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Modification of the Lamellar Phase in C₁₂E₅/Water System by a Random Hydrophilic-Hydrophobic Polyelectrolyte

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Abstract. — The microstructure of a ternary system of a non-ionic surfactant (n-dodecyl pentaoxyethylene glycol ether, C₁₂E₅), water and a water-soluble random heteropolymer, poly(styrene-r-Na styrene sulfonate) with degrees of sulfonation ranging from 30% to 90% is investigated at room temperature. Using small angle X-ray scattering and freeze-fracture electron microscopy we focus on the lamellar phase region and study samples along four different paths of constant surfactant-to-water ratio with increasing polymer content. We demonstrate that for samples with low water content (S/W ratios 3.4 and 4.9), regardless of the sulfonation degree of the polymer, the lamellar phase persists up to several weight percent of polymer and the polymer molecules are confined inside the surfactant bilayers. With increasing quantity of polymer the lamellar phase Bragg peak shifts in a manner consistent with the increase of the lamellar period, which indicates increased bilayer thickness. For mixtures less concentrated in surfactant first order phase transitions are observed for all polymer charge contents when the amount of polymer is increased. For low charge contents of polymer (30% and 45%) the lamellar stack of the polymer-doped surfactant bilayers coexists with either cubic or another lamellar phase and microscopic phase separation is only observed. For large charge contents (65% and 90%) a surfactant-rich lamellar phase coexists with a polymer-rich water solution. It is suggested that the observed phase behaviour is governed by the concentration number of free water molecules.

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1. Introduction

Nonionic surfactants in the binary water solutions form a rich variety of liquid crystalline phases ranging from a dilute micellar, dilute lamellar and disordered sponge phase to the more concentrated normal hexagonal, cubic, lamellar and reversed phases [1-3]. Phase diagrams of these systems have regions corresponding both to discrete aggregates and phases bicontinuous in water and surfactant. This offers an unique opportunity to study the properties of a variety of microstructural fluids in one class of systems and opens up a broad field for potential applications. Another important feature of nonionic surfactants is that the occurrence of phases and the phase transitions themselves are easily controlled not only by the surfactant concentration in the solution but also by the temperature [1-3]. This is especially important for studies of systems with a third component as, for example, a polymer or oil.

The behaviour of mixtures of surfactants and polymers in water has been studied for a long time, starting early this century with natural biological systems. It is well known that effects resulting from the polymer/surfactant interaction may alter both the volume and surface properties and these systems have broad applications in materials science, cosmetics, pharmacology, medicine and biology [4, 5]. During the last decade it has been postulated theoretically [6-8] and shown experimentally [9-16] that addition of even a relatively small amount of polymer molecules markedly affects both the microstructure and elastic properties of the interface via the specific polymer/surfactant interactions. In particular, the polymer-induced vesicle to micelle [9], rod micelle to spherical micelle [10] and microemulsion to gel [11, 12] transitions have been recently reported. Previous studies on the ternary surfactant/polymer/solvent mixtures with polymer molecules solubilised into a surfactant lamellar phase [13-16] have shown that the macromolecules can occupy a variety of sites in such systems. Indeed, polymer molecules were found to be confined into the water regions [13, 14], or to penetrate the bilayers between those regions [15] or to attach themselves to surfaces of the bilayers [16]. Also, a polymer induced phase transition was observed in an electrostatically stabilised surfactant lamellar phase [13]. Recent substantial progress in the calculations of free energy for different lateral arrangements of inclusions in mixed membrane systems [8, 17, 18] further accentuates the need for continuing detailed experimental studies.

As the starting point for our study we have chosen the binary C$_{12}$E$_5$/water mixture. Our choice has been motivated by the fact that this is a relatively well known non-ionic system characterised by various experimental techniques. It also has a very large lamellar phase region which is of special interest in this work. To this mixture we add a charged water-soluble polymer, poly(styrene-r-Na styrene sulfonate), of a particular property: it has an extremely hydrophobic backbone. This random heteropolymer contains both the highly hydrophobic styrene monomers and monomers of a typical polyelectrolyte: styrene sulfonate. Incorporation of these two different types of monomers into the same macromolecule also leads to a copolymer which exhibits more than one kind of affinity. This feature can be manipulated to great advantage in order to tune the behaviour of this copolymer in water solutions. In semi-dilute aqueous solutions, the hydrophobic interactions lead to an evolution of each chain conformation from extended to compact as the charge fraction decreases and to the presence of diffuse hydrophobic regions [19].

Our aim is to study a system in which the microstructure is determined by interactions between the surfactant and solvent molecules and the copolymer molecules are subjected to an anisotropic field. Short accounts of some aspects of this work have been published recently [20, 21]. The lamellar phase is especially interesting because of its structural simplicity and biological importance. It is well established that natural lipids spontaneously form lamellar structures in water and that lipid bilayers are the basis of biological membranes [22].
The lamellar phase in the binary C_{12}E_{5}/water system has been studied by many researchers. This system has a very large lamellar phase region extending (at various temperatures) from the extremely diluted mixture (about 2 wt% of surfactant) to 85 wt% of surfactant. This is a rare example of pure steric stabilisation of a long range lamellar order. Since there are no electrostatic interactions, it is an ideal system to study the elastic properties of bilayers. The fact that the L_3 phase exists at elevated temperatures offers an opportunity to investigate the order-disorder phase transitions at fixed composition. At room temperature the lamellar phase exists from about 64 wt% to 85 wt% of surfactant; at about 75 wt% of surfactant it undergoes an internal transition to a structure with no free water [23].

