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# Characterization of a complex dispersion of multilamellar vesicles

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**Abstract** Spherulites® are multilamellar vesicles made up of surfactant bilayers. These vesicles would potentially be very useful for the encapsulation and protection of molecules; however, traditional formulations of these vesicles are poor at retaining small hydrophilic molecules (below 1000 g/mol). In this study, we present new systems of Spherulites called complex dispersions. These are prepared by dispersing Spherulites in an oil medium, and then emulsifying this oily dispersion of Spherulites within an aqueous solvent. These new systems provide an additional oil barrier between encapsulated molecules and an external aqueous phase. We have used polarized light optical microscopy, X-ray diffraction and freeze–fracture electron microscopy to study a complex dispersion of Spherulites at all stages of its preparation. We first studied the sheared lamellar phase, followed by the dispersion of the multilamellar vesicles in the oily medium and finally the emulsification of the oily dispersion within the aqueous solvent. We compared our results on lamellar phases with previous results obtained with Spherulites directly dispersible in an aqueous medium. Since the formulation of our lamellar phase included a large percentage of oil as a component, we studied the localization of the oil in the lamellar structure. We also studied the influence of osmotic pressure on complex dispersions, because complex dispersions possess a double structure similar to that of water-in-oil-in-water emulsions and multiple emulsions are known to be sensitive to osmotic pressure. In conclusion, complex dispersions proved to be new potential carriers exhibiting some unique physical properties.

**Keywords** Complex dispersion · Multilamellar vesicles · X-ray diffraction · Freeze–fracture electron microscopy · Lamellar structure

## Introduction

Lyotropic phases are surfactant/solvent systems. In many cases, the component units are organized locally in the form of a membrane, i.e. a planar bilayer of surfactant molecules. A lamellar phase is an example of such a structure, composed of a stack of surfactant bilayers separated by water layers. It is a liquid-crystal phase. It was recently discovered that the shearing at intermediate shear rate of some lyotropic lamellar phases could lead to the formation of multilamellar vesicles called Spherulites [1], the overall structure of these multilayered vesicles being conserved upon dilution [2, 3, 4,5]. Spherulites appeared to be interesting systems in

pharmaceutics for controlled release and protection of encapsulated substances [6,7]. They were also evaluated for their ability to stabilize molecules in an internal pH different from the external one but showed some limitations for that application [8]. In fact, the efficiency of Spherulites in long-term stabilization and encapsulation is limited by the permeability of the surfactants bilayers. Therefore, the presence of an additional barrier, able to slow down the rate of release of encapsulated molecules and prevent the diffusion of ions from the external aqueous phase to the internal aqueous phase, would be highly desirable. Thus, complex dispersions, i.e. multiple compartmented systems of Spherulites dispersed in oil and further emulsified in an aqueous phase, were developed [9]. Complex dispersions are prepared by a two-step process. Multilamellar vesicles are first dispersed in an oily medium. This oily dispersion is then emulsified within an aqueous solvent. This particular way of combining oil, water and surfactants leads to metastable systems composed of an emulsion with large oil droplets in which small multilamellar vesicles are dispersed. With well-chosen proportions of suitable surfactants, their lifetime can exceed several months. These new systems are particularly interesting because the oil medium around the vesicles provides an additional diffusion barrier between encapsulated molecules and the external aqueous solvent.

The present study was conducted to characterize these new systems of complex dispersions. Polarized light optical microscopy, freeze–fracture electron microscopy and X-ray diffraction had been used previously to study the structure of Spherulites. We used these methods to study the structure of complex dispersions at all stages of their preparation. We first looked at the sheared lamellar phase, followed by the dispersion of the multilamellar vesicles within the oil medium and finally the emulsion of the oily dispersion within the aqueous solvent. We compared our Spherulites, which are dispersible in oil, with Spherulites dispersible in water studied by Gulik-Krzywicki et al. [2]. These previous experiments had shown the absence of any appreciable water core (in contrast to liposomes) inside the objects, as well as the absence of water between the objects, revealing that the lamellar structure was totally conserved after shearing, in the form of multilamellar vesicles. In the present study, the location of the internal aqueous phase in the multilamellar structure of the Spherulites was determined. A particularity of the formulation of our lamellar phase was the inclusion of a large percentage of oil as a component. It has been previously shown that only up to a few percent weight for weight of medium-chain triglycerides could be incorporated into the bilayer structure, between the phospholipid chains [10,11]. Our aim was therefore to determine whether a part of the oil component was entrapped inside the bilayers of the lamellar structure. For this purpose, we studied the effect of the oil dilution on the multilamellar vesicles. Finally, complex dispersions were studied. Complex dispersions have some points in common with water-in-oil-in-water emulsions as far as their structure is concerned, since the latter are oil-in-water emulsions in which the dispersed oil droplets themselves contain smaller dispersed aqueous droplets. It is well known that one major release mechanism for multiple emulsions is a swelling-breakdown process, which occurs when there is a higher solute concentration in the internal aqueous phase than in the external one [12, 13,14]. It therefore seemed interesting to study the influence of osmotic pressure on complex dispersions.

