Preparation of monodisperse multilayer vesicles of controlled size and high encapsulation ratio.

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Preparation of monodisperse multilayer vesicles of controlled size and high encapsulation ratio.

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Abstract. — The effect of shear on lyotropic lamellar phases is studied by light scattering and microscopic observations. We found that in a certain range of shear rate the lamellar phase organizes itself into spherical multilayer vesicles of well controlled size. The size is shown to vary as the inverse of the square root of the shear rate. A simple explanation in terms of a balance between shear stress and elastic forces is given.

Surfactant molecules in solution aggregate very often into two dimensional structures called membranes. These membranes are often organized in space into a long range ordered structure named the lamellar phase (lyotropic smectic A) [1]. The lamellar phases are usually found at relatively high concentrations in surfactant (20-60 %) but they may remain stable for very dilute systems (< 10 %) [2]. When lamellar phases are dispersed in an excess amount of solvent they might lead to metastable structures named vesicles. These vesicles are closed spherical objects which can encapsulate a certain amount of solvent with one membrane (Small or Large Unilamellar Vesicles: SUV or LUV) or several membranes (Multilamellar Vesicles: MLV, also called spherulites). When phospholipids are used they are also called liposomes and they have many very important applications for drug delivery [3] and cosmetology [4]. Sometimes MLV form spontaneously by dissolving dry phospholipids in water [5]. However, for practical uses, technological methods have been developed to improve the control of the size, the polydispersity and the encapsulation ratio (defined as the volume of solvent inside the vesicles over the total volume of solvent) [6]. These techniques are based on preparing a lamellar phase dispersed in an excess solvent and shearing this mixture either by extrusion or sonication. We have observed that, under certain conditions, a pure lamellar phase in the absence of excess solvent sheared at a constant rate leads to highly monodisperse multilamellar vesicles with a very high encapsulation ratio (90-95 %). The size can be controlled very precisely (better than 10 % in radius) by varying the shear rate (typical values for the vesicle diameter: from 0.5 μm to 10 μm) [7]. The novelty of this method has to be opposed to the classical techniques involving shearing in an uncontrolled way a mixture of lamellar phase with an excess solvent. In addition

to the fact that it leads to much better quality particles as a result, it brings a proper scientific perspective and enables us to understand better the basic mechanism involved in the vesicle formation.

Lamellar phases can be prepared according to the phase diagram of the particular system studied. To illustrate this, we will describe a phase made of AOT and brine (water + sodium chloride). Depending upon the degree of salinity, the lamellar phase is stable at room temperature from 70 % to 3 % of surfactant corresponding to a repeating distance d of the lamellar phase ranging from 30 Å to 700 Å [8].

We apply a constant shear rate to this phase by using a home made Couette cell which permits a light scattering observation. The cell consists in two concentric glass cylinders; for stability reasons the outer cylinder can rotate at a constant velocity while the inner one remains stationary. Depending upon the cylinder used we can choose the gap between the two cylinders in the range 0.5 mm to 4 mm. The shear rate is defined as the ratio between the velocity of the outer cylinder to the gap size. Light scattering observation is made by sending a laser beam through the cell perpendicularly to the cylinder walls and by observing at small angles on a screen situated at some distance (from 3 to 50 cm) from the cell.

