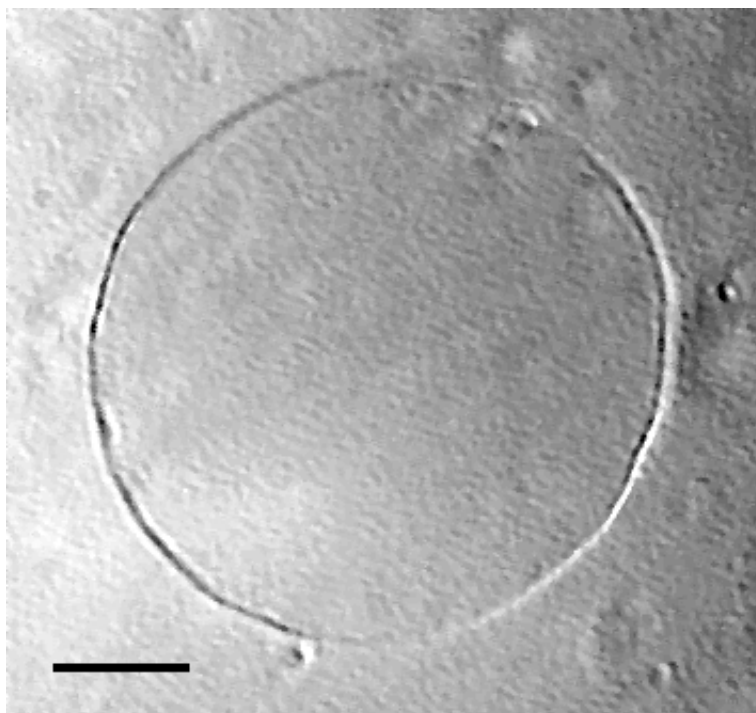

Fig. 1: A giant magnetoliposome without any magnetic field. The membrane is fluctuating as observed with differential interference contrast microscopy (DIC). Length of the bar is 20 µm.

3. Observations

Cells made of two glass slides separated by a 200 µm spacer are filled by aliquots of the magnetoliposomes suspension and observed under an optical microscope (Leica ×40, NA 0.65). Pictures are taken with a CCD camera and digitized on a computer.

The magnetoliposomes are sensitive to an applied magnetic field of low intensity, typically 400 G. Two types of deformations have been observed. An initially spherical magnetoliposome is either elongated by the field (Fig. 2) or on the contrary compressed at the poles (Fig. 3). The shape is always described by an ellipsoid with rotational symmetry along the field direction: a is the semi-axis parallel to the magnetic field $\vec{H}$ and b is the value of the two other semi-axes perpendicular to $\vec{H}$.
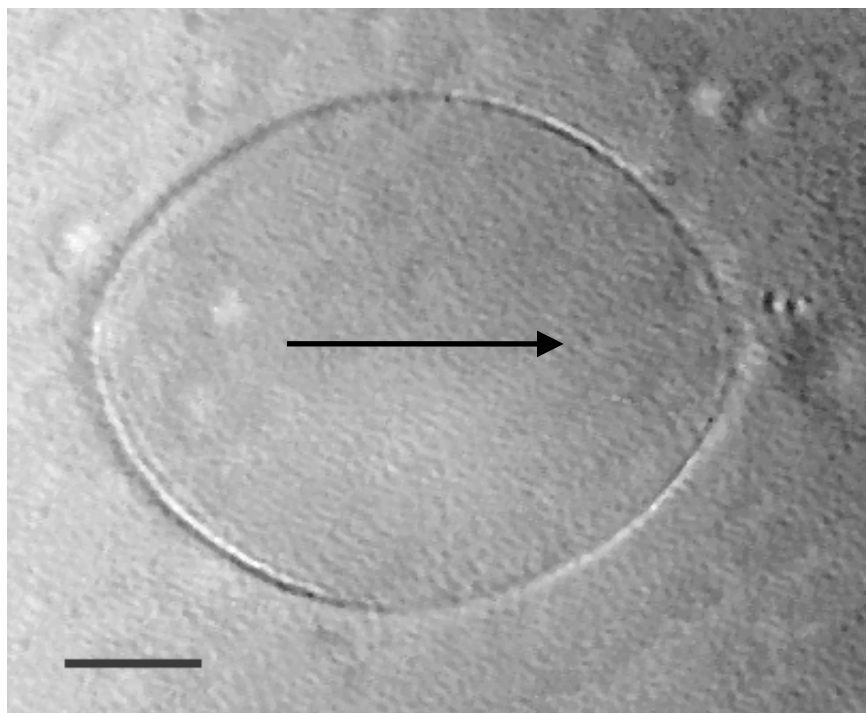
Fig. 2: The liposome shown in Fig.1 submitted to a magnetic field (direction given by the arrow). The deformation is prolate and the membrane fluctuations vanish. Please note that the ionic strength is high (Cs=85 mM). Length of the bar is 20 µm.
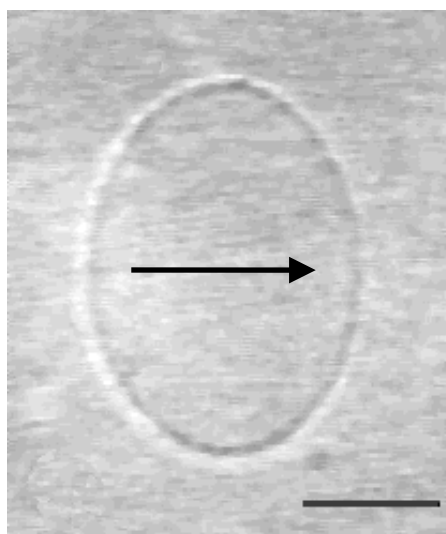
Fig. 3: A magnetoliposome filled with a low ionic strength magnetic fluid (Cs=7.75 mM) and submitted to a magnetic field (direction given by the arrow). The deformation is oblate. Length of the bar is 10 µm.

