

## Micellar growth in hexagonal phases of lipid systems

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Measurements are reported of the unit cell parameter  $a$  of the oleoyl-lyso-phosphatidyl-choline-water system in the hexagonal phase  $H$  as a function of the paraffinic volume concentration  $c_{v,\text{par}}$ . A functional behavior  $a \propto c_{v,\text{par}}^{-x}$  with  $x < \frac{1}{3}$ , has been derived. It is shown that this behavior characterizes micellar growth along the hexagonal domain, with nearly spherical micelles at the isotropic  $I$ - $H$  phase transition and practically infinite cylindrical micelles at the  $H$ - $Q^{230}$  (cubic bicontinuous, space group  $Ia3d$ ) phase transition. These results are compared with other systems for which  $x = \frac{1}{3}$  or  $\frac{1}{2}$  and with recent statistical-mechanical calculations for systems with self-association.

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Since the initial studies, the structure of the lyotropic liquid crystalline hexagonal  $H$  phase has been assumed to consist of "infinite" cylindrical micelles, packed in the plane perpendicular to the cylinder axes with a two-dimensional positional order [1,2].

However, the existence of finite rigid micelles has been recently demonstrated in the  $H$  phase of the sodium dodecyl sulfate (SLS)-water system [3]: after a direct isotropic  $I$ - $H$  phase transition, the hexagonal cell parameter  $a$  was found to vary with the volume concentration  $c_v$  as  $a \propto c_v^{-1/3}$ . For an  $H$  phase formed by "infinite" cylinders, it would be expected that  $a \propto c_v^{-1/2}$ . The  $\frac{1}{2}$  exponent was observed in the case of a deoxyguanosine derivative, which shows a cholesteric  $N^*$  phase intermediate between the  $I$  and  $H$  phases [4]. These results are in agreement with recent statistical-mechanical predictions for the liquid-crystalline phase transitions observed in reversibly self-assembling lyotropics [5-7]. In particular, exponents  $\frac{1}{2}$  and  $\frac{1}{3}$  are considered to be the fingerprints of respectively interparticle distance decreasing in a plane (long and flexible objects) and volume around particles decreasing in all three dimensions (short and rigid objects).

A detailed study of micellar growth in SLS in the vicinity of the isotropic to liquid-crystalline phase transitions [8] demonstrated that in this system micelles show only a small growth in the  $I$  phase. However, the question of micellar growth at the  $I$ - $H$  transition or within the domain of the  $H$  phase is still open.

In this paper, we analyze results obtained in the  $H$  phase in the oleoyl-lyso-phosphatidyl-choline (OLPC)-water system, which presents the sequence of phases  $I$ - $H$ - $Q^{230}$  (cubic bicontinuous, space group  $Ia3d$ ) [9,10]. The analysis of this system, and the comparison with previous results on SLS [3], provides evidence of the occurrence of micellar growth within the domain of the hexagonal phase.

OLPC was purchased from Sigma Co. (purity  $\sim 99\%$ ) and used without further purification. Controlled amounts of lipid and freshly bidistilled water were mixed

and kept at room temperature until equilibrium was reached. An Ital-Structures Co. 1.5-kW x-ray generator equipped with a Guinier-type focusing camera with a bent quartz monochromator was used. The samples were mounted in a vacuum-tight cell with thin mica windows; the cell was continuously rotated during the exposure in order to reduce spottiness. Measurements were taken at 20°C.

Figure 1 shows the variation of the hexagonal cell parameter  $a$  with  $c_{v,\text{par}}$ , which is the volume concentration of the paraffinic moiety given lipid volume concentration  $c_v$  times the volume fraction of paraffin in the OLPC molecule ( $c_{v,\text{par}} = 0.593c_v$ ). A good fit to the data is obtained with the equation

$$a = (44.7 \pm 0.1)(c_{v,\text{par}})^{-(0.226 \pm 0.002)}. \quad (1)$$

The experimental exponent is therefore quite distant from both  $\frac{1}{3}$  and  $\frac{1}{2}$ , which are theoretically expected [5-7] for "finite rigid" and "infinite flexible" rods, respectively. As reported above,  $\frac{1}{3}$  and  $\frac{1}{2}$  exponents were experimentally observed for SLS [3] and for a deoxyguanosine derivative [4], respectively.