For typical observations, we have used a lamellar phase prepared with 17 % of AOT in 83 % of brine (15 g/l of NaCl). This lamellar phase has a d-spacing of around 150 Å [8]. Once placed into the cell and sheared at a constant rate of about 1 to 400 s^{-1} one can observe on the screen a ring of scattering. A second ring corresponding to the second order peak is often observed. As the shear rate is increased, the size of the ring becomes larger indicating smaller and smaller sizes (see Fig. 1). The characteristic time to reach the steady state (ring size constant) depends a lot on the system and on the shear rate. Generally, the higher the shear rate, the faster the steady state is reached. The radius of the ring of scattering indicates that a characteristic size of the order of a few μm exists in the structure. This size, which is larger than the repeat distance of the smectic (lamellar) order, corresponds to the diameter of MLV formed under shear. The polydispersity in size, estimated by the width of the peak, leads to values that are usually better than 10 %.
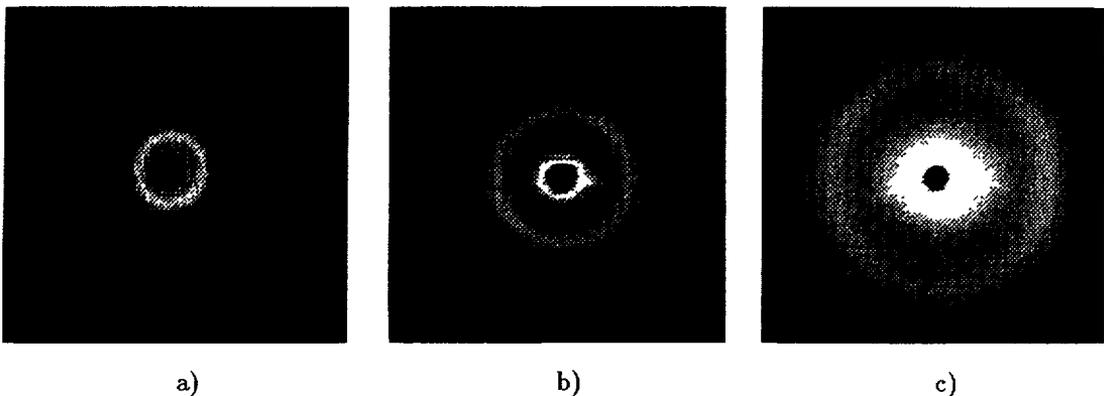

a)   b)   c)

Fig. 1. — Ring of scattering observed for AOT/brine sample at different shear rates a) 7 s^{-1}, b) 20 s^{-1}, c) 50 s^{-1}

After reaching a steady state, we stop the shear suddenly and observe that the ring persists with the same size. This shows that the structure has been quenched. Then, the sample (that

has now the appearance of a homogeneous cream) is removed from the cell and a transparent cell is filled. If the cell is put into a laser beam, the same ring of scattering is observed, indicating that the structure was isotropic and not destroyed by the treatment [9]. The cell can also be put in a regular light scattering set-up and the scattered intensity measured as a function of the scattering vector: a peak is observed at the position of the maximum observed on the screen. If the cream is observed under a phase contrast microscope (lens × 100) a uniform texture is observed with a characteristic length corresponding to the one observed with light scattering (see Fig. 2a). The structure can be further characterized by dilution of the cream formed under shear. Indeed, one may add some solvent (brine 13 g/l) to the cream (around 50 % in the case of Fig. 2b) and observe this solution under the microscope. We can clearly notice a dense phase of spherical droplets (see Fig. 2b), these droplets are multilayer vesicles of the same size as that determined by light scattering. The multilayer nature of the spherulite has been checked by X-ray and neutron scattering. We conclude that the phase that has been prepared under shear is a very dense phase of spherulites. The multilayer nature of the spherulites is easily revealed in diluting the initial cream with pure water instead of brine. In this case, microscopic observations (phase contrast) show that the droplets are swollen due to the difference in osmotic pressure (see Fig. 2c).
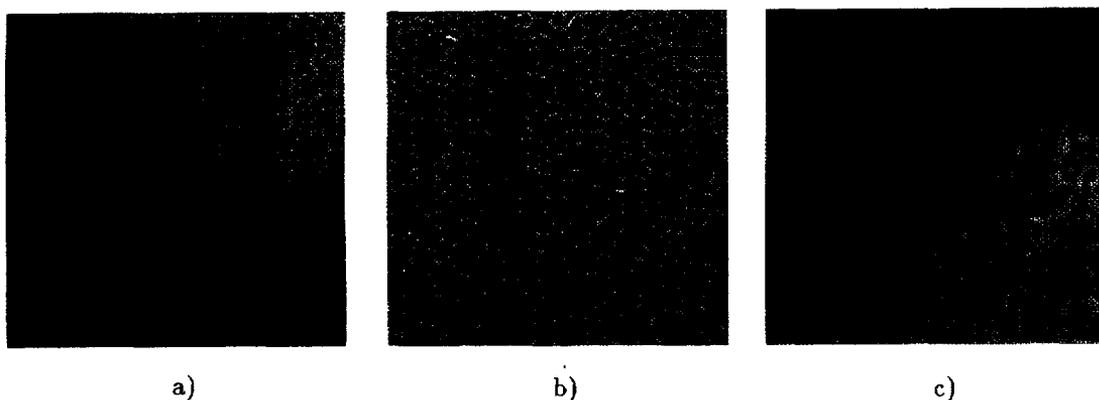

a)   b)   c)

Fig. 2. — Structure of the cream observed under phase contrast microscope (Normarsky, lens 100). The cross on figure c) corresponds to a length of 10 μm. a) Structure directly transferred from the shear cell (Couette) after shearing an AOT sample for 20 mn at 50 s^{-1}. b) Structure after a 50 % dilution with a brine solution (13 g/l of NaCl). c) Structure obtained by dilution with pure water.

