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Reversible size of shear-induced multi-lamellar vesicles

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Abstract We have investigated the reversibility in the shear-induced multi-lamellar vesicle (MLV) size during stepwise cycling of the shear rate by employing common rheometry, polarized light microscopy and rheo-optic techniques. We thus address the question whether there is a true MLV steady state, irrespective of history. The system studied, was the nonionic surfactant triethylene glycol decyl ether ($C_{10}E_3$) with a concentration of 40 wt.% in D_2O and a constant temperature of 25°C. It was found that the MLV size varies reversibly with varying shear rate, and hence there exists a true steady state in the presence of shear flow. The experimental observations of reversibility are however restricted to higher shear rates. Because the transformation of the size results

from the shear strain, the process is very slow at lower shear rates, where the steady state cannot be reached within a reasonable experimental time.

Keywords Multi-lamellar vesicles · Size reversibility · Rheo-techniques · Polarized light microscopy · $C_{10}E_3$

Introduction

The behavior of lamellar phases under shear is a subject that has been receiving a lot of attention in the recent years [1–4]. In fact, many different shear effects have been reported: transformation from lamellar phases to Multi-lamellar vesicles, MLVs (or “onions”), in different amphiphilic systems and followed by different techniques [5–20]; formation of MLVs in presence of submicron-size particles (“stuffed onions”) [21]; changes in lamellar orientation [17, 22, 23]; formation of intermediates with cylindrical structure between a lamellar and MLV phases [24–26]; reduction in lamellar spacing [27]; transitions from MLVs to unilamellar vesicles [28] and “layering” effects on onions [8, 29]. As a practical application, these

MLVs can be used, for instance, to encapsulate chemicals leading to a new kind of controlled micro-reactor [30] or as carriers for oligonucleotide delivery [31].

The size distribution of shear-induced multi-lamellar surfactant vesicles depends on the applied shear rate. In general, the MLV systems are shear thinning and the average MLV radius, R , decreases as the shear rate increases [5, 26, 32]. For higher surfactant concentrations, the inter-bilayer spacing is independent of the MLV size [33]. Hence, a decrease in the MLV size is accompanied by an increase in the MLV number density, N/V , where N is the number of vesicles and V the total volume. The MLVs at higher surfactant concentrations are polyhedral. They fill space with a total volume fraction which is essentially unity [8, 34] and N/V is approximately proportional to R^{-3} .

The question of reversibility has been addressed previously. Bergenholtz et al. [14] questioned the existence of a true steady state under continuous shear in the AOT-brine system. Escalante et al. [35] interpreted their rheology results as an irreversible transformation in MLVs with the increase of shear rate. Panizza et al. [6] found two different regimes in the size changes of MLVs that depended on the initial and final shear rates. Müller and collaborators [7] reported reversibility in a related system, $C_{12}E_4/D_2O$ and, in the present work, we investigated the reversibility in the MLV size distribution during stepwise cycling of the shear rate, focusing on the dynamic phase diagram of the system $C_{10}E_3/D_2O$ described recently by Oliviero et al. [32]; where the authors concluded that a steady-state viscosity could be reached independent of the shear history. The very existence of a history-independent steady state under continuous shear is an important issue and in this paper we present a more detailed investigation of this topic. We focus on the multi-lamellar vesicles, and investigate whether the steady states obtained at different shear rates can be reproduced when the shear rate is increased or decreased. Here we apply common rheometry to measure shear viscosity and viscoelastic properties and use rheo-small angle light scattering (rheo-SALS) to measure the MLV size. In addition, we have also used polarized light microscopy to image the MLVs directly.

Materials and methods

Tri-ethyleneglycol mono *n*-decyl ether ($C_{10}E_3$) was purchased from Nikko Chemical Co. (Tokyo, Japan) with a purity higher than 99.8%. Deuterium oxide (D_2O) was obtained from Sigma Chemicals (Steinheim, Germany). Dextran T500 was also purchased from Sigma Chemicals.

Rheometry

The rheology experiments were performed on a Physica UDS 200 rheometer using the cylindrical geometry Z3 DIN, (gap between the inner and outer cylinder of 1 mm) and in a Stress Tech rheometer, using the cylindrical geometry CC 25. The instruments are equipped with a temperature control unit that was calibrated to give a temperature in the sample chamber within 0.1°C of the set value.