In a study of polymer incorporation into a surfactant lyotropic lamellar phase the simplest situation to consider is that of a concentrated surfactant system at room temperature with practically no undulations and very little free water. The main issue is structural: what is the position and the conformation of polymer molecules in such a system? We have chosen a polymer with a certain degree of biological analogy which would mimic the basic hydrophobic/hydrophilic character of proteins without the complications due to the complex protein structure and experimental handling problems. The connection between the behaviour of random heteropolymers in water solutions and the protein folding problem has been recently addressed theoretically by Garel et al. [24].

2. Experimental

2.1. Materials. — n-dodecyl pentaoxyethylene glycol ether (abbreviated as C_{12}E_{5}) was obtained from Nikko Ltd., Tokyo, Japan (high purity grade > 99%) and used as received. It was shown by Schubert et al. [25] that the shape of a lower consolation curve in the binary water-surfactant system as well as surface tension values in the vicinity of cmc may be used as a test for the purity of the compound. In particular, close to cmc the miscibility gaps are drastically affected by the presence of impurities. For the purified C_{12}E_{5} the following critical compositions and temperature were reported: \( \gamma_{\text{cmc}} = 2.9 \times 10^{-3} \text{ wt\%} \), \( \gamma_c = 1.5 \text{ wt\%} \) and \( T_c = 32.0 \text{ °C} \) [25]. For the surfactant used in the present study \( \gamma_{\text{cmc}} = 2.6 \times 10^{-3} \text{ wt\%} \) as determined by surface tension measurements and the cloud point was found to be 32.0 °C in the vicinity of 1 wt% of the surfactant concentration in the binary system.

Poly(styrene-\( \rightarrow \text{ Na styrene sulfonate} \)) (abbreviated as PS_{1-\( x \)}NaPSS_{x}, \( x \) being the degree of sulfonation or charge content) was obtained by postsulfonation of polystyrene of weight average molecular weight 250 000 and a polydispersity \( (M_w/M_n) \) of 2 [26]. Poly(styrene-\( \rightarrow \text{ Na styrene sulfonate} \)) is water-soluble for degrees of sulfonation ranging from 30% to 100% [19,27]. The structural formulas of n-dodecyl pentaoxyethylene glycol ether and poly(styrene-\( \rightarrow \text{ Na styrene sulfonate} \)) are shown in Figure 1. The water was purified using millipore filtering.

2.2. Sample Preparation . — The binary and ternary mixtures were prepared by weight in glass ampoules. Immediately after preparation the ampoules were flame sealed. They were then heated to 90 °C and shaken, slowly cooled down and centrifuged several times in order to homogenise the sample. This procedure was repeated until the appearance of sample remained stable. In order to investigate the phase diagram the samples were stored in a thermostated water bath and visually inspected in transmitted light between crossed polarisers. The texture of observed phases was examined using a polarising microscope. Samples prepared for the small angle X-ray scattering studies were transferred to glass capillaries (diameter \( \approx \) 1 mm) and sealed.
2.3. SMALL ANGLE X-RAY SCATTERING. — Small Angle X-ray Scattering (SAXS) experiments were carried out on the beam line D22 at LURE, Orsay using the DCI synchrotron radiation source. Data were collected using pinhole collimation and recorded with a linear detector containing 512 elements. The scattered intensities are expressed versus the magnitude of scattering vector \( |Q| = \frac{(4\pi n)}{\lambda} \sin(\theta/2) \), where \( \lambda \) is the wavelength of incident radiation (\( \lambda = 1.46 \text{ Å}^{-1} \)) and \( \theta \) is the scattering angle. The experimental range of scattering vector was \( Q_{\text{min}} = 2 \times 10^{-2} \text{ Å}^{-1} \) to \( Q_{\text{max}} = 35 \times 10^{-2} \text{ Å}^{-1} \). Data were normalised to constant beam intensity and corrected for the transmission, sample thickness, parasitic and background scattering according to the standard procedure. Experimental resolution was \( 1.5 \times 10^{-3} \text{ Å}^{-1} \) HWHM. Octadecanol was used as the scattering vector calibration standard and the absolute intensity was calibrated using scattering from Lupolen. Complementary SAXS measurements were performed using a conventional Kratky camera at Collège de France. Some samples were analysed in the wide angle region \( (2\theta \leq 45^\circ) \) on the beam line D44 at LURE.