## Material and methods

### Preparation of the double dispersion of multilamellar vesicles

The following substances were used: deionized water, soybean phosphatidylcholine (PC90) (Nattermann, Germany), Simulsol 989 (hydrogenated ethoxylated castor oil, Seppic, France), Miglyol 812 N (medium-chain triglycerides, Condea, France), Lutrol F68 (poloxamer 188, polyoxyethylene/polyoxypropylene, BASF, France).

We first prepared a lamellar phase of surfactants. The formulation of the lamellar phase was PC90 38.9% w/w, hydrogenated ethoxylated castor oil 7.8% w/w, medium-chain triglycerides 23.3% w/w, aqueous phase 30% w/w. The lamellar phase was prepared as follows. PC90, oil (medium-chain triglycerides) and lipophilic surfactant (hydrogenated ethoxylated castor oil) were mixed and left at 37 °C for 12 h. Then, the mixture was stirred and the aqueous phase was added. After a further 12 h at 37 °C, Spherulites were obtained through shearing of the lyotropic lamellar phase [3, 4,5]. Since only small quantities were prepared (a few grams), shearing was done manually with a spatula.

These Spherulites, containing a significant proportion of rather hydrophobic surfactant, could be dispersed in oil. To obtain the oily dispersion, 40% w/w medium-chain triglyceride oil was added to 60% w/w multilamellar vesicles. After high-speed magnetic stirring, the oily dispersion was formed.

The oily dispersion of Spherulites could then be emulsified in an aqueous medium containing surfactant. The surfactants used in the aqueous external phase had to be rather hydrophilic in order to ensure emulsion stability [15]. To obtain the complex dispersion, 30% w/w oily dispersion was added to 70% w/w aqueous solution of a hydrophilic surfactant and emulsification was achieved by manual shaking. The various hydrophilic surfactant aqueous solutions used were a 0.1% w/w, a 0.2% w/w, a 0.5% w/w, a 1% w/w aqueous solution of Lutrol F68, a 0.5% w/w aqueous solution of Solutol HS15 [poly(ethylene glycol 660) hydroxystearate, BASF] and a 1% w/w aqueous solution of Elfacos OW100 [Poly(alkylene glycol), Laserson].

The final system was an aqueous emulsion of oil droplets containing dispersed Spherulites.

### Freeze–fracture electron microscopy

A thin layer of the sample (20–30 μm) was placed on a thin copper holder and then rapidly quenched in liquid propane. The frozen sample was fractured at −125 °C, under a vacuum lower than $10^{-6}$ torr, with a liquid nitrogen cooled knife in a Balzers 301 freeze-etching unit. The replication was performed using

unidirectional shadowing, at an angle of 35°, with platinum–carbon (1–1.5 nm of mean metal deposit). The replicas were washed with organic solvents and distilled water and observed with a Philips 410 electron microscope. All internal aqueous phases contained 30% of glycerol, after we had checked that the replacement of water with water–glycerol mixtures did not modify the structure of the samples but improved the aspect of the freeze–fracture electron micrographs. The glycerol permitted optimal preservation of the sample structure upon cryofixation.

### X-ray diffraction

Samples were prepared in X-ray quartz capillaries (1.5 mm outside diameter with 0.01 mm wall thickness) purchased from GLAS company (W. Muller, Berlin, Germany), which are specially designed for X-ray diffraction and minimize attenuation of the beam and parasitic scattering. About 20 µl of each sample was loaded into the bottom of the thin glass capillaries.

Synchrotron X-ray diffraction measurements were performed at the D-22 beam line (wavelength 1.5498 Å) at the Laboratoire pour l'Utilisation du Rayonnement Electromagnétique in Orsay, France. Two detectors covering the small-angle and the wide-angle diffraction regions were used, the sample-to-detector distances being respectively about 1774 and 300 mm. The samples were positioned in an oven (Peltier element) and held at a controlled temperature of 20 °C. The exposure time varied depending on the sample, but was always of the order of a few minutes. The crystalline $\beta$ form of high-purity tristearin was used as a reference for both small-angle and wide-angle channels for wave-vector calibration of the detectors. The scattered intensity was therefore obtained as a function of wave vector $\mathbf{q}$ (reciprocal angstroms). The repeat distance, $d$ , of the lamellar structure was determined from the maximum position of the first-order diffraction peak using the Bragg equation $d = 2\pi/q$.

## Results and discussion

### Characterization of the sheared lamellar phase

Spherulites are obtained through shearing of a lyotropic lamellar phase and the anisotropic liquid-crystal structure is conserved upon shearing; therefore, Spherulites are birefringent objects. So, polarized light optical microscopy was used to check for spherulite formation (Fig. 1).

Freeze–fracture electron microscopy confirmed the absence of any appreciable water core inside the Spherulites, as well as the absence of water between the multilamellar vesicles, as previously observed by Gulik-Krzywicki et al. [2], and the absence of oil between the multilamellar vesicles (Fig. 2). This means the lamellar structure was entirely conserved after shearing, in the form of multilamellar vesicles. One difference was that the Spherulites did not appear to be so closely packed and did not adopt a three-dimensional structure of a polyhedral type (foamlike) structure, and their size was much more polydisperse. This could be explained by the method of preparation of the Spherulites, as in our experiments Spherulites were not prepared at a controlled shear rate.