Rheo-SALS

Depolarized small angle light scattering experiments under shear flow were performed using a Bohlin-CVO HR rheometer equipped with quartz 3°-cone/plate shear

geometry in the strain-control mode. Using He–Ne laser as a light source ($\lambda = 632.8$ nm) causes an accessible q -range of 0.06–4 μm^{-1} . The incident beam was polarized parallel to the flow direction and passed the sample along the gradient direction. The time evolution of the SALS pattern was captured by a CCD camera with a certain time interval and recorded in tiff format on the computer.

Polarized light microscopy

Microscopic studies were performed with an Axioplan Universal (Carl Zeiss) polarizing light microscope equipped with a differential interference contrast (DIC) unit. Images were recorded with an Olympus digital camera. A sample was recovered from the rheometer, where it had been sheared at a given shear rate for 2 h, and placed on a glass slide. The sample was then put in contact with an aqueous dextran solution (10 wt%), covered with a cover glass and viewed in the microscope between crossed polarizers.

Results and discussion

Figure 1 shows a graphic representation of the viscosity versus the number of measurements (200 measurements

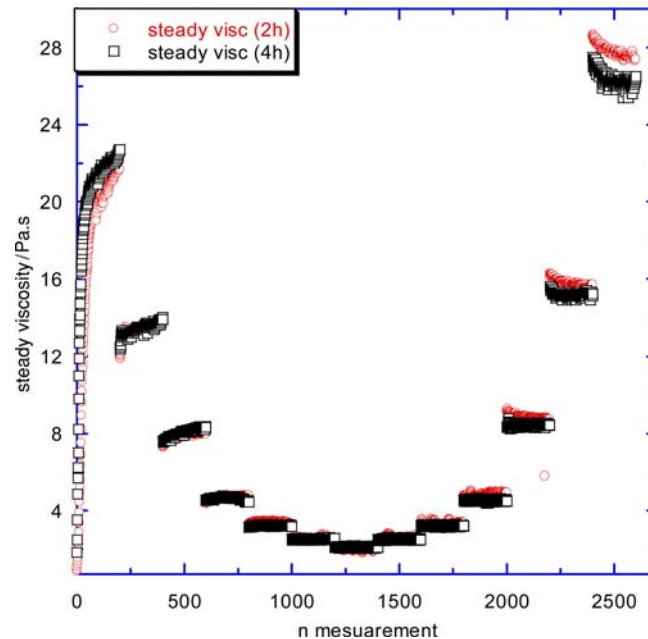


Fig. 1 Representation of steady viscosity during stepwise cycling of the shear rate (5, 10, 20, 40, 60, 80, 100, 80, 60, 40, 20, 10 and 5 s^{-1}). The *empty dots* represent the first test (each shear rate applied for 2 h) and the *empty squares* represent the second test (each shear rate applied for 4 h). Each shear rate is represented with 200 measurements equally spaced in time. The temperature is 25°C

for each shear rate), during stepwise cycling of the shear rate, as the following: 5, 10, 20, 40, 60, 80, 100, 80, 60, 40, 20, 10 and 5 s^{-1} , at 25°C . This cycle was performed twice; once keeping the sample for 2 h at each shear rate and once with 4 h. Except for the two lowest shear rates (5 and 10 s^{-1}), a steady state viscosity is reached within two hours. The viscosity is reproducible; both when comparing the two sets of cycles and when comparing increasing and decreasing shear rates with a cycle. Hence, from the viscosity experiments we conclude that a steady state can be reached, independent of the shear history, at least if the shear rate is $> 10\text{ s}^{-1}$. The MLVs are shear thinning and the viscosity values obtained here are similar to the ones obtained by Oliviero et al. [32] at slightly higher temperatures.

