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Double-Chain Cationic Surfactant and *n*-Pentanol: An L3 Phase in the Rich-Water Domain?

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A ternary system containing water, pentanol and a quaternary cationic surfactant, dioctadecyldimethylammonium bromide (DODAB) was investigated. We present the phase diagram and ESR studies that demonstrate the existence of the well-known L_3 or sponge phase in the water-rich domain of the diagram. The remarkable fact is the existence of some kind of order in such diluted conditions.

Introduction

Dioctadecyldimethylammonium bromide (DODAB) is one of the most investigated vesicle-forming cationic synthetic lipids.[1] Above its chain melting temperature (T_m), these double C18, very low soluble species spontaneously form closed stable bilayer structures[2−3] when dispersed in water. The thickness of vesicle membranes in the radial direction is bimolecular (3−5 nm), and they may have dimensions and structures similar to those of living cell membranes (∼1 μm).[4] In addition, they exhibit some functional characteristics of natural membranes such as permeability and the ability to accommodate some compounds. The presence of these compounds in the bilayer membrane affects properties such as viscosity, permeability, and curvature. Changing the composition, temperature, and preparation procedure, structures with different properties can be obtained for surfactant/cosurfactant/water systems.[5]

For swollen surfactant systems, two well-characterized phases exist, having a surfactant bilayer as their basic unit. The first, a lamellar phase (L_α), is a birefringent one-dimensional array of indefinite bilayers stacked with smetic order. The second, the sponge phase (L_3), in contrast, is an optically isotropic three-dimensional random multiple connected bilayer. Both were soon suspected to be a diluted phase of fluid bilayers.[6]

Since the pioneering observations of Per Ekwall, L_α and L_3 have been observed in a large number of systems, ranging from the simplest binary non-ionic surfactant solutions to the most complex system involving up to five components. The assessment of these structures is generally made by a combination of techniques such as neutron and X-ray scattering.[7]

Few studies exist on the behavior of standard organic compounds in bilayer vesicles in diluted colloidal domains (< 1 wt/wt amphiphile),[8−10] but instead most focus on relatively concentrated lipid phases.[11]

Here we report on phase evolution in the ternary system DODAB/water/pentanol in the water-rich corner of the phase diagram. With increasing amounts of *n*-pentanol, the following phases appear successively: vesicles (L_4), lamellar swelling (L_α), L_3, and a multiphase. The phases, characterized by a combination of techniques, have aggregate morphologies governed by the pentanol/DODAB ratio. This sequence has some analogy with other anionic surfactant−alcohol−water systems.[12−14] Evidence of the lamellar phase is currently under investigation.

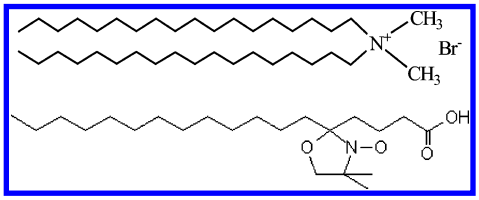

Figure 1. Synthetic cationic lipid DODAB and 5-SASL.

Experimental Section

Chemicals. DODAB (Figure 1a), with $a \geq 99\%$ and 5-doxyl stearic acid spin labels (5-SASL with the doxyl group at the fifth C-atom) (Figure 1b) were used as purchased from Sigma Chemical Co. Water (Milli-Q quality) and *n*-pentanol (spectroscopic grade; Merck), were used without further purification.

Phase Diagram. The three components were weighed in Falcon tubes with 50 mL of capability, gently stirring at 65 °C (above T_m) for 2 h and then cooled to 30 °C, the storage temperature. The phase behavior was determined by direct optical observations and by polarized light observation. All dispersions were prepared in a water-rich region (volume fraction of water > 99%). Equilibration for three weeks was necessary for an accurate phase boundary determination. Some samples were characterized by turbidity at 450 nm, in a UV/vis Perkin-Elmer Lambda 20 spectrometer.

Dynamic Light Scattering (DLS). Mean-average diameters (D) were carried out using Brookhaven equipment (Brookhaven Instruments Corp., Holtsville, NY), equipped with a 570-nm laser and DLS at 90°, for particle sizing. Samples were read without dilution, at 25 °C. Typically, five independent measure-

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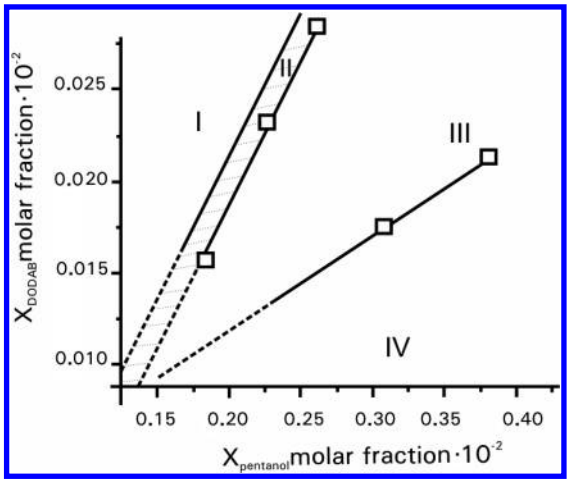

Figure 2. Phase diagram of the DODAB/*n*-pentanol/water system for a small surfactant concentration at 30 °C. Phase I: vesicles; phase II: assumed to be an L_α phase (under investigation); phase III: isotropic phase L_3; phase IV: multiphase.

