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## COMMUNICATION

## Collapse and coacervation of a lamellar phase by inter-headgroup bridging†

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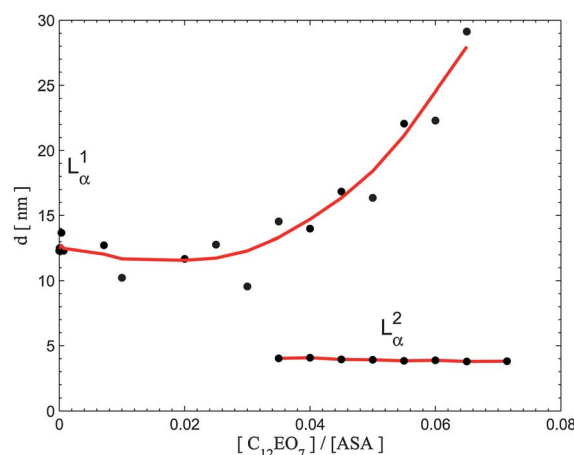
We report the collapse of an electrostatically swollen surfactant lamellar phase above a critical concentration of an added nonionic surfactant due to hydrogen bonding between the two types of headgroups. At still higher concentrations of the neutral surfactant a temperature-driven lamellar to coacervate transition is observed. Small-angle X-ray diffraction pattern of the coacervate can be modeled as arising from bilayers with short-range positional correlations, whereas cryo-scanning electron microscopy images show bilayer-like features riddled with pores. Very similar behavior is found when the nonionic polyoxyethylene alkyl ether surfactant is replaced by polyethylene glycol of sufficiently high molecular weight.

Coacervates are concentrated colloidal dispersions coexisting with a dilute aqueous solution and are of considerable technological importance in a variety of applications.<sup>1</sup> In the case of self-assembled amphiphilic systems the relationship between the coacervate phase and the vesicular phase has been, in particular, a topic of considerable importance in the context of the origin of life.<sup>2,3</sup> It has been shown that small changes in the molecular structure of the amphiphile can stabilize a coacervate at the expense of a vesicular phase, due to changes in the spontaneous curvature of the amphiphile–water interface.<sup>4,5</sup> Although some of the coacervates in amphiphilic systems are known to be in the sponge ( $L_3$ ) phase,<sup>6</sup> it is not clear if other microstructures are also prevalent.

In this report we introduce a novel class of coacervate forming system, which exhibits a temperature driven transition from a lamellar phase consisting of amphiphile bilayers to a coacervate. This system consists of the ionic surfactant  $C_{12}$ -alkenylsuccinic acid (ASA), doped with small amounts of a non-ionic polyoxyethylene alkyl ether ( $C_nEO_m$ ) surfactant. ASA forms a swollen lamellar phase in aqueous solutions; the lamellar periodicity can be made comparable to the wavelength of visible light, so that these solutions display iridescence.<sup>7</sup> The lamellar periodicity is found to abruptly decrease to a value comparable to the bilayer thickness above a critical concentration of the neutral surfactant. This collapse can be attributed to bridging interactions between the bilayers due to hydrogen bonds

formed between undissociated  $-COOH$  groups of ASA and oxygens in the polyoxyethylene headgroup of the nonionic surfactant. As the concentration of the non-ionic surfactant is increased beyond a second critical concentration, a lamellar to coacervate transition is observed on increasing the temperature. We have confirmed our results by showing that high molecular weight polyethylene glycol (PEG) also induces similar bridging interactions, leading to the collapse and coacervation of the swollen lamellar phase of ASA.

ASA is a weak acid with two carboxylic groups, whose  $pK_a$  values are  $\approx 4.2$  and  $5.6$  at room temperature. In aqueous solutions it self-assembles into bilayers that are charged due to the partial dissociation of the  $-COOH$  groups. As a result, the screened Coulomb repulsion dominates over other inter-bilayer interactions<sup>8</sup> and the ASA–water system exhibits a lamellar phase ( $L_\alpha^1$ ) over a wide range of ASA concentrations. The variation of the lamellar periodicity  $d$  with the surfactant volume fraction  $\phi$  is well described by the usual swelling behavior,  $d = \delta/\phi$ , with the bilayer thickness  $\delta = 2.9$  nm. On adding  $C_{12}EO_7$  to a 20 wt% ASA solution  $d$  initially remains constant at a value of about 12.0 nm, followed by the appearance of a lamellar–lamellar coexistence at a relative molar concentration  $\alpha$  ( $= [\text{surfactant}]/[\text{ASA}]$ ) of about 0.035. The second lamellar phase has a  $d$  of about 4.03 nm at  $\alpha = 0.035$  and decreases gradually to 3.87 nm at  $\alpha = 0.060$ , whereas  $d$  of the swollen phase increases sharply due to the water released from the collapsed phase (Fig. 1). Only the collapsed phase ( $L_\alpha^2$ ) coexisting with a very dilute aqueous solution, which does