The MLV size decreases with increasing shear rate [5, 26] as can also be viewed by light microscopy. The MLVs are kinetically stable and, after stopping the shear, a

certain amount of sample was recovered and observed in a light microscope after being put in contact with an aqueous solution of dextran. In the vicinity of the interface individual MLVs become dispersed in the polymer solution and can be viewed in the microscope. The polymer is used to approximately match the osmotic pressure inside the MLV so that they retain their composition and thereby their size. Figure 2 shows microscope images from three samples. Image 2a was taken after applying a shear rate of 20 s^{-1} and in Fig. 2b we show the viscosity trace. Image 2c was obtained after shearing at 60 s^{-1} and with the corresponding viscosity trace shown in Fig. 2d. Finally, image 2e, was again obtained at 20 s^{-1} . The corresponding viscosity trace at 20 s^{-1} is shown in Fig. 2f. As can be seen, the average MLV size decreases with increasing shear rate (from 20 s^{-1} to 60 s^{-1}) and returns again to the initial size when the shear rate is decreased (from 60 s^{-1} to 20 s^{-1}).

Fig. 2 On the left side, optical micrographs showing Maltese crosses using the contact line method and, on the right side, the respective behavior of steady viscosity. A dextran solution (10 wt% in water) was used to promote the MLVs, bulk dilution and reach a suspension of onions; magnification 40x at 25°C . **a** and **b** correspond to 20 s^{-1} ; **c** and **d** correspond to an increase to 60 s^{-1} ; and **e** and **f** correspond to a decrease again to 20 s^{-1} . The bar represents $50\text{ }\mu\text{m}$