In order to interpret these data, we assume that in the hexagonal phase the cylinders pack in a two-dimensional

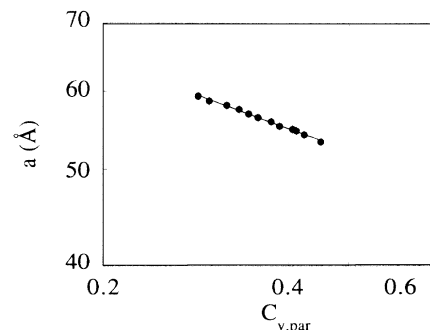


FIG. 1. Measured hexagonal cell parameter  $a$  as a function of paraffin volume fraction  $c_{v,\text{par}}$  with experimental fitting [Eq. (1)]. Both axes are in a logarithmic scale.

hexagonal cell with parameter  $a$ , and as a fluid in the third dimension (normal to the hexagonal plane) with an average distance  $C$  between micellar centers.

Let us consider initially a flat ended cylindrical micelle with radius  $R$  and average length  $L$ . The amphiphile-to-water volume ratio must be the same in the unit cell as in the whole sample, therefore

$$\frac{L}{C} = \frac{\sqrt{3}}{2\pi} c_v (a/R)^2, \quad (2)$$

where  $c_v$  is the volume fraction of the amphiphile (we shall in fact consider  $R$ ,  $L$ , and the volume concentration of the paraffinic core, since the polar region contains water [3]).

Considering a constant  $R$  value estimated by the length of the extended chain [10], it is possible to obtain from the experimental values ( $a$  and  $c_v$ ) the ratio  $L/C$  that gives essentially the fraction of water in the  $C$  direction.

For "infinite" cylinders, we expect  $L/C=1$  and  $a \propto c_v^{-1/2}$ . This condition is in fact reached for long flexible cylinders [7]. For finite hard cylinders, and using the condition of uniform decrease in interparticle distances in all three dimensions which comes from statistical mechanics arguments [5-7],

$$L/C = 2R/a, \quad (3)$$

we obtain [3,5]

$$a = 2R(2\sqrt{3}/\pi)^{-1/3} c_v^{-1/3}. \quad (4)$$

As discussed in a previous paper [3], the approach of flat ended cylinders may lead to inconsistencies in the case of finite objects. It is better in this case to consider a spherocylinder with radius  $R$ , cylinder length  $l$ , total length  $L=(l+2R)$ , and anisometry  $\mu=(l/2R)$ . The condition for the amphiphile-to-water volume ratio becomes then [instead of Eq. (2)]

$$\frac{L - (\frac{2}{3})R}{C} = \frac{\sqrt{3}}{2\pi} c_v (a/R)^2. \quad (5)$$

Rearranging in terms of the anisometry, we obtain

$$(L/C)_{sc} = \frac{\sqrt{3}}{2\pi} c_v (a/R)^2 \frac{(\mu+1)}{(\mu+\frac{2}{3})}, \quad (6)$$

where the index "sc" has been introduced to indicate that this ratio refers to spherocylinders. Compared with Eq. (2), which gives the  $L/C$  ratio for flat ended cylinders, Eq. (6) shows that  $(L/C)_{sc}$  grows with  $c_v$  increased by a factor that depends on the anisometry and reaches unity for very long spherocylinders.

Using the same condition of uniform decrease in interparticle distances in all three dimensions [Eq. (3)], results in [3,5]

$$a = 2R \left[ \frac{\pi}{2\sqrt{3}} \frac{(\mu+2/3)}{(\mu+1)} \right]^{1/3} c_v^{-1/3}. \quad (7)$$