To estimate the encapsulation ratio we measure the conductivity (χ) of the cream before dilution and compare it to the conductivity of the brine (χ_0). We have found an encapsulation ratio better than 90 % ($\chi/\chi_0 = 0.065$). We can also estimate this encapsulation ratio by diluting the basic preparation in a controlled way and measure the volume occupied by the spherulites by microscopic observation, we get about the same result as the conductivity. The very high value obtained indicates that the spherulites are deformed when packed together, this is confirmed by neutron scattering observation [9].

By light scattering, we have measured the evolution of the size as a function of shear rate (see Fig. 3). As clearly shown in figure 3, the typical size decreases as the inverse of the square root of the shear rate.
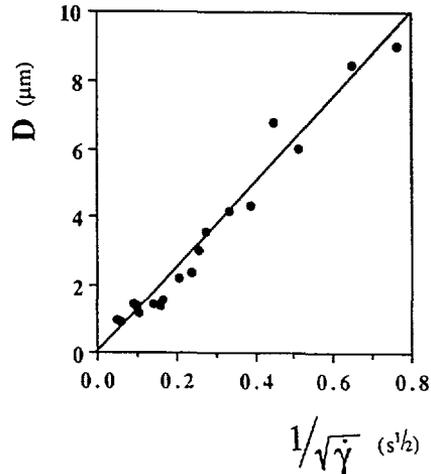
Fig. 3. — Measurements of the characteristic size (diameter D) of the spherulites obtained with the AOT/brine sample as a function of the inverse of the square root of the shear rate (see Eq. (3) in the text).

Many different systems have been studied including nonionic surfactant, ionic surfactant in brine, direct and inverted (water layers surrounded with surfactant diluted with an oil rich solvent [2]) bilayers and they all exhibit the same feature which is the appearance of a droplet structure in some regime of shear rate. In particular, we have applied this method to a lamellar phase made of soy lecithin (45 %), cholesterol (15 %) and water (40 %) and found that we can make liposomes with controlled sizes between 0.6 and 3 μm.

The region where the spherulites are formed is bounded by two transitions (instabilities) toward different states of orientation [10]. At a low shear rate (< 0.5 s^{-1} for the sample described previously), the lamellar phase is mainly oriented with the velocity in the plane of the layers and the gradient of the velocity is perpendicular to the layers; however, a lot of defects (probably dislocations) exist. At an intermediate shear rate (from 0.5 to 400 s^{-1}) the spherulites are formed. At a higher shear rate (> 400 s^{-1}) we usually find a phase with the same global orientation as that obtained at a lower shear rate [9] but with no defect in the direction of the velocity.

We can understand the formation and evolution of the spherulites within some very simple hand-waving arguments. Let us assume that under shear the preferred orientation is given by the velocity in the plane of the layer and the gradient of velocity perpendicular to the layers, as observed at very low and high shear rates. Within this orientation the gap in which the lamellar phase is moving is of the order of 1 mm with a precision which is of the order of 5/100 mm. The fact that the lamellar phase experiences dilation in the perpendicular direction that is much larger than the penetration length λ [11] (which is of the order of d) leads to the well known undulation instability [12]. Consequently, the lamellar phase develops defects that are only slightly anisotropic at a low shear rate [13]. This is the first state observed that has already been described in the literature for thermotropic liquid crystal (smectic A) [13]. In this state, the system flows by moving dislocations [14]. For intermediate shear rates, the sample is forced to move faster and the dislocations cannot follow. In this state the system bifurcates to another orientation and forms small spheres rolling on each other to allow the flow to proceed. At a higher shear rate the lattice of dislocations corresponding to an undulation unstability is

so anisotropic [13] that no dislocations are left in the direction of the velocity and the oriented state is becoming again the most stable one.