The type of deformation and its amplitude are described by the ellipsoid eccentricity: $e^2 = 1 - \dfrac{b^2}{a^2}$. For an elongated liposome (prolate ellipsoid), e^2 is positive, and for a compressed one (oblate ellipsoid), e^2 is negative. A combination of the two deformations is also observed and described as a 'spinning top' shape (Fig. 4).
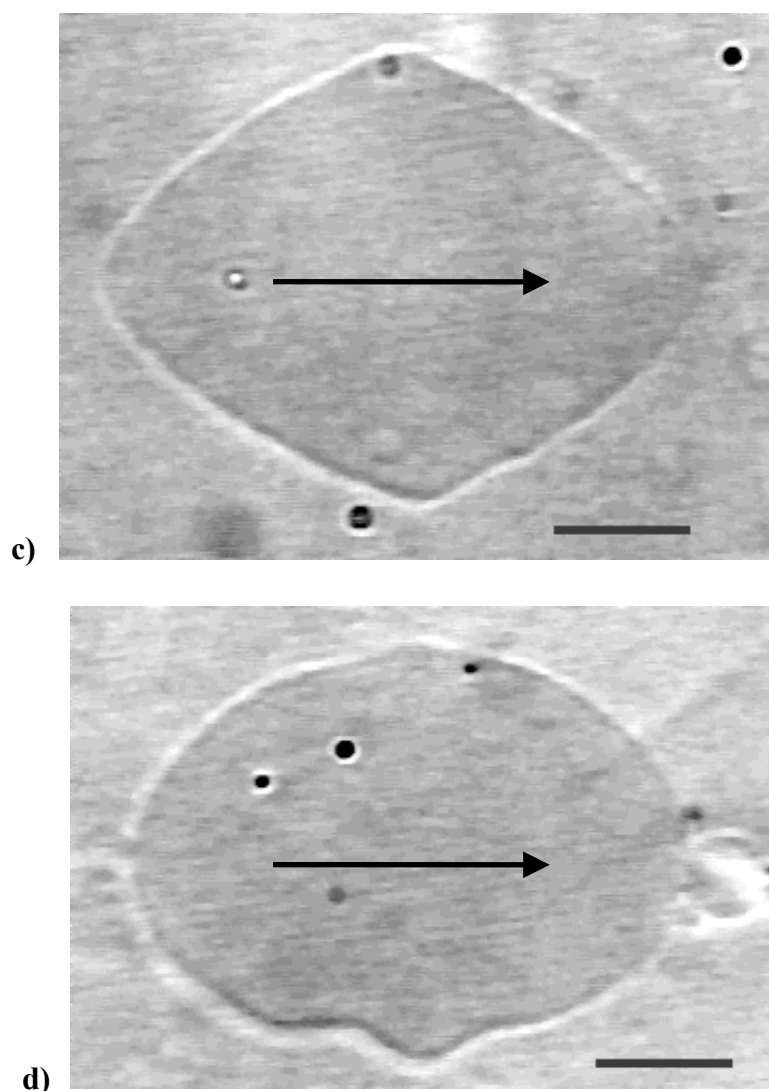
a)