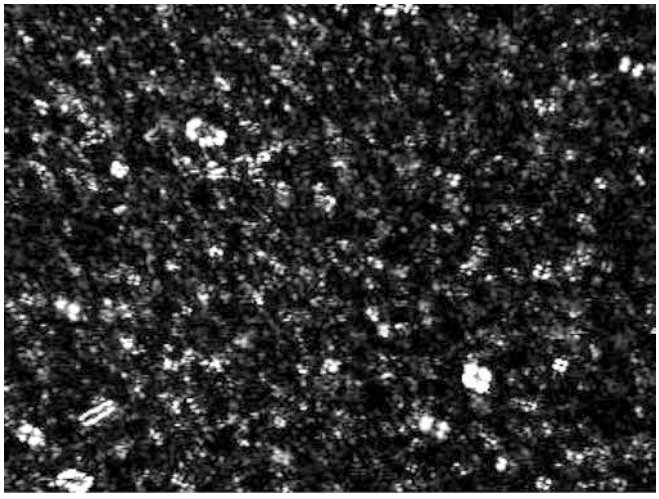

**Fig. 1** Example of a sheared lamellar phase observed with polarized light optical microscopy, showing the birefringent nature of the vesicles, which is a result of their lamellar internal structure

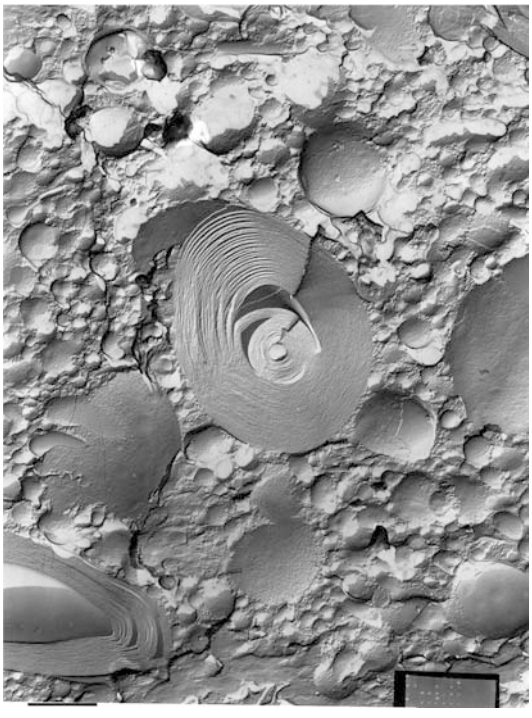

**Fig. 2** Freeze–fracture electron micrograph of multilamellar vesicles obtained after shearing a lyotropic lamellar phase. A 0.05 M saline solution is encapsulated. The central Spherulite was cross-fractured, revealing its multilayered nature. The diameter of the middle vesicle is of the order of 3 µm. A large polydispersity is observed, since the preparation was not made under a controlled shear rate. The bar represents 1 µm

The X-ray diffraction study was conducted to determine the swelling limit of the monophasic lamellar phase above which the system was biphasic with excess water.

Layer spacing values, $d$ , were plotted as a function of $1/\Phi$, where $\Phi$ is the weight fraction of lipophilic surfactant, PC90 and medium-chain triglycerides, in the formulation of the lamellar phase (Fig. 3). The layer spacing increased linearly up to 57.7 Å for around 32.5% w/w of water ($1/\Phi = 1.48$) indicating that the system was homogenous (monophasic lamellar phase) up to this percentage. Above 32.5% of water, $d$ was constant and equal to 57.7 Å. The system was therefore biphasic, consisting of the lamellar phase at the swelling limit and excess water. Since we had few experimental points below $1/\Phi = 1.48$, we represented the linear dilution law for a homogenous monophasic lamellar phase $d = \delta \cdot \frac{1}{\phi}$, whose slope is the surfactant thickness, $\delta$. This straight line passes through the point (0,0) and the point (1,$\delta$). The value of $\delta$ was deduced from the linear part of our experimental graph ($1/\Phi = 1$).

## Influence of the presence of oil on the sheared lamellar phase

X-ray diffraction studies were conducted to determine whether the oil was entrapped between the membranes of the lamellar structure. We prepared various samples of sheared lamellar phases with different percentages of oil (Table 1). The percentage of each component in the formulation was calculated with the following equations:

$$\frac{PC}{Sim} = 4.99,$$
$$\frac{W}{Sim} = 3.85,$$
$$S = PC + Sim,$$
$$\phi = S + O,$$
$$\phi + W = 100,$$

where PC is the percentage weight for weight in PC90, Sim is the percentage weight for weight in lipophilic surfactant Simulsol 989, $S$ is the percentage weight for weight in lipophilic surfactants PC90 and Simulsol 989, $W$ is the percentage weight for weight in water, $O$ is the percentage weight for weight in Miglyol 812 N oil and $\Phi$ is the percentage weight for weight in lipophilic surfactant Simulsol 989, PC90 and medium-chain triglycerides (Miglyol 812 N oil).