2.4. FREEZE FRACTURE ELECTRON MICROSCOPY. — A 20 to 50 \( \mu \text{m} \) thick layer of liquid sample was deposited on a thin copper holder, and the holder was either directly rapidly quenched in liquid propane, or the sample was first squeezed between the holder and a thin copper plate and then quenched in liquid propane [28, 29]. Both types of preparations were fractured \( \textit{in vacuo} \) (about \( 10^{-7} \text{ torr} \)) with a liquid-nitrogen-cooled knife inside a Balzers 301 freezeETCHING unit. The squeezed preparations were fractured by removing the upper plate with the cold knife.

The replication was done using unidirectional shadowing with platinum/carbon at the angle of \( 35^\circ \). The mean thickness of the metal deposit was 1 to 1.5 nm. The replicas were washed with organic solvents and distilled water. The images were observed using a Philips EM 301 electron microscope. The contrast in images is related to the depth fluctuations of the metal deposit.

A number of case studies using low temperature X-ray scattering and freeze fracture electron microscopy has been devoted to assess the degree of structure preservation during cryofixation [29–31]. It has been demonstrated for a variety of protein/lipid/water lamellar, hexagonal and cubic phases that the freeze fracture images of quenched samples are in excellent agreement with the structure determined by X-ray scattering [31–35]. The ambient temperature structure is preserved without artefacts by the freeze fracture process and the high resolution of the method enables one to resolve the molecular organisation details down to the size of about \( 30 - 40 \text{ Å} \). Therefore, one can use this technique in order to determine the position of the
polymer molecules in microstructure. If water is present in the system, it is often necessary to use a cryoprotectant (e.g. 25 wt% solution of glycerol in water) in order to prevent the formation of water crystals upon rapid quenching. This was found impractical in our case because of the significant influence of glycerol on the phase diagram. However, because of the relatively low water content of samples studied in this work, and the consequent interpenetration of water region by the headgroups, there is no danger of ice crystal formation upon rapid quenching. Therefore, no crystal-formation preventive agents were used and the TEM images correspond to samples of exactly same composition as those studied by SAXS.

3. Results and Discussion

In this study we focus on the microstructure of the lamellar phase region ($L_\alpha$) containing polymer inclusions. We chose four different paths of constant surfactant-to-water ratio ($S/W = 1.7, 2.4, 3.4$ and $4.9$). Starting within a concentrated lamellar phase of a binary system we progressively increased the concentration of $PS_{1-x}NaPSS_x$. This method allows one to determine the role of all three components in the formation of the microstructure. In order to isolate the conformational behaviour of the $PS_{1-x}NaPSS_x$ molecules in the system versus their charge content we used four degrees of the polymer sulfonation: $x = 0.30, 0.45, 0.65$ and $0.90$.

After initial equilibration samples were stored in a thermoregulated bath and observed either visually between crossed polarisers, or, when necessary, with a polarising microscope. Both methods are extremely useful in the studies of phase diagrams and are routinely applied to identify the phases and determine their extent. Both samples containing the polymer and these in the binary system revealed optical anisotropy and a characteristic mosaic pattern of a lamellar phase when observed with a polarising microscope (Fig. 2). Samples containing the polymer are in general more turbid and more viscous than the corresponding samples in the binary system. In order to determine the microstructure of phases one needs a combination of the visual observation methods, optical and electron microscopy and the scattering techniques. Precise study of a phase diagram is very time-consuming and requires a large number of samples. Often the equilibration times are very long, in particular for systems containing polymers. At the current stage of our knowledge about the ternary system we have only mapped the phase diagrams, leaving the precise determination of the phase boundaries for the future.

The binary mixture of $C_{12}E_5$ with water has been previously studied [1, 3] and its phase diagram is well known. However, it was important to precisely characterise in the binary system the fragment of the $L_\alpha$ phase region of interest to this study. The small angle X-ray scattering studies were carried out in the range of surfactant concentrations from $62.9$ wt% to $85.2$ wt% at the temperature $22$ °C. The lamellar phase is very well defined as confirmed by the presence of first and second order Bragg peaks in the scattering spectrum. It has been verified using a synchrotron source and a wide angle experimental configuration that higher harmonics are not detectable in the scattering spectrum.

Assuming a simple geometric model of an ordered stack of water and surfactant bilayers, the lamellar repeat distance $D$ can be calculated from the position of the first order Bragg peak, $D = 2\pi/Q_B$, and expressed by the surfactant volume fraction in the sample. It has been shown by Strey et al. [36] that SANS data for the $L_3$ phase in the $C_{12}E_5$/water system can be interpreted assuming a Gaussian density profile of the bilayers, characterised by the thickness parameter $t = 10$ Å. This phenomenon is caused by penetration of water molecules into the head group region and the presence of a hydrocarbon core only about $10$ Å thick. The authors conclude that the hydrophobic tails of the surfactant molecules in the bilayer
Fig. 2. — Polarisation micrograph of the lamellar phase in the ternary $C_{12}E_5$/water/ $PS_0 \gamma NaPSS_0$ 3 system (S/W=1.7) with low polymer content. The micrograph was taken between crossed polarizers at magnification 400× at the temperature 22 °C. a) reference sample, no polymer added, b) 0.4 wt% of polymer.

interpenetrate each other. Because of similar extension of the hydrophobic chain and hydrophilic polyoxyethylene group in $C_{12}E_5$ one can make the assumption that volumes of the hydrophobic and hydrophilic parts of the molecule are nearly equal [37]. Thus, the hydrophobic layers occupying volume fraction $\Phi_{hc} = \Phi_s/2$, (where $\Phi_s$ is the surfactant volume fraction), are
intercalated with the polar layers built of hydrophilic headgroups mixed with either bound or (for more diluted samples) bound and free water molecules. It follows that

\[ D = 2\delta_{hc}/\Phi_s \]  

(1)

where \( \delta_{hc} \) is the thickness of hydrocarbon interior of the bilayer.