If experimentally  $a = Ac_v^{-x}$  is observed, the anisometry can be obtained from

$$\mu = \frac{Kc_v^{(1-3x)} - 2/3}{1 - Kc_v^{(1-3x)}}, \quad (8)$$

where  $K = (2\sqrt{3}/\pi)(A/2R)^3$ . For spherocylinders with constant radius,  $K$  is a constant. If  $x = \frac{1}{3}$ , the spherocylinders have a constant average anisometry:

$$\mu = \frac{K - 2/3}{1 - K}. \quad (9)$$

If  $x < \frac{1}{3}$ , Eq. (8) gives a law for the growth of the spherocylinder with concentration. The condition

$$1 \geq (Kc_v^{(1-3x)}) \geq \frac{2}{3} \quad (10)$$

corresponds to  $\infty \geq \mu \geq 0$ . Therefore, the analysis of the function

$$B = Kc_v^{(1-3x)} \quad (11)$$

in the hexagonal domain, and particularly at transition points to neighboring phases, may clarify the process of growth of spherocylinders and the nature of the phase transitions.

We also stress that the failure of condition (10) (that would lead to  $\mu < 0$ ), must be interpreted as the failure of the admitted condition of uniform decrease in interparticle distances in all three dimensions [Eq. (3)]. As the spherocylinders become "infinite,"  $(L/C)_{sc}=1$  and Eqs. (3) and (8) are no longer valid. In fact, these equations are also no longer valid for long flexible spherocylinders.

Let us now analyze the OLPC results admitting  $R_{par} = (21.0 \pm 1.0) \text{ \AA}$ , as obtained from the projection of the extended chain length [11], which takes into account the bend of the hydrocarbon chain due to the double bond, and from comparison with molecular models.

Figure 2 shows  $2R/a$  and  $L/C$  obtained from  $a$  values and Eq. (2) plotted as a function of the paraffinic volume concentration  $c_{v,par}$ . As can be seen by comparing Eqs. (2) and (6), the correct  $(L/C)_{sc}$  value for spherocylinders is always larger than the value given by Eq. (2) (and shown in Fig. 2): the deviation increases as far as the spherocylinders become smaller. Thus,  $L/C < 2R/a$ , as for lower concentrations in Fig. 2, indicates short spherocylinders. But  $L/C > 2R/a$  indicates a breakdown of Eq. (3), as expected for very long and flexible spherocylinders: this seems to occur for the last three points, at the highest investigated concentrations, but the difference

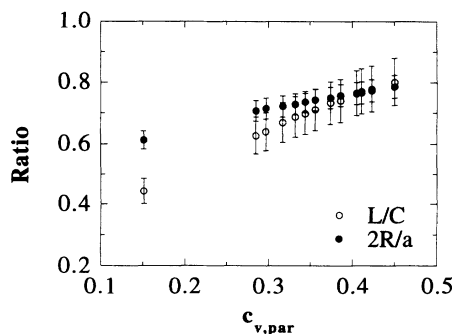


FIG. 2. Ratios  $L/C$  [Eq. (2)]—valid for flat ended cylinders and  $2R/a$  as a function of paraffin volume concentration  $c_{v,par}$ , giving the fraction of paraffin in the  $C$  direction and in the hexagonal two-dimensional plane. Errors are due essentially to the imprecision in the estimate of the  $R$  value.

is within experimental errors. Thus, there is no clear indication of deviations from Eq. (3) within the hexagonal domain. It should be noted that details of the shape of the particles, such as differences between cylinders and spherocylinders, might not accurately be captured by Eq. (3), making it not strictly correct.

Figure 3 shows function  $B$  and the corresponding anisotropies [Eqs. (11) and (8)] plotted as a function of  $c_{v,\text{par}}$ . These results give evidence of OLPC micellar growth along the hexagonal phase, with nearly spherical micelles at the  $I$ - $H$  transition and practically infinite micelles at the  $H$ - $Q^{230}$  phase transition. This conclusion is clear, even if both  $B$  and  $\mu$  are critically dependent on the  $R$  value adopted.