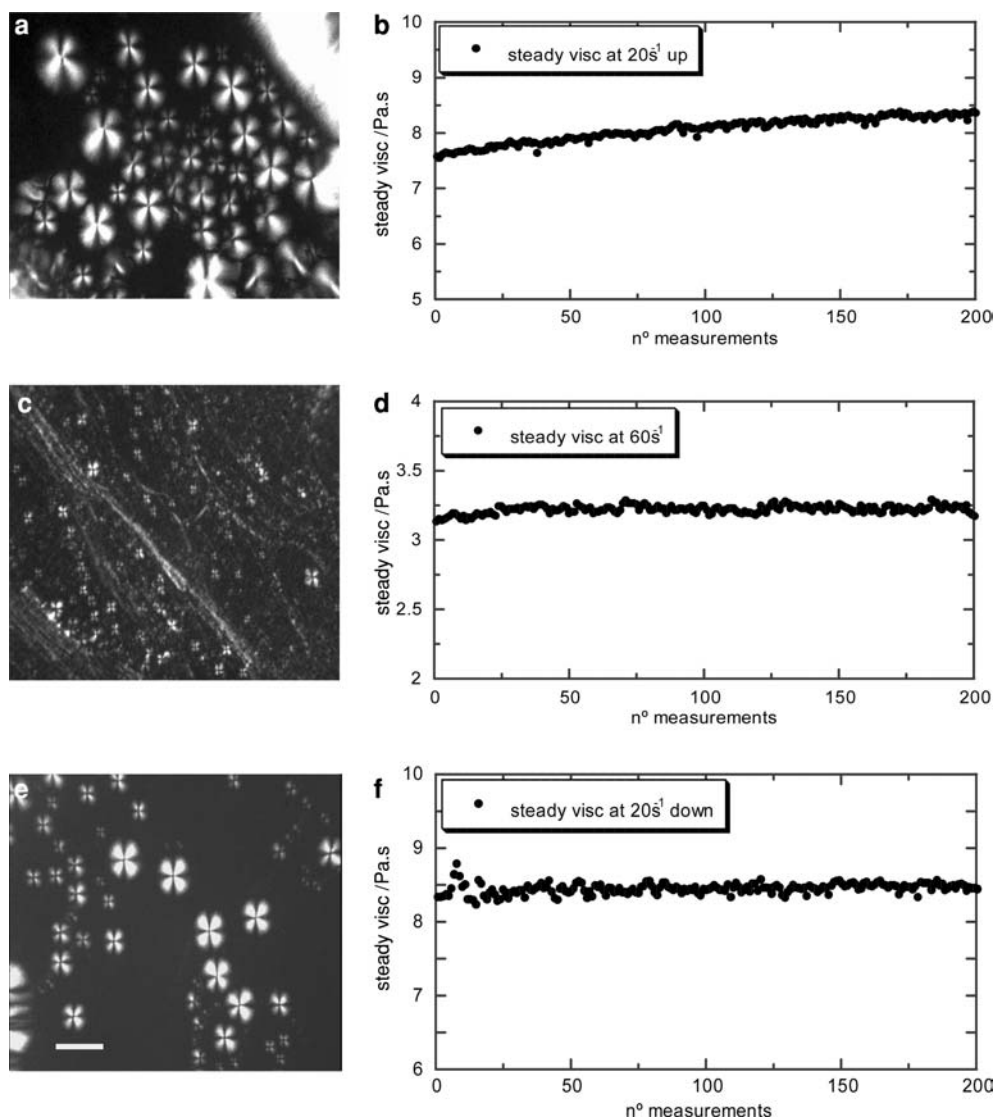


Figure 3 shows the in-situ determinations of size of the MLVs using time-resolved rheo-SALS. In Fig. 3a we present the time evolution of the average MLV radius, R , when first increasing the shear rate from 10 to 20 s^{-1} and then decreasing it back to 10 s^{-1} , while in Fig. 3b we show the corresponding results when the shear rate was varied as $20 \text{ s}^{-1} \rightarrow 40 \text{ s}^{-1} \rightarrow 20 \text{ s}^{-1}$. Detector images obtained at the end of each shear period are shown as inserts. In depolarized SALS the MLVs show a characteristic scattering pattern [17] modulated by a structure factor which gives an intensity maximum at a given q -value, q_m . The vesicle radius is estimated from $R = \phi/q_m$ [34]. In Fig. 3a we see that the same vesicle size is obtained at 10 s^{-1} also after the sample had been sheared at 20 s^{-1} . Figure 3b shows similar results, however, with slightly different sizes at 20 s^{-1} .

The average sizes observed in the light microscope appears to be several times larger than the sizes obtained from the SALS experiments, in particular for the 20 s^{-1} samples. This may be due to non-steady-state conditions

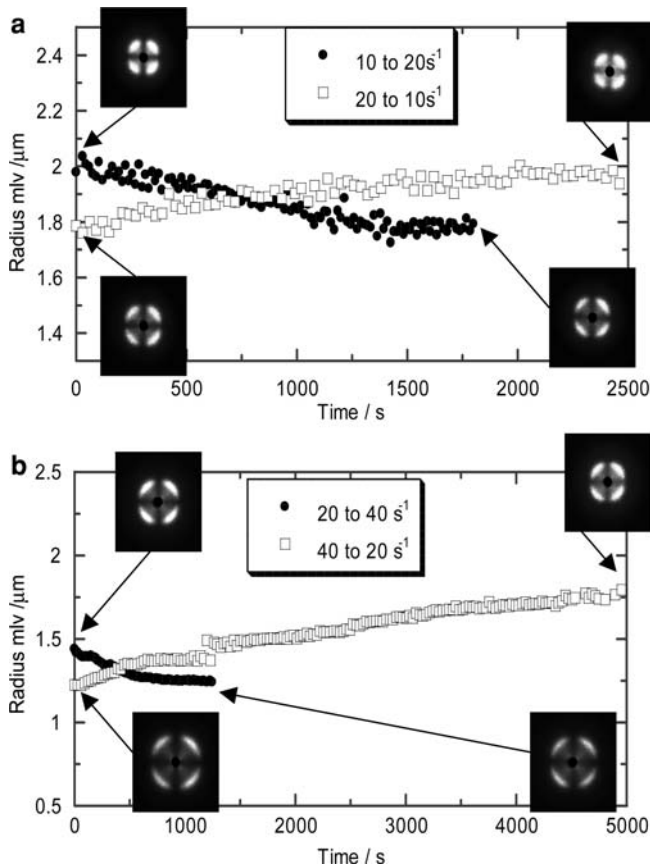


Fig. 3 **a** Representation of the MLVs, radius evolution in time and respective initial and final scattering patterns recorded in depolarized SALS, when increasing the shear rate from 10 to 20 s^{-1} and decreasing again to 10 s^{-1} . **b** Representation of the MLVs, radius evolution in time and respective initial and final scattering patterns when increasing the shear rate from 20 to 40 s^{-1} and decreasing again to 20 s^{-1} .

or the way the microscope samples have been treated. There could be some osmotic swelling during the dilution process. Further work is necessary to understand this discrepancy.