Figure 3 shows the lamellar distance \textit{versus} the inverse of surfactant volume fraction in the binary system. From the linear fit to data we calculate \( \delta_{hc} = 11 \pm 1 \) Å, in good agreement with previous result [36]. In order to calculate the surfactant volume fraction we use the following density values: \( \rho_{water} = 0.998 \text{ g cm}^{-3} \) and \( \rho_{surfactant} = 0.9665 \text{ g cm}^{-3} \).

The ratio of the number of bound to free water molecules for the water mixtures of oligo(ethylene oxide) type non-ionic surfactants determines their microstructure. It has been shown that in aqueous solutions at high concentrations the C_{10}E_5 behaves essentially as a block-copolymer melt [38, 39]. There are large orientational correlations between the adjacent amphiphile molecules. Furthermore, water molecules are hydrogen bound to the polyoxyethylene head groups with a proportion of 1.5-2 water molecules per oxyethylene group. Raman scattering measurements have shown that for volume fraction of surfactant larger than 0.75 all water molecules present in the system are bound to the head groups. By contrast, for more dilute systems some water molecules remain free [23]. One would expect a similar behaviour of the C_{12}E_5/water system because of the same surfactant headgroup.

SAXS results obtained for the two high surfactant-to-water ratios, 3.4 and 4.9, with various amounts of polymer, are shown in Figures 4 and 5. The lamellar phase is well defined up to the highest polymer concentrations for all the polymer sulfonation degrees. Two harmonics are visible in the X-ray scattering spectrum. The characteristic features of the scattering by a lamellar phase, namely the number and position of Bragg peaks, shape of the harmonics and background scattering, have been extensively discussed in the literature [40-45]. The structure and form factor have been calculated for several specific cases and information about the elastic constants, domain size and the interactions stabilising the lamellar structure has been obtained.
In our case the quantitative applicability of existing theoretical models may be limited due to the high degree of complication of the ternary system [8,17,18]. but the lineshape analysis has given results consistent with the predictions of the theory for the thermally undulating lamellae, with additional line broadening caused by the random strain introduced into the system by the presence of the polymer molecules. We have addressed this issue in detail elsewhere [20].

Certain features of the scattering pattern systematically change upon addition of the third component (polymer). Firstly, the Bragg peaks shift slightly with the polymer concentration towards smaller values of the scattering vector, indicating an increase of the lamellar period. The shift of Bragg peaks is more pronounced for the polymer sulfonation degree \( x = 0.90 \). For \( x = 0.30 \), the calculated lamellar period changes from \( D = 41.40 \pm 0.05 \) Å in the binary system to \( D = 42.60 \pm 0.05 \) Å at the polymer content 0.9 wt% and remains constant up to 2.3 wt% of the polymer. For \( x = 0.90 \) the maximum observed value of the lamellar period is \( D = 43.50 \pm 0.05 \) Å at the polymer concentration 2.2 wt%. Figure 6 shows the measured lamellar period for different amount of polymer. Secondly, the scattered intensity in the small-Q region markedly increases for high polymer concentrations at large charge contents. Based only on these results one cannot infer the possible position of \( \text{PS}_{1-x}\text{NaPSS}_x \) molecules within the lamellar structure. For this purpose, the freeze-fracture electron microscopy is best suited [20].
Fig. 5. — SAXS spectra for the C$_{12}$E$_{6}$/PS$_{1-x}$NaPSS$_{x}$/water system for samples of surfactant-to-water ratio equal to 4.86 and two degrees of polymer sulfonation. (a) $x = 0.30$, polymer concentrations 0, 0.6 and 1.7 wt%. (b) $x = 0.90$, polymer concentrations 0 and 1.7 wt%.