**Fig. 1** Variation of the lamellar periodicity ( $d$ ) of the ASA–water system with  $C_{12}EO_7$  concentration.  $L_\alpha^1$  and  $L_\alpha^2$  are the swollen and collapsed lamellar phases, respectively.

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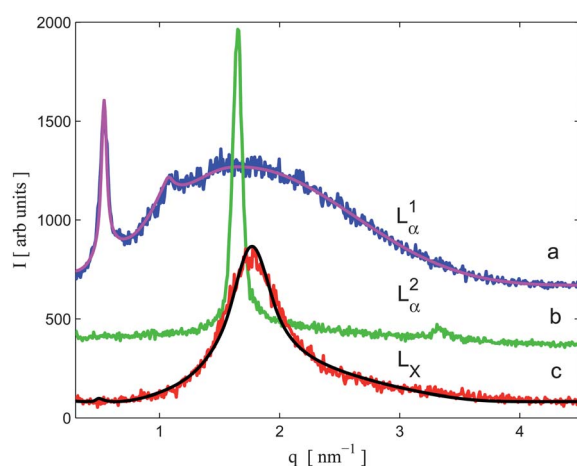
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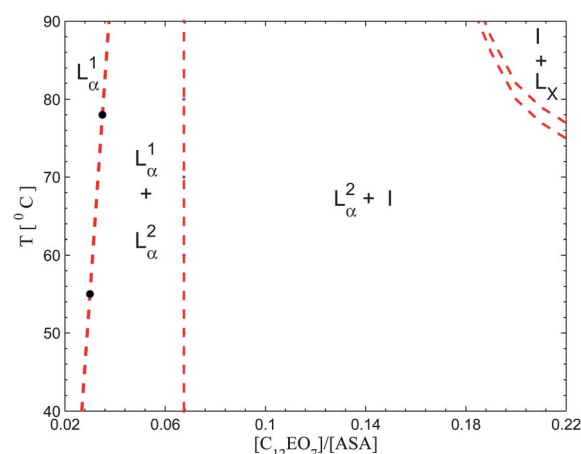
not give rise to any scattering signal, is observed for  $\alpha > 0.065$ . At  $\alpha \sim 0.2$ , the  $L_\alpha^2$  phase is converted into an isotropic coacervate ( $L_X$ ) on heating beyond 80 °C. This transition is first-order, as indicated by the narrow range of  $L_\alpha^2$ - $L_X$  coexistence. Fig. 2 shows typical diffraction patterns of the phases. The broad peak observed in the  $L_X$  phase has an average spacing comparable to the  $d$ -spacing of the  $L_\alpha^2$  phase. A partial phase diagram of the system determined from the X-ray data is shown in Fig. 3.

The ASA- $C_{12}EO_9$  system behaves in a manner very similar to ASA- $C_{12}EO_7$ . However,  $C_{12}EO_9$  is more efficient in inducing the collapsed lamellar phase as compared to  $C_{12}EO_7$ ; the critical value of  $\alpha$  at 60 °C being 0.030 for the former as compared to 0.035 for the latter. X-Ray diffraction studies show that the collapsed phase has a periodicity of 4.08 nm at  $\alpha = 0.035$ , which gradually decreases to 3.70 nm at  $\alpha = 0.60$ .

The collapse of the lamellar phase can be understood in terms of hydrogen bonding between the undissociated -COOH groups of ASA and the oxygens in the  $EO_7$  headgroup of the surfactant; hydrogen bonding between these groups is known to occur in polyacrylic acid (PAA)-polyethylene glycol (PEG)<sup>10,11</sup> and PAA-ethoxylated surfactant complexes.<sup>12,13</sup> When  $C_{12}EO_7$  is added to the ASA-water binary system, the hydrocarbon chain of the surfactant inserts into the bilayer, with the bulky  $EO_7$  headgroup protruding out; it is essentially a short polymer whose one end is anchored in the bilayer. The resulting configuration of the  $EO_7$  headgroups facilitates hydrogen bonding between the oxygens in the headgroup of  $C_{12}EO_7$  molecules residing in one bilayer with undissociated -COOH groups of ASA in the adjacent bilayer (*trans* bonds). The thickness of the water layer in the collapsed phase ( $\sim 1$  nm) is consistent with such *trans* H-bond formation. On the other hand, in the absence of such anchoring, the short  $EO_7$  chains would lie flat at the bilayer surface and form H-bonds with -COOH groups within the same bilayer (*cis* bonds). The *trans* H-bond mediated bridging leads to an effective attractive interaction between the bilayers, which can overcome the screened Coulomb repulsion if sufficient number of hydrogen bonds