A simple geometric model for lamellar phases describes a lamellar phase as a periodic stack with repeat distance $d$ of planar membranes of thickness $\delta$ and hydrophilic layers of thickness $\delta_w$. The relationship between layer spacing $d$, $\delta$ and $\delta_w$ is

$$d = \delta + \delta_w.$$

Values of $d$ are given in Table 1. We observed that $d$ remained the same whatever the formulation. So, $d$ remained constant even when the percentage in water was reduced, i.e. when $\delta_w$ decreased. Considering that $d = \delta + \delta_w$, we assumed that $\delta$ increased simultaneously. Therefore, we assumed that the oil incorporated within the membranes of the lamellar structure, causing a linear increase of the hydrophobic layer $\delta$, obeying the relationship $\delta = d \times \Phi$, where $\Phi$ is the weight fraction of surfactants and oil in the formulation of the lamellar phase. Furthermore, the extrapolation to zero percentage oil of the linear relationship $\delta = f$ (%oil) permitted us to verify that the thickness for the surfactant bilayer was around 40 Å. Thus, the difference found between the surfactant bilayer thickness and the hydrophobic layer thickness calculated

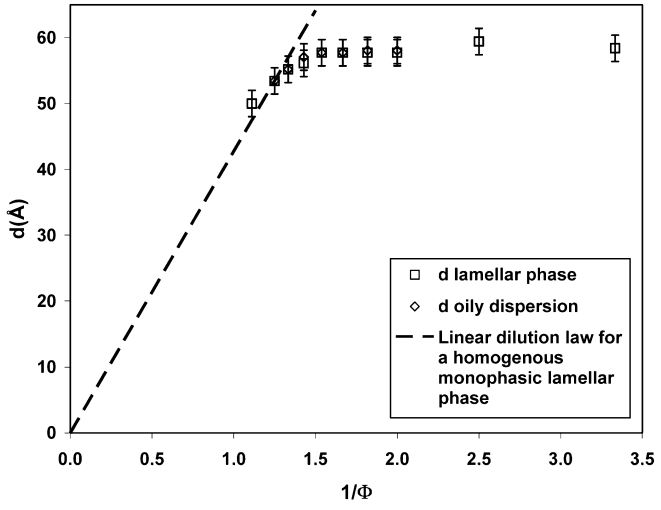


**Fig. 3** Layer spacing values, $d$ , for the sheared lamellar phase and oily dispersion of Spherulites as a function of $1/\Phi$, where $\Phi$ is the weight fraction in hydrophobic components, with a 0.05 M saline solution being encapsulated in the Spherulites. Because the layer spacing was conserved upon dilution of the sheared lamellar phase in oil, i.e. the same values of the lamellar spacing were obtained as a function of $1/\Phi$, the values for the oily dispersion appear buried under the sheared lamellar phase values in this figure. Up to about 32.5% w/w of aqueous phase ($1/\Phi = 1.48$) could be encapsulated in the Spherulites and the maximum spacing value was 57.7 Å. The linear dilution equation for a homogenous monophasic lamellar phase $d = \delta/\Phi$ is represented. The slope gives the thickness of the hydrophobic layer: $\delta = 43$ Å

**Table 1** Samples of sheared lamellar phases encapsulating various percentages of oil. The total spacing value, $d$, remained the same, whatever the percentage of oil

| Samples | Percentage oil | Percentage water | Percentage surfactants | $d$ (Å) |
|---|---|---|---|---|
| A | 1.5 | 38.5 | 60 | 61 |
| B | 10 | 35.2 | 54.7 | 63 |
| C | 20 | 31.3 | 48.7 | 62 |
| D | 30 | 27.4 | 42.6 | 63 |
| E | 50 | 19.6 | 30.5 | 62 |
| F | 70 | 11.7 | 18.3 | 62 |

from the relationship $\delta = d \times \Phi$ was explained by the presence of the oil incorporated within the membranes. However, the increase of $\delta$ could only occur up to a limiting percentage of oil above which the system became biphasic with excess of oil. We did not determine in our study the percentage above which the system became biphasic with excess of oil, so we could not determine the maximum oil content which could incorporate within the membranes.

## Characterization of the oily dispersion of Spherulites

Polarized light optical microscopy was used to confirm the presence of Spherulites after dispersion in the oily medium. Indeed, when large enough, Spherulites are birefringent objects showing characteristic Maltese crosses.

The X-ray diffraction study of the oily dispersion of Spherulites revealed that the layer spacing was conserved upon dilution of the sheared lamellar phase in oil, i.e. the same values of lamellar spacing were obtained as a function of $1/\Phi$, where $\Phi$ is the weight fraction of hydrophobic components (Fig. 3).