Examples of freeze-fracture TEM micrographs for the binary C$_{12}$E$_{6}$/water mixture with S/W=3.4 and for ternary systems with the same surfactant-to-water ratio and containing polymers with two different degrees of sulfonation are shown in Figures 7, 8 and 9. All of the micrographs clearly show a well-defined lamellar structure, in agreement with the SAXS data. The interlayer distance can be estimated from these parts of replicas where the fracture surface is roughly perpendicular to the bilayers (see, for example, bottom part of Fig. 8b). This distance is about 50 Å, in very good agreement with the SAXS-determined value. The detailed freeze-etching electron microscopy studies on biological membranes and membrane model systems have proved that the plane of cleavage-induced fracture propagates through the center of lipid hydrocarbon layer [32, 46–49]. The stair-step effect represents points where the fracture plane has jumped to adjacent bilayers. Thus, the surfaces exposed by cleavage (and visible on the freeze fracture EM images) are mid-sections through the hydrophobic centre of the bilayer. These surfaces appear smooth for the binary C$_{12}$E$_{6}$/water mixture (Fig. 7). For the ternary C$_{12}$E$_{6}$/water/PS$_{1-x}$NaPSS$_{x}$ systems additional objects are clearly visible on the images (Figs. 8 and 9).
Fig. 6. — The lamellar repeat distance $D$ calculated from the position of the first order Bragg peak for the C$_{12}$E$_5$/PS$_{1-x}$NaPSS$_x$/water system for samples with S/W=3.39 versus the polymer concentration. Data for two polymer sulfonations, $x = 0.30$ and $x = 0.90$, are shown. The lines are guide for the eye only. The volume fraction of surfactant and polymer was calculated assuming the specific density of 0.9665 $g$ $cm^{-3}$ and 1.0 $g$ $cm^{-3}$, respectively.

Fig. 7. — Freeze-fracture electron microscopy image of lamellar phase in the binary C$_{12}$E$_5$/water system. S/W=3.39. Note the very smooth appearance of the oblique fracture surfaces. The bar length represents 500 nm.

In order to interpret these images we rely again on the published microphotographs of biological specimens, showing the freeze-fractured, water-soluble protein-lipid (vesicular or lamellar) associations and comparing them with the associations of lipids with the integral membrane proteins [32,34]. In the former case large, smooth fracture planes are observed, whereas in the latter case the planes are spotted with particles which have been identified as the membrane-
Fig. 8. — Freeze-fracture electron microscopy images for samples of surfactant-water ratio 3.39 with PS$_{1-x}$NaPSS$_x$ of sulfonation degree $x = 0.30$. Images for three different concentrations of polymer are shown: (a) 0.2 wt%, (b) 2.3 wt%, (c) 5.2 wt%. Arrows indicate the images of polymer molecules in (a) and (b). Note the increasing amount of particles on the oblique fracture surfaces. The bar length represents 250 nm.

penetrating proteins. Therefore, we infer that in our study the microphotographs actually show images of PS$_{1-x}$NaPSS$_x$ molecules confined inside the lamellar phase bilayers. As the concentration of polymer is increased in the sample, the polymer molecules remain contained
Fig. 9. — Freeze-fracture electron microscopy images for samples with S/W=3.39 and PS$_{1-x}$NaPSS$_x$ of sulfonation degree $x = 0.90$. Images for four different concentrations of polymer are shown: (a) 0.9 wt%. (b) 2.2 wt%. (c) 2.7 wt%. (d) 4.8 wt%. Note the presence of some particles on otherwise smooth oblique fracture surfaces. The bar length represents 250 nm.

inside the bilayer as separate objects. We have shown before that the concentration number of these objects scales with the concentration of polymer molecules present in the sample [21].

The inclusions appear as randomly distributed, compact objects. There is no direct evidence of the possible contact (or lack of it) of parts of the polymer chain with water layers.
Three schematic structural models of the polymer inclusion in a bilayer are shown in Figure 10. Depending on the extent of the hydrophobic part of the polymer, the average bilayer thickness may decrease (A) or increase (B or C) compared to the binary system. Note that the hydrophobic/hydrophilic interface increases for (A) and (B) and remains unchanged in (C). Geometries more complex than those shown in (A), (B) and (C) are also possible. For instance, the charged part of the polymer chain may be adsorbed on the surfactant/water interface or the surfactant molecules can be enclosed inside the polymer coil. To address such structural details one needs specifically designed experiments.

For samples with a constant ratio of surfactant to water molecules, the assumption that the polymer molecules contribute only to the bilayer thickness (and not surface area) of the bilayer (Fig. 10C) leads to the following expression for the lamellar period:

\[ D = \frac{\delta}{\Phi_s + \Phi_p} \]  

which is analogous to equation (1). In contrast to the dilution curve for a binary system (Fig. 3), the bilayer thickness is not constant but systematically increases with the increasing polymer content as shown in Figure 6. One should note different swelling behaviour for the two extreme polymer sulfonations. The calculated swelling is \( \Delta \rho = 1.20 \ \text{Å} \) for 2.2 wt% of the \( x = 0.30 \) polymer content, and \( \Delta \rho = 2.10 \ \text{Å} \) for 2.3 wt% of \( x = 0.90 \) polymer content.

In contrast to the above discussed behaviour, for the surfactant-to-water ratio 2.4 and 1.7 the initial single-phase lamellar system eventually exhibits a first order transition to a two-phase mixture as more and more polymer is added (Figs. 11 and 14). The character of these coexisting phases as well as concentration at which the transition occurs depend on the degree of polymer sulfonation. However, it is difficult to study this aspect in detail because usually there is no macroscopic phase separation. The application of freeze-fracture EM to identify locally mixed phases in a system where the total volume of one of the phases is about thirty times larger than that of the other one is very difficult. Furthermore, the procedure of rapid quenching and obtaining good replicas of samples containing relatively large amounts of water without cryoprotectants is complicated. So far we have not been successful and, therefore, are only able to propose the structural models of phase transitions in limited cases.