**Fig. 2** Typical diffraction patterns of the swollen lamellar ( $L_\alpha^1$ ), collapsed lamellar ( $L_\alpha^2$ ) and isotropic ( $L_X$ ) phases at  $\alpha = 0.02$  and  $T = 60$  °C (a),  $\alpha = 0.07$  and  $T = 60$  °C (b),  $\alpha = 0.22$  and  $T = 80$  °C (c), respectively. The solid lines through curves a and c are fits to a lamellar stack with long-range and short-range positional correlations, respectively (see ESI†). The very broad peak in (a) at  $q \sim 1.5$  nm<sup>-1</sup> arises from the form factor of the bilayer.<sup>9</sup>



**Fig. 3** Partial phase diagram of the ASA- $C_{12}EO_7$ -water system.  $L_\alpha^1$  and  $L_\alpha^2$  denote the swollen and collapsed lamellar phases, respectively.  $L_X$  is the isotropic coacervate and  $I$  the dilute aqueous solution.

are formed. This results in the formation of the collapsed lamellar phase, presumably containing a large fraction of the non-ionic surfactant, above a critical concentration of  $C_{12}EO_7$ . This conclusion is confirmed by the fact that  $C_{12}EO_9$  with two more EO groups than  $C_{12}EO_7$  is more effective in inducing the collapsed phase. In order to confirm the formation of H-bonds we have studied the influence of increasing pH on the collapsed lamellar phase of the  $C_{12}EO_7$ -ASA system. This phase is found to swell on the addition of NaOH and to fully disperse at around 0.5 M NaOH concentration. Thus the deprotonation of the -COOH groups destabilizes the collapsed phase by preventing the formation of H-bonds.

We can estimate the strength of the bridging interaction by calculating the total interaction potential per unit area  $U_l$  at the observed bilayer separation.  $U_l$  is the sum of the electrostatic, van der Waal's and hydration interactions.<sup>8</sup> From the measured pH of the ASA solution,  $U_l$  is found to be  $-1.02 \times 10^{-3}$  J m<sup>-2</sup>, for a bilayer separation of 1.0 nm and typical values of the interaction parameters (see ESI†). Taking the energy of a hydrogen bond to be  $8k_B T$  and the cross-sectional area of the surfactant to be 0.35 nm<sup>2</sup>, this corresponds to 0.01 H-bonds per ASA molecule. The complete collapse of the lamellar phase is found to occur at a  $C_{12}EO_7$  to ASA molar ratio of about 0.07. Our estimate shows that only a fraction of the headgroups need be involved in *trans* H-bond formation to lead to the collapse of the lamellar phase. We should note here that the reported values of many of the parameters vary typically within a factor of 2 to 3 and hence the above numbers should be taken only as order of magnitude estimates. The observed lower critical concentration of  $C_{12}EO_9$  required to induce the collapsed phase is consistent with increased probability of *trans* H-bond formation in the case of the longer headgroup. The higher lamellar periodicity of the collapsed phase near the onset could again be a consequence of the longer length of  $EO_9$ , whereas the shorter periodicity at higher concentrations might reflect the larger number of *trans* H-bonds formed in this system.

To check this hypothesis further, we have replaced the polyoxyethylene alkyl ether surfactant with PEG in the ASA-water system. We find that PEG of molecular weight 1500 can induce bridging interactions and lead to the formation of  $L_\alpha^2$  and  $L_X$  phases. The critical concentration to induce  $L_\alpha^2$  is  $[PEG1500]/[ASA] = 0.001$ . Since

the degree of polymerization  $n \sim 35$ , this corresponds to a relative EO monomer concentration of about 0.035, which is almost an order of magnitude higher than the monomer concentration in the case of the two ethoxylated surfactants. This difference arises from differences in the chain conformations, with the much longer non-tethered PEG1500 chains being in a less extended conformation and hence being able to form fewer hydrogen bonds per EO group with the undissociated  $-\text{COOH}$  groups of ASA. A partial phase diagram of the ASA–PEG1500 system obtained from the diffraction data is shown in Fig. 4. Here the lamellar–lamellar coexistence region shrinks to a narrow concentration range beyond which the  $L_\alpha^2$  phase coexists with a dilute aqueous solution. At higher PEG1500 concentrations a  $L_\alpha^2 \rightarrow L_X$  transition occurs as in the case of  $\text{C}_{12}\text{EO}_7$ , with the broad peak observed in the  $L_X$  phase having an average spacing comparable to the  $d$ -spacing of the  $L_\alpha^2$  phase.<sup>‡</sup>