b)

c)

d)
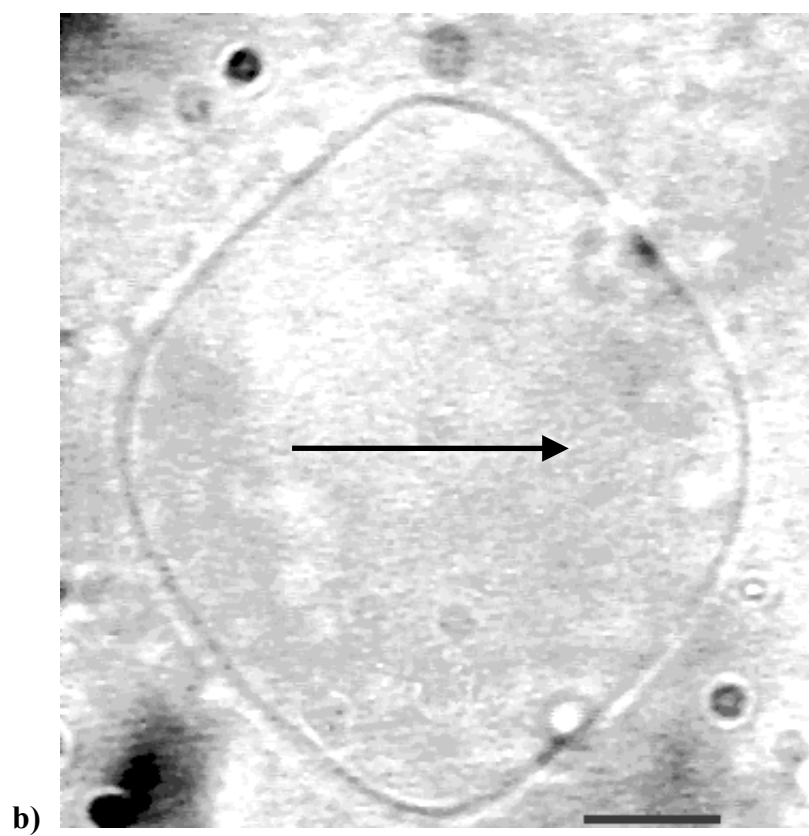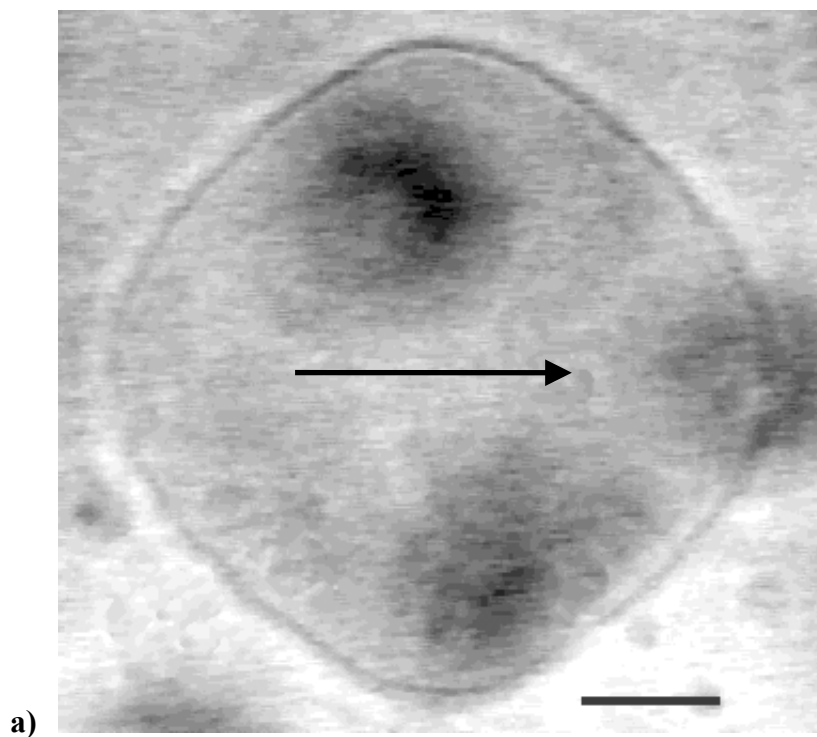
Fig. 4 (a to d): Magnetoliposomes inside which salt concentration is intermediate (Cs=25 mM) and submitted to a magnetic field (direction given by the arrow). The liposomes are always axisymmetric around the field direction but combine elongations both at the poles and at the equator ('spinning top' shapes). The values of can be nearly zero (a), negative (b) or positive (c). In that last case the sharp shape is only transient and relaxes to a more rounded one, but with membrane buckling (d). Length of the bar is always 10 µm.