Figure 11 shows SAXS data for a series of samples in the ternary surfactant/polymer/water system with the surfactant-to-water ratio of 2.4. The shift of the first order Bragg peak for
Fig. 11. — SAXS spectra for the C$_{12}$E$_5$/PS$_{1-x}$NaPSS$_x$/water system for samples with S/W=2.42 and for four sulfonation degrees of polymer. The polymer concentrations are as follows: (a) $x=0.30$: 0 and 2.8 wt%.  (b) $x=0.45$: 1.2 and 2.9 wt%.  (c) $x=0.65$: 1.1 and 2.9 wt%.  (d) $x=0.90$: 0.3 and 2.6 wt%. Arrows in (a) indicate the calculated positions of the first four Bragg peaks in the Pn3m cubic phase.

the main L$_\alpha$ phase versus the PS$_{1-x}$NaPSS$_x$ concentration is illustrated in Figure 12. For the polymer sulfonations 0.30, 0.45 and 0.65 a small amount of polymer (0.4 wt%) is initially incorporated into the existing lamellar phase without altering neither the peak width nor its position. For $x=0.30$ and a sample containing 1.2 wt% of polymer the Bragg peak shifts towards larger $Q$-values ($\Delta Q = 0.002 \text{ Å}^{-1}$). Also, scattering in the small-$Q$ region increases and the peak broadens, thus indicating a phase transition. For even more concentrated sample (2.8 wt% of polymer, $x=0.30$) two coexisting phases are detected in the SAXS spectrum. The existence of a second phase is evidenced by two or possibly three sharp lines, the first one being the most intense (about 3% of the first Bragg peak of the coexisting lamellar phase). Unambiguous identification of the second phase is difficult because of its relatively small volume, resolution limitations of the experiment and the overlap of lines from the two coexisting phases. The best fit seems to be to a primitive cubic lattice (space group Pn3m, No 224 [50]),
giving reflections with $Q$-values in the ratio $\sqrt{2} : \sqrt{3} : \sqrt{4} : \sqrt{5} : \sqrt{6}$. Positions of the first four peaks are indicated with arrows in Figure 11a.

The cubic structure with space group Pn3m can be modelled as two interwoven bicontinuous networks of short rods connected in fours at each end [51]. As the hydration increases, the lamellar phase in the binary $C_{12}E_6$-water system first transforms into a cubic phase and then into a normal hexagonal phase [3]. It has been shown by Rançon and Charvolin [52, 53] that in the binary system of a homologous surfactant $C_{12}E_6$ and water the lamellar phase is fragmented by cylindrical dislocations of local hexagonal order prior to the formation of the cubic phase. By analogy, we can postulate that in the ternary system studied by us the deformations introduced into bilayers by the polymer molecules trigger the lamellar-to-cubic phase transition, which therefore occurs at the water content smaller than for the corresponding binary system without polymer.

For samples with the polymer charge content 45% the scattering spectra indicate similar phase behaviour as for $x = 0.30$, but the increase of scattering in the small-$Q$ range is more pronounced and only the first peak of the second liquid crystalline phase is visible with the same value of scattering vector as in the former case (at $Q_1 = 0.069$ Å$^{-1}$). The case of 65%
of polymer sulfonation is yet more complex. Initially, the PS$_{1-x}$NaPSS$_x$ is fully incorporated into the Lα phase. Next, at 1.1 wt% of polymer, a cubic and a surfactant-rich lamellar phase (of the same period as in previous cases) coexist, as shown in Figure 11c. For the cubic phase only two Bragg peaks are visible, namely the second and the third one. At even higher polymer concentrations water is being removed from the lamellar phase, as documented by the Bragg peak moving towards larger Q-values. Samples containing polymer of sulfonation degree $x = 0.90$ show macroscopic phase separation starting at the polymer concentration 2.6 wt%. In the corresponding SAXS spectrum a large, broad band is present (Fig. 11d).

All the replicas for S/W=2.4 show well defined lamellar structure, with the images of additional objects embedded in the hydrophobic part of the bilayer, similar to those visible in Figures 8 and 9. Typical images are shown in Figure 13. We could not detect the presence of a second liquid crystalline phase in the micrographs.

Figures 14 and 15 show small angle X-ray scattering spectra for samples with S/W=1.7 and the shift of the lamellar phase first harmonic versus the polymer concentration, respectively. In the binary (surfactant/water) system a sample with this composition (1/Φ$_s$ = 1.57) is already in the two-phase region (cubic-lamellar). However, for all samples, irrespective of the degree of polymer sulfonation, one observes that the addition of a small amount of polymer (in the range 0.2 wt% to 4.4 wt%) to the binary mixture shifts the position of the first order Bragg peak towards smaller Q-values. This effect can be explained by postulating that the presence of polymer stabilises the lamellar phase and moves the phase boundary towards the smaller concentrations of surfactant compared to the phase boundary in the binary (polymer-free) system. The lamellar period ($D = 47.85\ \text{Å}$) corresponding to the surfactant volume fraction in samples containing small amount of polymer agrees very well with the dilution curve (see Fig. 3).