Although the solubility and hydration of EO groups decrease with increasing temperature, both the phase diagrams presented above show that the critical concentration of the  $\text{C}_{12}\text{EO}_7$ /PEG1500 at which the collapsed phase forms is only weakly dependent on temperature. Similar insensitivity to temperature has been observed in the complexation of PAA and ethoxylated surfactants driven by H-bond formation,<sup>13</sup> which shows that the orientation of the H-bonding moieties is not adversely affected by temperature in these macro-molecular systems. On the other hand, the  $L_\alpha^2 \rightarrow L_X$  transition temperature decreases substantially with increasing  $\text{C}_{12}\text{EO}_7$ /PEG1500 concentration. It is very likely that the dehydration of the EO groups with increasing temperature is responsible for this behaviour, which can lead to a negative spontaneous curvature of the interface leading to a change in the morphology of the bilayer.

In contrast, with PEG of molecular weight 400 ( $n \sim 9$ ) only the swollen  $L_\alpha^2$  phase was observed up to a concentration of  $[\text{PEG400}]/[\text{ASA}] = 3.7$ , although the degree of polymerization is comparable to that of the headgroups of the two surfactants. We believe that this difference is related to the fact that the two headgroups are anchored at the bilayer surface, which allows them to span across the narrow water layer and bridge adjacent bilayers. On the other hand, PEG400 chains are more likely to be adsorbed on to a single bilayer surface, even if their conformation in water is not very different from that of the headgroups.<sup>16,17</sup>

Although some of the coacervates formed from amphiphilic molecules are known to be sponge ( $L_3$ ) phases,<sup>5,18</sup> the microstructure

of many others have not been probed in detail. Diffraction data from the  $L_X$  phase of both ASA– $\text{C}_{12}\text{EO}_7$  and ASA–PEG1500 systems do not correspond to that from a  $L_3$  phase. However, they can be fitted to a bilayer stack with very short-range positional correlations (see ESI†). One possibility would be a dispersion of oligo-lamellar vesicles having a few bilayers. However, the observation of a well-formed meniscus between the coacervate and the supernatant would suggest a more connected structure. Another possibility is an isotropic phase similar to the one observed by Beck *et al.*<sup>19</sup> in an ionic surfactant system containing a cosurfactant. The diffraction pattern of this phase, which the authors call a ‘novel sponge’ phase, is somewhat similar to the one obtained from the  $L_X$  phase of the present system. However, diffraction data from their system has not been analyzed and its detailed microstructure remains unknown. Cryo-scanning electron microscopy images of the  $L_X$  phase are somewhat similar to those reported earlier from coacervates of amphiphiles; some of them show surfaces riddled with pores (Fig. 5), whereas others show oriented bilayer-like features probably produced due to the shearing of the sample<sup>6</sup> (see ESI†). These images suggest that the  $L_X$  phase is made up of bilayers, consistent with the scattering data. However, further work is needed to elucidate the detailed microstructure of this phase.

As discussed earlier, the collapse of the swollen lamellar phase can be understood in terms of bridging interactions due to hydrogen bonding. On the other hand, it is not clear as to what is driving the  $L_\alpha^2 \rightarrow L_X$  transition. The  $L_\alpha \rightarrow L_3$  transition can be understood in terms of the Gaussian rigidity ( $\bar{\kappa}$ ) of the bilayer becoming positive,<sup>20</sup> and a transition from  $L_\alpha$  to an inverted phase can be driven by the spontaneous curvature  $C_0$  becoming negative.<sup>21</sup> Adsorption of polymers is known to affect  $\bar{\kappa}$ ,  $C_0$  and the bilayer bending rigidity ( $\kappa$ ).<sup>22,23</sup> However, the contributions from the adsorbed layer to  $\kappa$  and  $\bar{\kappa}$  are predicted to be rather small. These studies, however, do not consider the influence of the adsorbed polymers on the lateral pressure profile  $\pi(z)$  in the membrane, which determines bilayer parameters such as  $\kappa$ ,  $\bar{\kappa}$  and  $C_0$ .<sup>24,25</sup> It is very likely that adsorbed polymers alter the effective inter-headgroup interactions and hence  $\pi(z)$ ; a similar mechanism has been proposed to explain the action of anesthetics.<sup>26</sup> This can result in changes in the bilayer parameters mentioned above, and lead to changes in the bilayer morphology.