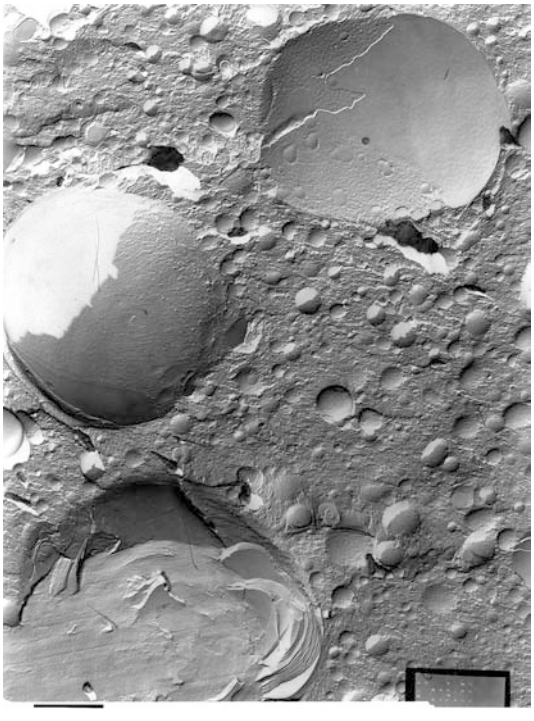

**Fig. 4** Freeze–fracture electron micrograph of an oily dispersion encapsulating a 0.05 M saline solution. Vesicles (60% w/w) were diluted in oil ( 40% w/w). The diameter of largest vesicles is around 4 μm. The basic lamellar structure and the layer spacing were preserved, as verified with an X-ray diffraction study. The bar represents 1 μm

Freeze–fracture electron microscopy demonstrated that the overall structure of the Spherulites was conserved (Fig. 4). While the presence of smaller vesicles had previously been observed after dilution of Spherulites in an aqueous medium [2], probably owing to the fragmentation of the external layers of concentrated Spherulites, this has not been noted after dispersion in the oil medium.

## Characterization of the complex dispersion

Polarized light optical microscopy was used to confirm the presence of Spherulites after emulsification of the oily dispersion of Spherulites within the aqueous solvent. When they were observed between crossed polarizers, birefringence inside the droplets revealed the presence of Spherulites.

The complex dispersion was observed by freeze–fracture electron microscopy (Fig. 5). The presence of smaller vesicles was observed after emulsification within

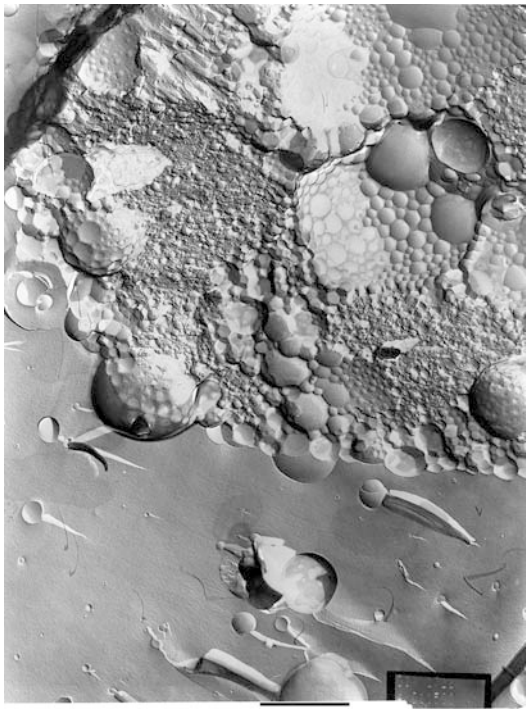

**Fig. 5** Freeze–fracture electron micrograph of a complex dispersion encapsulating a 0.05 M saline solution. An oily dispersion of Spherulites (30% w/w) was emulsified in a hydrophilic surfactant aqueous solution (70% w/w). The diameter of the oil globule is about 7 μm. The basic multilayered structure was preserved, but the X-ray diffraction study revealed swelling of the bilayers. The vesicles appeared packed together in the oil globules. Smaller vesicles, probably unilamellar, appeared in the external aqueous phase, probably owing to the presence of some external amphiphiles that were released during the dilution process. The bar represents 1 μm

the external aqueous phase, probably owing to the fragmentation of some released Spherulites.

X-ray diffraction of the complex dispersion revealed that the lamellar structure was conserved upon emulsification (Fig. 6, Table 2). However, the layer spacing values increased by a few angstroms, reaching approximately 61 Å, a value higher than the maximum spacing of the lamellar phase, which was 57.7 Å (when the percentage of water in the lamellar phase was above the swelling limit), which could possibly be linked to an osmotic effect. The swelling of the layers of the complex dispersion was the same whatever the percentage of initial water fraction in the lamellar phase. Furthermore, the increase in spacing was the same regardless of the hydrophilic surfactant used in the external aqueous phase, and even when the emulsification was performed in water alone.

Since a saline solution (NaCl) was encapsulated between the bilayers of the Spherulites, this suggested that an osmotic phenomenon could be responsible for this increase. An osmotic water flow could have occurred from the external to the internal phase, in the direction of the concentration gradient. So, the complex dispersion could be sensitive to osmotic pressure, in the same way as multiple emulsions. Since osmotic sensitivity could greatly influence drug release, as has been previously observed for multiple emulsions [12,13], it seemed interesting to study the effect of osmotic pressure on the complex dispersion.