On further addition of polymer the phase behaviour is as follows. For the degree of sulfonation $x = 0.30$ the position of the first order Bragg peak does not change up to a polymer concentration of 1.5 wt%. Adding yet more polymer leads to a first order phase transition. This is demonstrated by the appearance of additional sharp peaks in the small Q-range (at $Q = 0.065$) for samples containing 3.0 wt% and 3.6 wt% of polymer. The two coexisting
Fig. 13. — Freeze-fracture electron microscopy images for samples with S/W = 2.42 with and without polymer. The polymer concentrations are as follows: (a) binary C_{12}E_{5}/water system, (b) $x = 0.45$: 2.9 wt%, (c) $x = 0.65$: 2.9 wt%, (d) $x = 0.90$: 2.9 wt%. Arrows indicate small aggregates in (c). The bar length represents 250 nm.

phases are probably both lamellar. The first one is a surfactant-rich phase, $L_{\alpha'}$, with a scattering spectrum corresponding to a lamellar period only slightly different from that of the $L_{\alpha}$ phase prior to the transition. The other one would be a surfactant-depleted phase [5]. The
Fig. 14. — SAXS spectra for the C_{12}E_6/PS_{1-x}NaPSS_x/water system for samples with S/W=1.70 and for four sulfonation degrees of polymer. The polymer concentrations are as follows: (a) x = 0.30: 0, 0.2, 0.4, 1.5, 3.0 and 3.6 wt%, (b) x = 0.45: 0, 0.4 and 3.4 wt%, (c) x = 0.65: 0, 2.2 and 3.7 wt%, (d) x = 0.90: 0, 0.3, 1.5 and 3.6 wt%.

The second order Bragg peak of this phase coincides with the first order Bragg peak of the L_{α'} phase (Fig. 14a).

For the degree of sulfonation 0.45, the phase behaviour of the ternary system is similar to the case x = 0.30 described above, as illustrated in Figure 14b. The system consists of two distinct lamellar phases, L_{α'} and L_{α'"}. The scattering spectra show first order Bragg peaks at Q = 0.065 Å^{-1} (for L_{α'}) and Q = 0.136 Å^{-1} (for L_{α'"}) for the polymer concentration 3.4 wt%. The increase of the polymer charge content to 65% and 90%, however, induces a change in the phase behaviour. After the initial incorporation of polymer molecules into
a single, well-defined lamellar structure (up to about 2.2 wt% for 65% sulfonation and less than about 1.5 wt% for the 90% sulfonation) a phase separation is detected. In this region, the scattering spectra show large increase of the scattering intensity in the small-Q range (Fig. 14c) for the polymer content 3.7 wt% ($x = 0.65$) as well as 1.5 wt% and 3.6 wt% ($x = 0.90$) (Fig. 14d). The increase of the polymer concentration in both cases causes a decrease of the lamellar period in the ternary L$_\alpha$ phase, as indicated by the corresponding shift of the first order Bragg peak. This effect may indicate formation of a second phase, containing polymer and water, and little or no surfactant. As the polymer concentration increases, water is drained away from the L$_\alpha$ phase region.

Indeed, the freeze-fracture EM replicas of a sample with surfactant-to-water ratio 1.7 and containing 3.7 wt% of polymer (sulfonation degree $x = 0.65$) shown in Figure 16 clearly show
Fig. 15. — The shift of the first order Bragg peak for main lamellar phase in the $C_{12}E_5/PS_{1-x}NaPSS_x$ /water system versus the polymer concentration for samples with $S/W=1.70$. Data for two different polymer sulfonations are shown: (a) $x = 0.30$, (b) $x = 0.65$. The lines are guides for the eye only.

Fig. 16. — Freeze-fracture electron microscopy image for sample with $S/W=1.70$ and with 3.7 wt% of polymer of sulfonation degree $x = 0.65$. Note the presence of particle aggregates between the large domains of the lamellar phase. The bar length represents 500 nm.

the presence of two phases: a lamellar phase almost free from inclusions and a phase containing large, randomly-distributed groups of small, collapsed polymer molecules. The two phases are possibly visible also in a more surfactant-rich system ($S/W = 2.4$, 2.9 wt% of polymer with sulfonation degree $x = 0.65$), as indicated by arrows in Figure 13c. We notice that the quality of the replicas of samples with large water content is not as good as for surfactant-concentrated material. For the two largest degrees of sulfonation (65% and 90%),
the presence of a second, isotropic and macroscopically separated phase was visually observed. Despite the long equilibration time, however, the phase separation was not complete and the scattering spectra of both phases are superimposed in our experimental data.