4. Analysis of the deformations under a magnetic field

The experimental parameters that are relevant for the observed shape transitions are (a) the initial radius R_0 of the liposome measured on the video frame and (b) the two characteristics of the encapsulated solution, namely its magnetic susceptibility χ and the free salt concentration C_S.

An *in situ* measurement of χ is provided by magnetophoresis, *i.e.* the migration of a liposome submitted to a controlled gradient of magnetic field. A magnetic field gradient is performed perpendicularly to an applied magnetic field with the set-up described in Fig. 5.
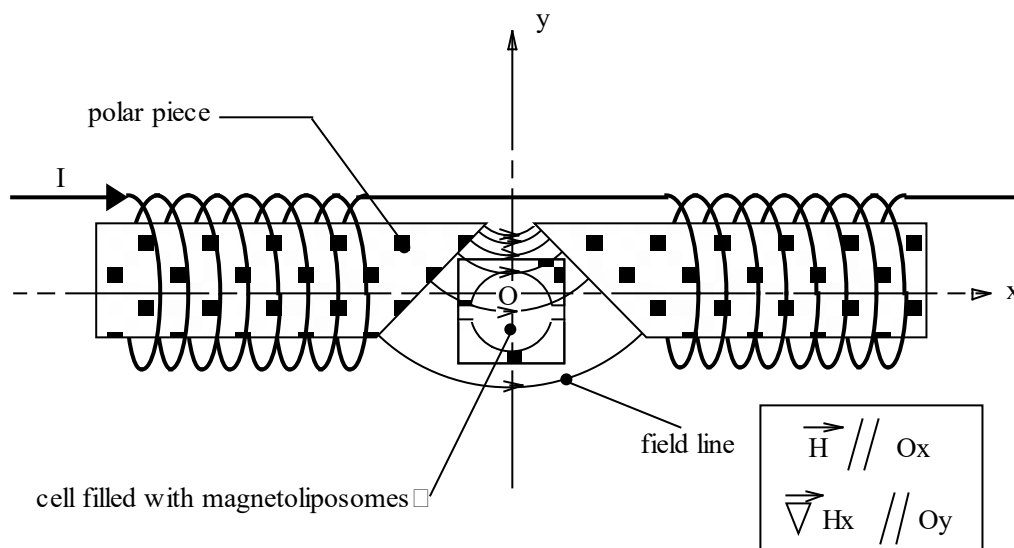
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Fig. 5: Experimental set-up to produce a controlled magnetic field gradient (the field lines get closer toward increasing y coordinate).The sample containing the magnetoliposomes is placed between the two coils in the cell described in the text.

The field lines of two coils in serial are deviated by polar pieces edged at 45°. The sample is in the middle region where the field lines are parallel to the O-x axis and the field intensity gradient is in the O-y direction. The gradient is 110±2 G·cm^{-1} (measured with Hall effect probes) with a field equal to 410±2 G in the sample place.