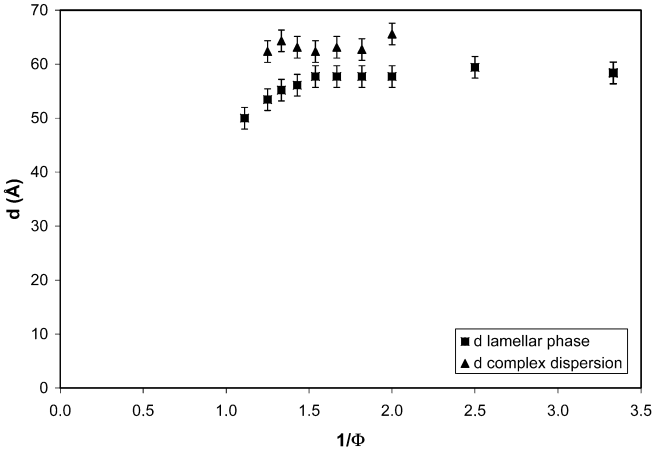


**Fig. 6** *d* for the sheared lamellar phase and the complex dispersion as a function of $1/\Phi$, where $\Phi$ is the weight fraction in hydrophobic components, with a 0.05 M saline solution encapsulated in the Spherulites. Emulsification led to swelling of the lamellar structure

**Table 2** Layer spacing values for the sheared lamellar phase, the oily dispersion and complex dispersions after emulsification in various aqueous solutions of hydrophilic surfactant

| Samples | *d* (Å) |
|---|---|
| Sheared lamellar phase encapsulating a 0.05 M saline solution | 57 |
| Oily dispersion of Spherulites | 57 |
| Complex dispersion after emulsification in deionised water | 60 |
| Complex dispersion after emulsification in a 0.1% aqueous solution of hydrophilic surfactant Lutrol F68 | 61 |
| Complex dispersion after emulsification in a 0.2% aqueous solution of hydrophilic surfactant Lutrol F68 | 60 |
| Complex dispersion after emulsification in a 0.5% aqueous solution of hydrophilic surfactant Lutrol F68 | 60 |
| Complex dispersion after emulsification in a 1% aqueous solution of hydrophilic surfactant Lutrol F68 | 60 |
| Complex dispersion after emulsification in a 0.5% aqueous solution of hydrophilic surfactant Solutol HS15 | 61 |
| Complex dispersion after emulsification in a 1% aqueous solution of hydrophilic surfactant Elfacos OW100 | 61 |

## Effect of osmotic pressure on the complex dispension

Various samples were prepared in order to vary the osmotic pressure between the internal aqueous phases of Spherulites and the aqueous media of complex dispersions. An X-ray study was conducted to determine the layer spacing values *d* of the lamellar structure of sheared lamellar phases and of complex dispersions (Tables 3, 4). As far as the sheared lamellar phase was concerned, higher concentrations of salt led to smaller layer spacing values (Table 3). So, the salt could reinforce the resistance of the layers by a salting-out effect.

**Table 3** Layer spacing of the lamellar phase for various concentrations of encapsulated salt. Higher concentrations of salt yielded smaller layer spacing values. Furthermore, dilution in oil did not modify the structure of the lamellar phase

| Salt concentration in the aqueous part of the lamellar phase ($mol \cdot l^{-1}$) | *d* (Å) |
|---|---|
| $10^{-3}$ | 62 |
| $5\times10^{-2}$ | 58 |
| $5\times10^{-1}$ | 53 |
| 1 | 52 |

**Table 4** Spacing of the lamellar structure of the complex dispersion for various concentrations of encapsulated salt and after emulsification in external aqueous phase of various salt concentrations

| Concentration of salt encapsulated in the complex dispersion ($mol \cdot l^{-1}$) | Concentration of salt in the external aqueous phase ($mol \cdot l^{-1}$) | Spacing of the lamellar structure of the complex dispersion *d* (Å) |
|---|---|---|
| $5\times10^{-1}$ | No salt | 63 |
| $5\times10^{-1}$ | $5\times10^{-1}$ | 54 |
| 1 | No salt | 80 |