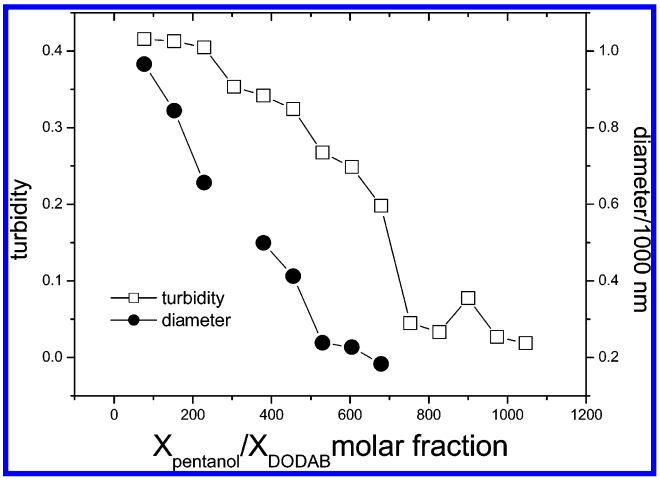

Figure 3. DODAB dispersion mean diameter (*D*) decreases in water as a function of the ratio between molar fractions of *n*-pentanol and DODAB $X_{pentanol}/X_{DODAB}$. In ratios higher than 700, the transparency of the samples prevents light scattering measurements. Circle: mean diameter (*D*) in nm; squares: turbidity at 450 nm.

ments were taken to obtain a mean hydrodynamic diameter and a mean variance. Data analyses were carried out by second-order cumulant analysis according to the software.

Electron Spin Resonance Spectroscopy (ESR). Measurements were performed with a Bruker EMX spectrometer with field-modulation amplitude of 1 G and microwave power of 5 mW. Temperature was controlled to ±0.2 °C with a Bruker BVT-2000 variable temperature regulator. The magnetic field was measured with a Bruker ER 035 NMR Gaussmeter. Spin-labeled sample preparations: films were formed from chloroform solutions, dried under a N_2 stream, and left under reduced pressure for at least 2 h to remove all traces of the organic solvent. Spin-labeled dispersions of DODAB were prepared by adding the amphiphilic dispersion to the dried film (to a final concentration of no more than 0.8 mol % of label relative to amphiphile, to avoid spin−spin interactions), and the sample was smoothly stirred for 5 min. ESR measurements were initiated within 1 h of the spin-label addition to the mixture dispersions.

Results and Discussion

Phase Diagram. A preliminary phase diagram characterization was done by changing the composition of the ternary system water/DODAB/pentanol. The water-rich region was initially mapped on the basis of visual inspection of the macroscopic properties (phase number, homogeneity, and birefringence) at 30 °C. Figure 2 shows a phase diagram where only molar fractions of DODAB and pentanol are shown. Four different phases were observed. The first (region I) is the well-known classical vesicles, obtained in the absence of alcohol. Preparations with higher alcohol content produce transparent samples (absorbancy measurements close to zero), which were stable for several weeks (region III). These morphological changes with increasing *n*-pentanol addition in a constant DODAB concentration can be observed in Figure 3: the variations of the apparent hydrodynamic diameter (DLS) and turbidity indicate very strong transformations in the size and shape of the structures. The diameter decreases to a limit value of ca. 200 nm, from which the transparency of the samples prevents any DLS measurements.

ESR. The ESR spectra yielded by the 5-SASL incorporated in DODAB vesicles (region I), in the isotropic phase (region III), and in DODAB vesicles saturated with pentanol, at three temperatures, are shown in Figure 4.