Let us now compare results for OLPC with previous data on SLS [3]. For the SLS-water system, a good fit to the  $a$  versus  $c_{v,\text{par}}$  curve was obtained with the exponent  $x = \frac{1}{3}$ ; leaving the exponent free, the data reported in [3] give  $x = (0.32 \pm 0.01)$ . Therefore, micelles are finite and have a constant or nearly constant anisotropy in the  $H$  phase, with no evidence of micellar growth within the  $H$  phase. The value of the SLS anisotropy was not determined in [3], due to its critical dependence on the  $R$  value adopted. Analyzing the data reported in [3], we notice that in the SLS system the condition  $L/C = 2R/a$  [Eq. (3)] is satisfied in the whole  $H$  domain for  $R_{\text{par}} = 17.4$  Å, a value about 4% larger than the length of the SLS extended chain. Such a value for  $R_{\text{par}}$  corresponds to a very large anisotropy,  $\mu = 106$ . Thus the picture for the SLS-water system would be of very long rigid micelles that behave as “finite” regarding water distribution in the three dimensions. In SLS, micellar growth would occur at the  $I$ - $H$  phase transition and not along the  $H$  domain. It is also to be stressed that the exponent  $x = \frac{1}{2}$  is expected for “infinite flexible rods” [7], and the flexibility is in fact responsible for the two-dimensional behavior of the interparticle distance. It is to be remarked that, in principle, a combination of flexibility (which increases the value of  $x$ ) plus micellar growth (which decreases the value of  $x$ ) could also account for the  $x = \frac{1}{3}$  value observed for SLS in phase  $H$ ; but in such a case Eq. (3) would be no longer exactly valid.

The results presented here for OLPC give clear evidence of micellar growth within the range of the  $H$  phase for a lipid system. Therefore, the main difference between SLS and OLPC is the very quick micellar growth which occurs at the transition for the SLS system (probably still in the coexistence region between  $I$  and  $H$  phases), while the OLPC is characterized by a slower growth along the range of existence of the  $H$  phase. We conclude that the growth in length does not seem to be a general property of the  $H$  phase, but is must be correlated with the characteristics of the lipid system.

Results for OLPC and SLS [8] can now be compared with recent statistical mechanical theories for systems with self-association [6]. Table I gives results for the  $I$ - $H$  transition in the SLS-water and OLPC-water systems, and for the  $I$ - $N$  (nematic) transition in the ternary SLS-water-decanol system. In Table I,  $c_v$  is the amphiphile volume fraction at which the transitions occur,  $\langle n \rangle$  is the average number of “monomers” or spherical micelles

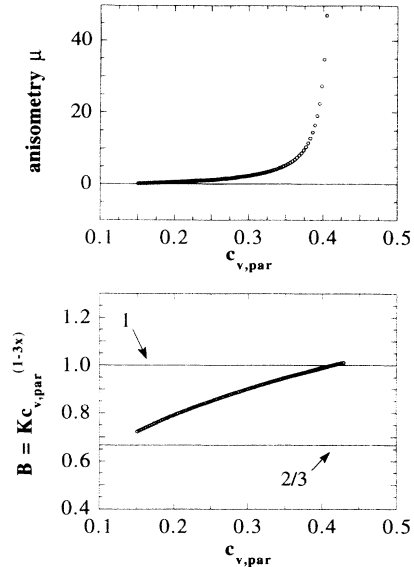


FIG. 3. Functions  $B = Kc_v^{(1-3x)}$  and anisotropy [Eq. (8)] as a function of lipid volume concentration  $c_{v,\text{par}}$ .

with radius  $R$  equivalent in volume to a spherocylindrical micelle of cylinder length  $l = 4(n-1)R/3$ , and  $\Phi$  is the free energy of association for “monomer-monomer” contact within the aggregate in units of  $k_B T$  (energy for “monomer fusion” in the micellar case). Values for the SLS systems have been derived from data reported in [8]. The experimentally determined  $(c_v, \langle n \rangle)$  pairs have been used to obtain  $\Phi$  values from [6]. Note that  $\langle n \rangle$  values for SLS correspond to experimental data obtained in the  $I$  phase just before the transition [8], while the  $\langle n \rangle$  value for OLPC has been obtained here in the  $H$  phase just after the transition.

By analyzing Table I, it appears that the theory developed for systems with self-association works reasonably well for the  $I$ - $H$  and  $I$ - $N$  transitions in the SLS systems. It explains the occurrence of the direct  $I$ - $H$  transition in the SLS-water system, and of the  $I$ - $N$  transition in the SLS-water-decanol system: the introduction of decanol increases the  $\Phi$  value, leading to a greater micellar growth in the  $I$  phase, and therefore entry into the  $N$  phase at a lower  $c_v$  value.