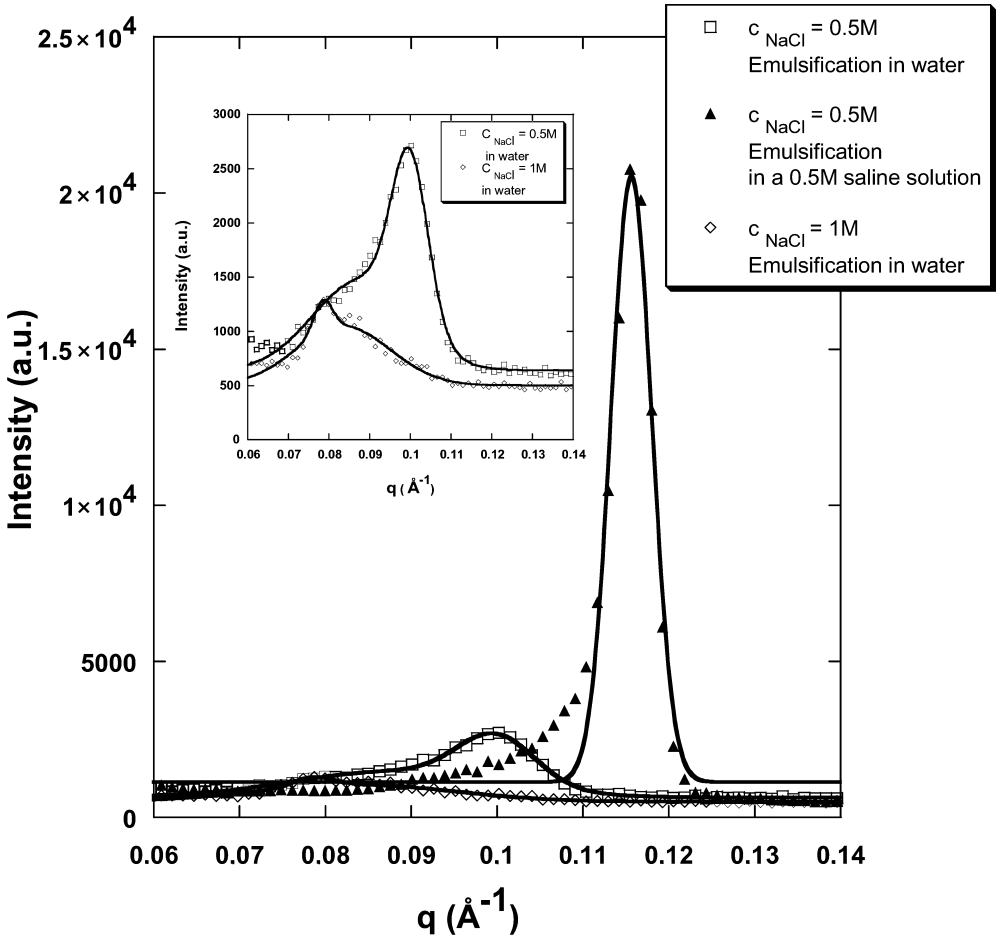


**Fig. 7** X-ray diffraction pattern for a complex dispersion encapsulating salt at various concentrations and emulsified in water or in a saline solution. The scattered intensity is expressed as a function of the wave vector **q**. Intensity values for second-order peaks were weak, so only the first-order peak position is represented here. The continuous curves correspond to fits with one (when $c_{NaCl} = 0.5$ M and emulsification was done in a 0.5 M saline solution) or two (when $c_{NaCl} = 0.5$ or 1 M and emulsification was done in water) Gaussian functions. The necessity of using two Gaussian functions to describe the curves revealed the presence of a biphasic system

Indeed, the ions from the encapsulated salt solution could attract the water molecules of the hydrophilic part of the membrane surfactants in an effort to solvate the ions, leading to the stiffening of the membrane. The lamellar structure and spacing between the layers were conserved upon dilution in oil. On the other hand, the study of complex dispersion samples proved the influence of osmotic pressure on the swelling of the layers of the lamellar structure, the swelling increasing with the osmotic pressure difference (Table 4, Fig. 7—X-ray diffraction spectra of complex dispersions show first-order Bragg peaks, the second-order peaks are not presented because the intensities were weak).

Both a complex dispersion encapsulating water and emulsified in water and a complex dispersion encapsulating a 0.5 M saline solution and emulsified in a 0.5 M saline solution showed no swelling. On the other hand, the highest swelling was observed when a 1 M saline solution was encapsulated and emulsification was performed in water. Even at such a high osmotic difference, the objects were not destroyed and they conserved a lamellar structure; however, the osmotic difference caused a biphasic system to appear. The curves in Fig. 7 were fitted with one (when $c_{NaCl} = 0.5$ M and emulsification was done in a 0.5 M saline solution) or two (when $c_{NaCl} = 0.5$ or 1 M and emulsification was done in water) Gaussian functions. Therefore, although the system conserved a lamellar structure, the smectic order was disturbed by the osmotic difference.

Sensitivity to osmotic pressure could have an impact on drug release. Results to be published have shown that the release rate of salt encapsulated in a complex dispersion depends on the difference between the osmolality of the internal aqueous phase and that of the dispersing aqueous medium; but to a much lesser extent than observed with multiple emulsions. And complex dispersions have permitted the release of encapsulated substances to be slowed compared with multiple emulsions after dilution under hypo-osmotic conditions.

Freeze-fracture electron microscopy of complex dispersions showed a tendency of the Spherulites dispersed in the oil globules to migrate to the interface between the oil and the external aqueous phase, and this tendency was more pronounced when water was encapsulated compared with when a saline solution was encapsulated (Fig. 8).