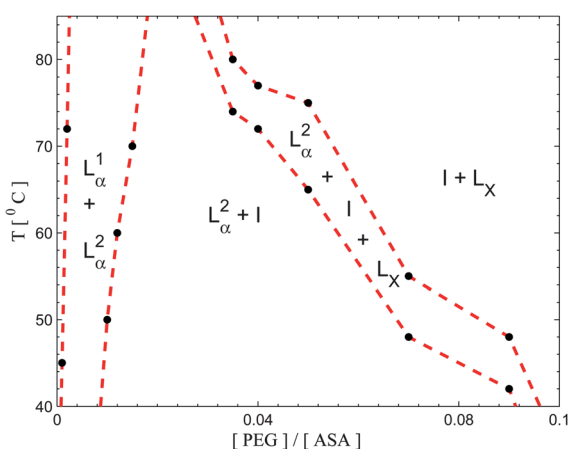


Fig. 4 Partial phase diagram of the ASA–PEG1500–water system.

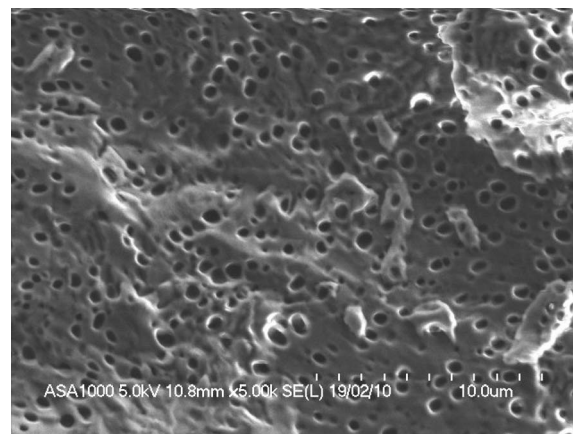


Fig. 5 Cryo-SEM image of the coacervate in the ASA–PEG1500–water system showing pore-like features.  $[\text{PEG1500}]/[\text{ASA}] = 0.1$ .

The ASA- $C_{12}EO_7$  system mimics the behavior of ligand–receptor mediated adhesion of biomembranes.<sup>27</sup> The anchoring of the short polyethylene chain at the membrane surface ensures that bonding occurs between complementary groups in apposing membranes, leading to *trans* membrane attraction. Theoretical studies have shown that membrane adhesion can take place above a critical concentration of such anchored sticker molecules and the observed critical concentration is in very good agreement with the theoretical predictions.<sup>28</sup> The observed phase separation of the bilayers on adhesion has also been theoretically predicted.<sup>29</sup> Thus the ASA- $C_{12}EO_7$  system can serve as a very good model to study membrane adhesion driven by specific interactions.

In conclusion, here we have introduced a novel amphiphile system that exhibits a temperature and concentration driven lamellar to coacervate transition. SAXS and cryo-SEM studies indicate that the coacervate is made up of bilayers; however, the detailed microstructure of this phase remains to be elucidated. The surfactant system presented here mimics ligand–receptor mediated adhesion of biomembranes. Our results suggest that the local morphology of these membranes could be modified by the high concentration of the adhesion molecules.

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## Notes and references

‡ The influence of PEG on the lamellar phase seen in the present system is very different from that reported in the case of lamellar phases of some charged surfactants, such as sodium dodecylsulphate (SDS) and sodium di-2-ethylhexyl-sulfosuccinate (AOT).<sup>14,15</sup> In the lamellar phase containing SDS high molecular weight PEG is found to lead to a  $L_\alpha$ - $L_\alpha$  coexistence over a narrow range of water content. On the other hand, PEG is found to be incorporated into the lamellar phase of AOT only for lamellar periodicities much larger or smaller than the radius of gyration of PEG; for intermediate periodicities a  $L_\alpha$ -isotropic coexistence is observed. However, in none of these cases does the polymer induces a collapsed phase and hence the mechanisms proposed in these contexts are not relevant in the present case.

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