For OLPC, the  $\langle n \rangle$  value obtained falls between curves  $d$  and  $e$  of Fig. 3 in Ref. [6], and then it corresponds to a lower value of  $\Phi$ , as shown in Table I. However, it should be stressed that the  $I$ - $H$  phase transition for such an  $\langle n \rangle$  value should occur at a particle volume fraction ( $v_p$  in [6]) of about 0.45. In the SLS case, good agreement was obtained considering  $c_v = v_p$ ; but in reality

TABLE I. Amphiphile volume fraction  $c_v$  and average aggregate size  $\langle n \rangle$  for phase transitions in SLS [8] and OLPC systems, with the corresponding free energy of association  $\Phi$  [6] (see text for more details).

System	Transition	$c_v$	$\langle n \rangle$	$\Phi$
OLPC/water	$I$ - $H$	0.253	1.3	16
SLS/water	$I$ - $H$	0.343	2.4	19
SLS/water/decanol	$I$ - $N$	0.265	3.0	22

$v_p$  should include the volume of water bound to the micelle. For OLPC, it is clear that  $c_v$  underestimates  $v_p$ , showing that in this case the water effect is more important.

From [6] it appears that the smaller the  $\Phi$  value, the larger the cylinder growth after entering the  $H$  phase. But it should also be stressed that the theory, while working for the transition from  $I$  to liquid-crystalline phases, fails to account for transitions between liquid-crystalline phases at higher concentrations. Thus neither the  $H$ - $M$  (distorted hexagonal) transition in the binary SLS-water system, nor the  $H$ - $Q^{230}$  (cubic bicontinuous) transition in the binary OLPC-water system are predicted by the theory [6] developed to account for the  $H$  phase. Essentially the particular interactions with water and the special properties of the water-amphiphile interface have not been taken into account in the model [6], and these fac-

tors are very important in lyotropic systems, particularly in the highest concentration region.

The results obtained in the present study suggest that the entrance in phase  $Q^{230}$  is defined exactly by the "extra" micellar growth that occurs in the  $H$  phase for OLPC. SLS, that does not have such an "extra" growth, does not show the  $Q^{230}$  phase, entering instead in a hexagonal distorted phase with increasing  $c_v$ .

The method developed for analysis of micellar growth in the  $H$  phase may be applied to other systems, and may help to clarify the nature of phase transitions to and from  $H$  phases in lyotropic systems.

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- [1] V. Luzzati, M. Mustacchi, and A. Skoulios, *Nature* **180**, 600 (1957).
  - [2] V. Luzzati, M. Mustacchi, A. Skoulios, and F. Husson, *Acta Crystallogr.* **13**, 660 (1960); F. Husson, M. Mustacchi, and V. Luzzati, *ibid.* **13**, 668 (1960).
  - [3] L. Q. Amaral, A. Gulik, R. Itri, and P. Mariani, *Phys. Rev. A* **46**, 3548 (1992).
  - [4] L. Q. Amaral, R. Itri, P. Mariani, and R. Micheletto, *Liq. Cryst.* **12**, 913 (1992).
  - [5] R. Hentschke, M. P. Taylor, and J. Herzfeld, *Phys. Rev. A* **40**, 1678 (1989); *Phys. Rev. Lett.* **62**, 800 (1989).
  - [6] M. P. Taylor and J. Herzfeld, *Phys. Rev. A* **43**, 1892 (1991).
  - [7] R. Hentschke and J. Herzfeld, *Phys. Rev. A* **44**, 1148 (1991).
  - [8] R. Itri and L. Q. Amaral, *Phys. Rev. E* **47**, 2551 (1993).
  - [9] P. O. Eriksson, G. Lindblom, and G. Arvidson, *J. Chem. Phys.* **91**, 846 (1987).
  - [10] P. Mariani, V. Luzzati, and H. Delacroix, *J. Mol. Biol.* **204**, 165 (1988).
  - [11] C. Tanford, *J. Phys. Chem.* **76**, 3020 (1972); **78**, 2469 (1974).