The magnetic force which causes the migration of a magnetoliposome submitted to a magnetic field gradient is:

$$\vec{F}_m = \chi V H_x \left(\vec{\nabla}_y H_x \right) \qquad \text{(Eq. 1)}$$

where $V = \dfrac{4}{3}\pi a b^2$ is the volume of the liposome and $\vec{\nabla}$ is the gradient operator. It is balanced by a viscous force, which was calculated by J. Perrin [5] in the case of an ellipsoid elongated in the O-x direction and moving along O-y in a medium of viscosity η :

$$\vec{F}_v = 16\pi\eta \frac{a}{2\ln\left(\dfrac{2a}{b}\right) + 1}\,\vec{v} \qquad \text{(Eq. 2)}$$

The viscosity of the suspending solution of the vesicles is $\eta = 10^{-2}$ P. The magnetophoretic velocity results from the balance of the two forces:

$$\vec{v} = \frac{b^2 \left[2\ln\left(\dfrac{2a}{b}\right) + 1 \right]}{8\eta}\,\chi H_x\left(\vec{\nabla}_y H_x \right) \qquad \text{(Eq. 3)}$$

The measurement of the liposome velocity leads to the value of χ characterizing the ferrofluid encapsulated in a given liposome. The concentration C_P of nanoparticles inside the liposome is then calculated using Langevin's law of superparamagnetism :

$$\chi = \frac{C_p V_p^2 m_s^2}{3kT} \qquad \text{(Eq. 4)}$$

where m_s=360 G is the magnetization at saturation of γ–Fe_2O_3 and V_P is the volume of a nanoparticle [6]. In the following we use V_P corresponding to a particle diameter of 12 nm, taking into account that the ferrofluid is polydisperse and that χ is ruled by the tail of the distribution.

Please note that R_0 and C_P vary a lot among the population of liposomes due to the swelling process. Their values are measured thereafter. The concentration of tri-sodium citrate salt in the ferrofluid is adjusted to a known value by dialysis through a cellulose membrane (Spectra/Por MWCO 12-14000 g·mol^{-1} from ROTH, France). Final concentrations C_S ranging from 5 mM to 85 mM are measured from the electrical conductivity of the ferrofluid.

The experimental values of R_0 and χ are measured on a large amount of magnetoliposomes prepared with ferrofluids of different C_S. Some of them are given as examples in Table 1. On Fig. 6 experimental data illustrate the influence of the three parameters R_0, χ and C_S on the observed deformations. Vesicles which are larger, more magnetic and filled with a high ionic strength magnetic fluid exhibit prolate deformation under a magnetic field, whereas smaller vesicles filled with a low ionic strength magnetic fluid and less magnetic become oblate. Two domains corresponding to both types of deformation are well defined on this 'topological' phase diagram, the oblate one being in the region of low values of R_0, χ, and C_S.

C_S (mol·L^{-1})	χ	C_P (cm^{-3})	R_0 (µm)	$e^2 = 1 - \dfrac{b^2}{a^2}$
5×10^{-3}	7.1×10^{-5}	8.2×10^{13}	25.2	-0.397
7.75×10^{-3}	1.1×10^{-4}	1.3×10^{14}	13.3	-0.596
7.75×10^{-3}	7.5×10^{-5}	8.7×10^{13}	21.4	-1.17
2.5×10^{-2}	7.3×10^{-5}	8.4×10^{13}	24.6	-0.845
2.5×10^{-2}	1.2×10^{-4}	1.4×10^{14}	22.5	0.496
2.5×10^{-2}	6.5×10^{-5}	7.6×10^{13}	50.4	0.343
3.5×10^{-2}	3.6×10^{-5}	4.2×10^{13}	21.1	-1.31
4.5×10^{-2}	5.5×10^{-5}	6.4×10^{13}	31.6	0.466
6.5×10^{-2}	1.1×10^{-3}	1.2×10^{15}	14.1	0.536
8.5×10^{-2}	1.4×10^{-4}	1.7×10^{13}	13.0	0.586

Table 1: Examples of experimental data about magnetoliposomes. C_S is the concentration of tri-sodium citrate electrolyte in the ferrofluid, R_0 the radius of the initial spherical shape, e the eccentricity of the ellipsoidal deformation and χ the magnetic susceptibility. The concentration C_p of magnetic particles *in situ* is calculated from χ using Eq. 4. The shadowed lines correspond to prolate deformations ($e^2>0$), the other to negative ones ($e^2<0$).