After shearing to a steady state we also performed small amplitude oscillatory experiments in the frequency range 0.5–5 Hz, remaining in the linear viscoelastic regime. In this frequency range, the MLV sample behaved as an elastic body. Both the storage (G') and the loss (G'') moduli were independent of the frequency and G' was significantly larger than G'' . In Fig. 4 we have plotted G' as a function of the shear rate. This shear rate here corresponds to the shear rate at which the sample first had been sheared for a period of 2 h, after which the shear was stopped and the oscillatory test was performed. The shear rate was varied between 20 and 100 s^{-1} . As can be seen, similar G' values are obtained when increasing and decreasing the shear rate. Some deviation is observed for the lowest shear rate (20 s^{-1}), for which 2 h may not be sufficient to reach a steady state.

The elastic modulus decreases with increasing MLV size according to $G' = a + b/R$, where b involves an effective interfacial tension and the offset a results from an excess elastic energy stored in the disordered MLV arrangement [34, 37]. Since we approximately have $R = c\dot{\gamma}^{(-1/2)}$, we expect

$$G' = a + (b/c) * \dot{\gamma}^{1/2}. \quad (1)$$

A best fit with the square root law is included in Fig. 4 as a solid line. As can be seen, the square root law describes the data reasonably well, although the range of shear rates is too small to accurately confirm the power law. From the best fit we obtain $a = 31.4 \text{ Pa}$ and $b/c = 7.9 \text{ Pa s}^{1/2}$, where the latter value can be compared with a theoretical estimate. For the present sample composition it has previously been found that $c = 9 \cdot 10^{-6} \text{ ms}^{-1/2}$ [26]. The effective interfacial tension

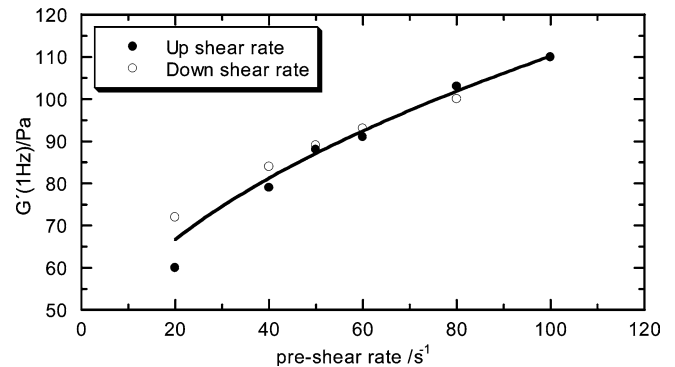


Fig. 4 Plot of storage modulus as a function of shear rate. The *full circles* represent an increase in shear rate. The *empty squares* represent the decrease in shear rate. In this oscillation test, G' was measured in a frequency range from 0.5 to 5 Hz maintaining constant stress (2.5Pa). The temperature is 25°C .

term can be approximately expressed as $b \approx (K\bar{B})^{1/2}$, where K and \bar{B} are the bulk compression and bending modulus, respectively, of the lamellar stack [34, 36–38]. For uncharged membranes stabilized by the Helfrich undulation force we have

$$(K\bar{B})^{1/2} = (3\pi/8)k_B T/d^2, \quad (2)$$

where k_B is Boltzmann's constant, T is the absolute temperature and d is the lamellar spacing [39]. For the present composition, $d=6$ nm [8, 25, 26]. With these values for c and d and the temperature $T=298$ K, we obtain $b/c=14.9$ Pa s^{1/2}, which is approximately twice the measured value. Similar reasonable agreement has been found in other systems [34, 38].

Here we have demonstrated by systematic rheology and small angle light scattering measurements that a well defined steady state is obtained in the limit of large strains, when a lamellar phase sample of the surfactant C₁₀E₃ in water is sheared into a MLV state. The steady state can be reproduced from different shear histories. This is at least true for higher shear rates. For lower shear rates, reproducibility is more difficult to prove due

to the long time required to obtain the strain necessary to reach a steady state. The MLV radius decreases with increasing shear rate. Hence, when increasing the shear rate the number of MLVs in the gap increases while a decrease in the shear rate results in a decrease. The local molecular mechanisms involved in the changes of the MLV size are still far from understood. The transition from planar lamellae to a phase of MLVs has been proposed to be a consequence of a mechanical flow instability of the planar lamellae at higher shear rates [40–42]. It remains to be shown how the reversibility of the MLV size, as demonstrated here, as well as the reversibility of the lamellar-MLV transition [32] fit into the picture of flow instabilities.

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