The only difference between the four sets of samples whose SAXS behaviour has been described above is the concentration of free water in the system. Therefore, there is a strong indication that the phase behaviour is governed by the concentration number of free water molecules. Polymer molecules remain confined inside the lamellar bilayers, regardless of the polymer charge content, for all samples containing bound water only. As soon as free water molecules are present, the phase behaviour becomes more complicated and depends on both the polymer concentration and the polymer sulfonation degree. Polymer molecules with a low charge content (and, consequently, highly hydrophobic) are randomly distributed in the hydrophobic environment of the bilayer interior. By contrast, highly charged polymer molecules added to a lamellar phase with abundance of free water form aggregates (Fig. 16). Inclusions embedded in fluid membranes locally change the elastic and structural properties of the membrane and spatially modulate the bending rigidity and spontaneous curvature. It has been predicted theoretically that both the direct inclusion-inclusion interaction as well as the membrane-mediated interactions may lead to phase transformations [17].

4. Conclusions

We have investigated supra-self assembly in the liquid crystalline phases of ternary mixtures containing a nonionic surfactant, a water-soluble heteropolymer and water. Our conclusions can be summarised as follows.

(1) For the surfactant-rich mixtures, the polymer macromolecules subjected to the anisotropic force field of lamellar structure remain within the hydrophobic interior of the bilayer up to several weight percent of polymer, regardless of the charge content and the related hydrophobicity of the polymer. However, this may not be a correct description of the actual kinetics of phase formation since all three ingredients are initially mixed and only the final structure (after equilibration) is known. It is likely that surfactant molecules would initially aggregate in water into a hydrophilic-hydrophobic matrix woven around the large polymer molecules. Next, these molecules would diffuse in the anisotropic medium (because of the layered environment and different local interactions dominating in every zone) and form objects preferred by free energy considerations. There is a question about the dominating interactions between the large polymer molecules and the system. Are these the long range forces between the organised layers (layers are defined by local interactions responsible for the local curvature of the interface) or short range interactions between the polymer molecule and nearby surfactant molecules, such a complex being incorporated into the lamellar phase?

(2) A special feature of this system is the high degree of water penetration of the headgroup region (in general characteristic for C_{12}E_{5}). This facilitates a transition from the system with bound water only to a system where free water exists as well. The highly hydrophobic backbone of the polyelectrolyte combined with lack of electrostatic interactions in the surfactant matrix make this a model system to study elastic properties of surfactant/polymer bilayers.

(3) We demonstrate that for highly hydrophobic polyelectrolyte at the limit of solubility in water ($x = 0.30$), addition of considerable amount (5.2 wt%) of macromolecules into the surfactant lamellar matrix with no free water ($S/W=3.4$) does not alter the long range symmetry. Mixtures containing even a few weight percent of free water ($S/W=1.7$) respond to the addition of about 1.5-2.0 wt% of the same polyelectrolyte ($x = 0.30$) with local change of
microstructure. Polymer molecules remain confined inside surfactant bilayers as individual objects within the lamellar phase. So far we have not been successful in our attempts to identify the microcomposition and its relation to the polymer presence in the matrix of microdomains of different microstructures.

(4) The phase behaviour of ternary systems containing polymer molecules with low charge content \((x = 0.30\) and \(x = 0.45\)) is very similar to each other. The polymer molecules are always embedded within the hydrophobic part of the lamellar phase bilayer and as soon as free water becomes available in the system, the addition of about 2 wt% of polymer induces a first order phase transition to another liquid crystalline phase. One should notice that the new phases formed at the large polymer content and coexisting with the lamellar phase may be different, depending on the number of free water molecules in the system. For samples with surfactant-to-water ratio 2.4 the lamellar phase coexists with a cubic phase, whereas for samples with larger amount of free water two lamellar phases of different pitch are simultaneously observed. One may speculate that different amounts of free water molecules constrain the interaction between the hydrated headgroups in a different way, thus allowing for two different spontaneous interface curvatures at equilibrium. Therefore, our results suggest that free water is necessary to facilitate change of the interfacial curvature, which in turn controls the phase transformation process. This is a very significant result since it directly demonstrates the role of interface curvature in colloidal microstructure.

(5) Ternary mixtures with surfactant-to-water ratios 2.4 and 1.7 and containing highly charged, less hydrophobic copolymer \((x = 0.65\) and \(x = 0.90\), initially behave like their less sulfonated counterparts (item 4 above). However, as the polymer concentration increases, the polymer molecules concentrate into an aggregated phase, which eventually starts to separate macroscopically. This is demonstrated by the presence of a wide band in the small-\(Q\) region of the small angle X-ray scattering data (Figs. 11c, d and 14c, d) as well as in the freeze fracture images (Fig. 16).

(6) For compositions at the boundary between the lamellar and cubic phase in the \(\text{C}_{12}\text{E}_5/\text{water} (S/W=1.70)\) system, addition of small amount of polymer (in the range 0.2 wt\% to 0.4 wt\%, irrespective of the charge content) stabilises the lamellar phase and moves the phase boundary towards smaller concentrations of surfactant compared to phase boundary in the binary (polymer-free) system.

(7) We infer that the phase formation process is dominated by the local interactions between the hydrophilic surfactant headgroups and with water molecules as well as between the polymer and surfactant molecules. This point is supported by the occurrence of a local change of phase behaviour (microscopic phase separation). Macromolecules seem to be immobilised in the liquid crystalline surfactant matrix. The processes of conformational evolution and migration of the polymer molecules, leading to the macroscopic phase separation, occur at a much longer time scale.

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