5. Interpretation

When submitted to an applied magnetic field, a liposome encapsulating a ferrofluid of susceptibility χ gets a bulk magnetization (proportional to R_0^3); as a consequence, the interface between inner magnetic and outer non-magnetic media (i.e. the bilayer) is stressed. The deformation of an initially spherical object by a magnetic stress should always be a prolate ellipsoid, whatever the sign of χ, so that magnetic droplets and magnetic holes (eg. air bubbles) have the same behavior [7]. This was observed for magnetoliposomes and used in [8] in order to determine the bending modulus K_c of the bilayer from the analysis of the liposomes deformation as a function of the magnetic field intensity.

Therefore the observation of an oblate deformation by a slight change of the experimental parameters is a tricky result. Please note that the inner compartment of the magnetoliposomes is a complex colloidal solution. It includes both a 3:1 electrolyte (tri-sodium citrate) with concentration C_S and a colloidal suspension of nanometric $\gamma-Fe_2O_3$ particles (giving χ). The latter are both nanoscopic magnets and macro-ions, the net charge per nanoparticle being $Z \approx -20$ [4]. The importance of decreasing C_S to obtain an oblate deformation suggests that there are electrostatic interactions between the particles and the bilayer. Indeed some electrophoresis experiments on large unilamellar liposomes (diameters about 200 nm) made of phospholipids with a phosphocholine head group have reported values of the ζ-potential ranging from -4 mV for natural egg-lecithin [9] to about -10 mV for synthetic lipids like 1,2-dimysristoyl-*sn*-glycero-3-phosphocholine (DMPC) [10]. The ζ-potential varies with temperature, ionic strength and specific adsorption of anions [11]. We retain for the present study that the bilayer and the magnetic nanoparticles are both negatively charged and so repel each other.

The shape of magnetoliposomes submitted to a magnetic field results from a competition between the magnetic energy of the bulk and the bending energy of the bilayer. The magnetic energy always favors the prolate shape, but we shall show that the bending energy can stabilize the oblate deformation. The contribution to the bending energy of the bilayer due to a surface charge density has been extensively studied theoretically [12, 13, 14], but to our knowledge only experiments on small unilamellar vesicles (diameters about 10 nm) have been reported [15]. The shape of liposomes is known to be strongly dependent on the spontaneous curvature of the bilayer (c_0). In the case of magnetoliposomes, c_0 originates from the asymmetry between the aqueous compartments (ferrofluid inside, non-magnetic solution outside). Winterhalter and Helfrich [12] have introduced a method to derive the elasticity constants of a bilayer bearing two different charge densities on its both sides. If we use the same approach, considering that the screening length of the electrostatic potential is κ_i^{-1} inside the liposome and κ_o^{-1} outside, the spontaneous curvature can be written:

$$c_0 = \frac{\kappa_o^3}{3}\left(\frac{1}{\kappa_o^2} - \frac{1}{\kappa_i^2}\right) \qquad \text{(Eq. 5)}$$

The effect of the magnetic field (intensity H_0) on the spontaneous curvature c_o is due to the modification it implies on the particles concentration profile in the vicinity of the bilayer. Qualitatively the depletion in nanoparticles is reduced near the magnetic poles because of magnetic attraction. The effect of this phenomenon on c_0 can be quantitatively described introducing an angular dependence in the expression of the decay factor of the electrostatic potential away from the bilayer:

$$\kappa_i^2(\theta) = \kappa_o^2 \left(1 + \frac{d}{1 + \dfrac{4\pi\chi^2 H_0^2}{C_p kT} \cos^2(\theta)} \right) \qquad \text{(Eq. 6)}$$

where θ is the angle between the field direction and the normal to the bilayer and d is the ratio $\dfrac{Z^2 C_p}{12 C_s}$ [16]. Thus, when a magnetic field is applied, c_0 becomes lower near the poles ($\theta = 0$ and $\theta = 180°$) than at the equator. But the angular contribution of κ_i according to Eq. 6 depends on the amount of magnetic materials through C_P and χ, and on the ionic strength through d. This explains the driving role of these parameters in the experiments (Fig. 6).