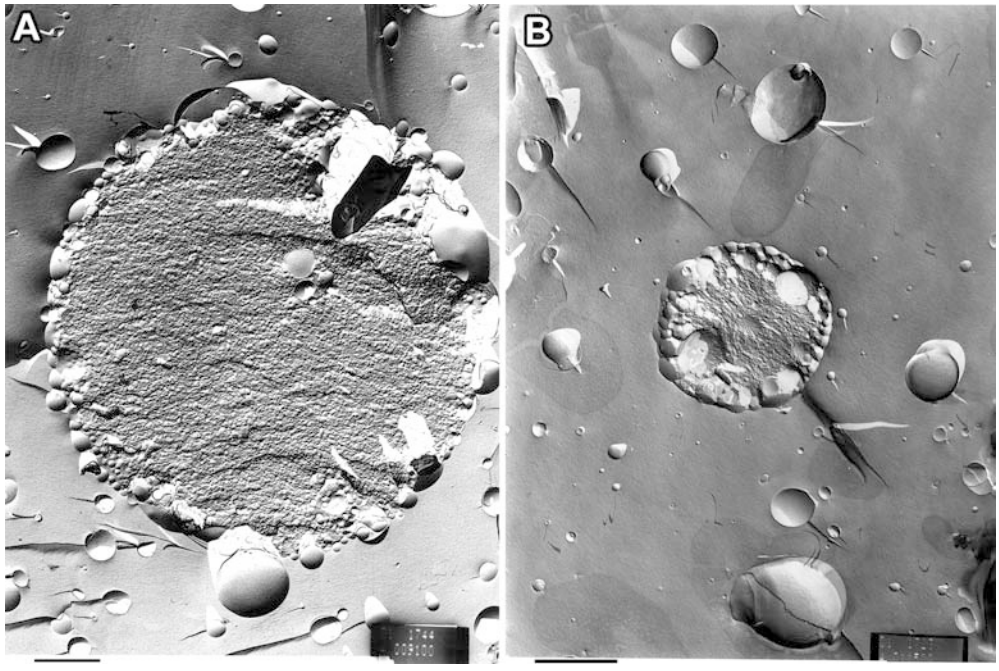


**Fig. 8** Freeze–fracture electron micrographs of a complex dispersion encapsulating **A** water and **B** a 0.05 M saline solution. The diameter of the oil globule in **A** is 7 μm and that of the oil globule in **B** is 2 μm. Spherulites dispersed within the oil globules appeared to migrate to the oil/external aqueous phase interface, and this to a higher extent when water was encapsulated. The bars represent 1 μm

A study by Ficheux et al. [16] has demonstrated a similar tendency for multiple emulsions. Indeed, it was shown that a major factor responsible for the instability of multiple emulsions was the coalescence of the small inner droplets with the oil globule interface, which led to a complete release of the small inner droplets into the external phase. Moreover, the kinetics of the release of the small inner droplets was clearly related to the concentration of hydrophilic surfactant in the external phase. Similarly, in the case of our complex dispersions, the fact that the aqueous external phase is a solution of hydrophilic surfactant could be one cause of instability which leads to the expulsion of the Spherulites and their contents into the external phase. Furthermore, the presence of salt could reduce this tendency as a result of improved stability from a salting-out effect. This could be a determining parameter for the optimization of the formulation of complex dispersions. Therefore, the release of the Spherulites and of their contents into the external aqueous phase could be slowed down if this migration to the interface could be reduced or if the interface itself was reduced by increasing the size of the oil globules. Therefore, it is probable that more work on the complex dispersion formulation could further improve the efficiency of this new system for controlled release applications.

## Conclusions

In this work, we have studied the structure of a new kind of Spherulites which can be dispersed in the oil droplets of an emulsion, which are called complex dispersions. We used polarized light optical microscopy, X-ray diffraction and freeze-fracture electron microscopy to study a complex dispersion at all stages of its preparation. We first studied the sheared lamellar phase. We confirmed the absence of any appreciable water core (in contrast to liposomes) inside the objects and between them, which proved that the lamellar structure was conserved after shearing. Furthermore, we demonstrated that a large percentage of the oil component in the formulation was also entrapped within the lipophilic bilayers of the lamellar structure. We next studied the oily dispersion of the multilamellar vesicles. We observed that dilution in the oil medium did not modify the overall lamellar structure. On the other hand, emulsification of the oily dispersion within an aqueous medium to obtain the complex dispersion led to a swelling effect on the bilayers when the osmolality of the internal aqueous medium was higher than that of the external aqueous medium. Nevertheless, the multilamellar objects showed a high resistance to swelling. This resistance could be an advantage for complex dispersions compared with multiple emulsions, which are known to be very sensitive to osmotic pressure. Freeze–fracture electron microscopy revealed that the Spherulites had a tendency to migrate to the oil/external aqueous phase interface and then to be released from there, and that this tendency seemed more pronounced when the encapsulated solution was water than when it was a saline solution. Therefore, the formulation of a complex dispersion could be further improved to slow down the migration of the

Spherulites and their subsequent release. In conclusion, complex dispersions are new multicompartmented systems which could significantly reduce the release of small hydrophilic molecules compared with simple Spherulites, owing to the additional presence of an oil layer. Complex dispersions could also limit the release of encapsulated molecules compared with multiple emulsions, owing to their reduced sensitivity to osmotic pressure. Complex dispersions could also prevent the diffusion of ions from the external aqueous phase towards the internal aqueous phase and thus protect encapsulated molecules from the external pH. So, these new systems could offer a very interesting alternative for encapsulation applications.

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