In the intermediate region, we can calculate the characteristic size in balancing two forces: an elastic one f_{el} and the viscous force f_{vis} [15]. The elastic force stored in a spherical droplet with a density of membranes per unit length $1/d$ is:

$$f_{el} = 4\pi \left(2\,\kappa + \bar{\kappa}\right)/d \tag{1}$$

where κ and $\bar{\kappa}$ are the mean and Gaussian elastic constant of the membrane, respectively [16].

The viscous force that a droplet experiences in a flow is [15]:

$$f_{vis} = \eta\,R^2\dot{\gamma} \tag{2}$$

with $\dot{\gamma}$ being the shear rate, η the viscosity of the solution at the relevant frequency (measured with a dynamic viscometer [10]) and R the spherulite size. Balancing (1) and (2) we can calculate the equilibrium size for the steady state:

$$R = \sqrt{\frac{4\pi\left(2\kappa + \bar{\kappa}\right)}{\eta\,d\dot{\gamma}}} \tag{3}$$

the shear rate dependence of equation (3) can be quantitatively checked by measuring the diameter $D = 2\,R$ as a function of the shear rate as is shown in figure 3. Knowing κ for AOT ($\kappa = 3\ k_BT$) [8] and taking $\eta = 180$ cp, d being 150 Å, we can estimate the value of $\bar{\kappa}$ from the slope of figure 3, we found: $\bar{\kappa} = -4\ k_BT$.

In summary, applying a constant shear to a lamellar phase leads in a definite range of shear rate to an orientation state composed of very dense spherical particles of controlled size. This method can be used to prepare liposomes in order to encapsulate drugs for medical or cosmetical purposes [7] but it can also be used to prepare well controlled colloidal particles. Indeed, it is possible to polymerize lamellar phases [17] and then stabilize the spherical object. Starting with a lamellar phase in which the solvent or the membrane is doped with other material (such as the ferrosmectics [18]), one could imagine preparing colloidal particles loaded with specific products.

Acknowledgements.

References

[1] EKWALL P., in Advances in Liquid Crystals, (Eds Brown, G.M. Academic, New York, 1975).
[2] LARCHE F., APPELL J., PORTE G., BASSEREAU P. and MARIGNAN J. *Phys. Rev. Lett.* **56** (1985) 1700.
[3] GREGORIADIS G., Liposome Technology (CRC Press Boca Raton, FL, 1984).
[4] SZOKA F. and PAPAHADJOPOULOS D., *Ann. Rev. Biophys. Bioneg.* **9** (1980) 467.
[5] BANGHAM A.D. and HORNE R.W., *J. Mol. Biol.* **8** (1964) 660-668.

[6] LASIC D.D., *Biochem. J.* **256** (1988) 1-11.

[7] ROUX D. and DIAT O., French patent number 92-04108.

[8] SKOURI M. and MARIGNAN J., APPELL J. and PORTE G., *J. Phys. II France* **1** (1991) 1121-1132.

[9] This has been confirmed by X-ray and neutron scattering: DIAT O., ROUX D., NALLET F., SAFINYA C. and SIROTTA E., in preparation.

[10] ROUX D., NALLET F. and DIAT O., in preparation.

[11] DE GENNES P.G., The Physics of Liquid Crystals (Clarendon Oxford, 1974).

[12] CLARK N. and MEYER R., *Appl. Phys. Lett.* **22** (1973) 493.

[13] OSWALD P. and BEN-ABRAHAM S.I., *J. Phys. France* **43** (1982) 1193-1197.

[14] OSWALD P. and KLÉMAN M., *J. Phys. Lett.* **43** (1983) L411-L415.

[15] TAYLOR G.I., *Proc. R. Soc. A* **138** (1932) 41-48; *Proc. R. Soc. A* **146** (1934) 501-523.

[16] HELFRICH W., *Z. Naturforsch.* **28a** (1973) 693.

[17] a) ANDERSON D.M. and STÖRM P., Polymer association structures: Microemulsion and liquid crystals, Nokaly M.E. Ed., *ACS symposium series* **384** (1989) 204;

b) LAVERSANNE R., *Macromolecules* **25** (1992) 489;

c) FRIBERG S.E., WOHM C.S. and LOCKWOOD F.E., *Macromolecules* **20** (1987) 2057.

[18] FABRE P., CASAGRANDE C., VEYSSIE M., CABUIL V. and MASSART R., *Phys. Rev. Lett.* **64** (1990) 539.