a)

Electrolyte concentration C_s (mol citrate ions per Liter)

b)

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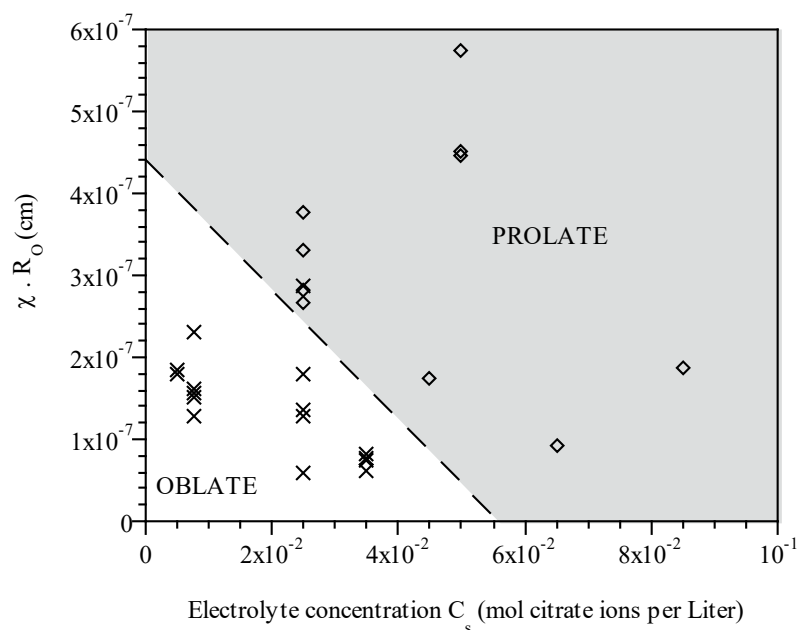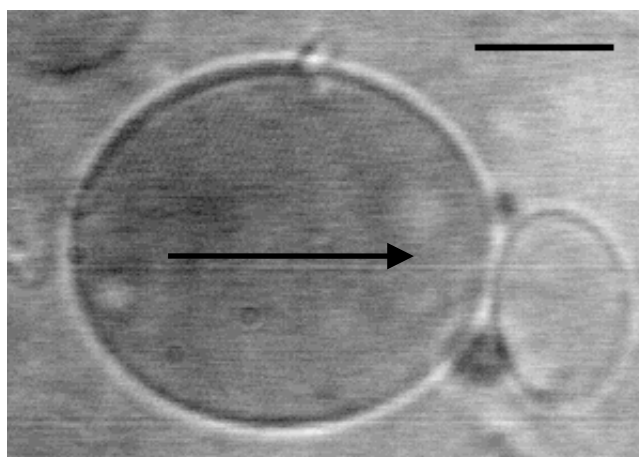
Fig. 6: (a) 'Topological' phase diagram of the magnetoliposomes under a magnetic field where product χR_0 is plotted versus tri-sodium citrate salt concentration C_S in the ferrofluid. The dotted line between the two domains of prolate and oblate deformations is only a guide for the eye; **(b)** Effect of product χR_0 at given salt concentration (C_S=25 mM) on two neighboring liposomes with opposite deformations under a magnetic field. The prolate one is large and highly colored (high χR_0) while the oblate one is smaller and appears more dilute in magnetic particles (low χR_0). Length of the bar is 10 μm. The arrow indicates the direction of the field.

For example a decrease of C_S (by dialysis of the ferrofluid) raises up the anisotropy of the spontaneous curvature. Hence the oblate shape minimizes the bending energy because it develops a less curved bilayer at the magnetic poles where c_0 is decreased. If the concentration of magnetic oxide is not too high, so that the bending energy prevails over the magnetic one, then the oblate shape is favored. This explains the tendency of oblate deformation under a magnetic field for magnetoliposomes encapsulating a ferrofluid diluted both in electrolyte and in nanoparticles.

We have described in this article a new shape transition of magnetoliposomes. We interpret this experimental result by a coupling between magnetostatics and electrostatics. A complete theoretical model will be proposed in a forthcoming paper. The main point of our results at the present time is that even a small charge density on the phospholipidic membrane can induce a coupling with charged encapsulated species.

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