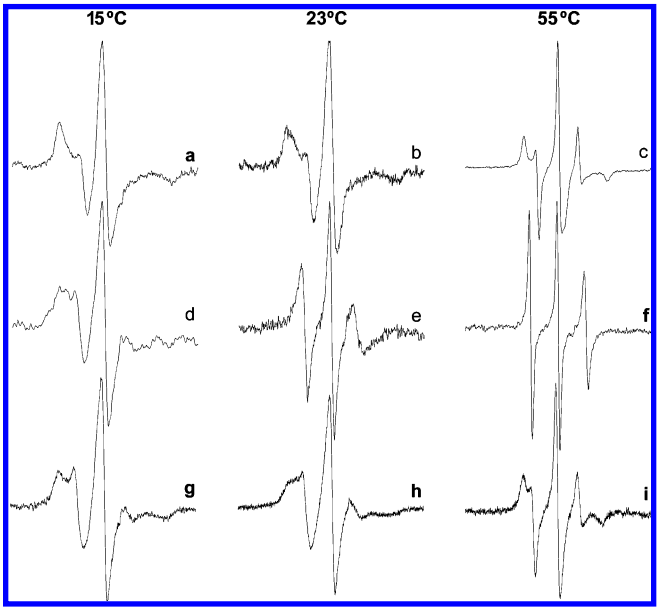

Figure 4. ESR spectra of 5-SASL incorporated in a DODAB aqueous dispersion: signals indicate the presence of lipid bilayer gel and fluid phases, both in DODAB vesicles (a and b, gel; c, fluid) and in the optically isotropic phase (g and h, gel; i, fluid). In the middle, for comparison, in a sample with 5 mM DODAB saturated with *n*-pentanol, relatively isotropic 5-SASL ESR signals are observed at 23 and 55 °C, indicating the presence of micelle-like aggregates.

The approach of comparing ESR spectra at three temperatures in defined compositions of the phase diagram allows the assessment of the degree of organization of DODAB chains in the membrane, the membrane bilayer fluidity state, and the occurrence of fluidity states transitions, an event possible only in ordered bilayers. Bilayers of DODAB in water at temperatures below 42 °C (T_m) are in a rigid gel state; in other words, the mobility of chains in the membrane is very low.[15] Above a given temperature T_m (melting temperature) DODAB chains have a higher mobility; the membrane is in a fluid state. The transition from gel to fluid can only be observed in organized systems, and cannot be detected in structures such as mixed micelles, for instance. The gel and fluid vesicle bilayer phases can be

well characterized by the ESR spectra of the 5-SASL incorporated in the membrane. At 15 and 23 °C (Figure 4a,b), the broad features of the 5-SASL ESR signals indicate the presence of a highly packed environment, typical of membranes in the gel phase.[15] At 55 °C (Figure 4c), the ESR is rather anisotropic, but with much narrower features, indicating fast movement for the probe, characteristic of the highly ordered but much less packed microenvironment of DODAB in the membrane fluid phase at the fifth C-atom position.[16] With isotropic samples, the 5-SASL signals at 15 and 23 °C (Figure 4g,h) are typical of a membrane gel phase, whereas the signal at 55 °C (Figure 4i) clearly indicates the presence of fluid bilayers.[17] In the middle (Figure 4e,f) turbid samples (DODAB saturated with pentanol), relatively isotropic 5-SASL ESR signals, at 23 and 55 °C, indicate the presence of micelle-like aggregates, probably DODAB/pentanol mixed micelles.

The combination of visual observations, turbidity, size particle measurements, and ESR signals of a probe allow us to assign Region III to the following characteristics: low viscosity, optical isotropy, and ordered surfactant arrangement. This group of properties points to an L_3 phase. The argument that pentanol is a reasonably water-soluble molecule, and thus could act as a cosolvent for DODAB, is not compatible with the highly packed environment of an ESR probe. Another important observation is that dilution of any sample with composition lying in Region III does not cause changes in its optical properties, indicating that the existence of this phase is governed by the ratio of DODAB/pentanol. When observed between crossed polarizers with gentle stirring, the phase displays a Tyndall effect. Samples with compositions lying in a narrow band (region II) show a subtle opalescence. Careful observation is necessary at this point because of the discreteness of the effects, which we believe is a consequence of the low surfactant concentration. Compositions lying in Region IV display multiphase behavior. It is possible to observe in the turbid samples skin-like structures, sediments, and many indications of the presence of several types of phases.

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References and Notes

(1) Carmona-Ribeiro, A. M. *Chem. Soc. Rev*. **2001**, *30*, 241.
(2) Feitosa, E.; Karlsson, G.; Edwards, K. *Chem. Phys. Lipids* **2006**, *140*, 66.
(3) Feitosa, E.; Barreleiro, P. C. A.; Olofsson, G. *Chem. Phys. Lipids* **2000**, *105*, 201.
(4) Laughlin, R. G. *Colloids Surf. A* **1997**, *128*, 27.
(5) Feitosa, R.; Bonassi, N. M.; Loh, W. *Langmuir* **2006**, *22*, 4512.
(6) Mahjoub, H. F.; McGrath, K. M.; Kléman, M. *Langmuir* **1996**, *12*, 3131.
(7) Porte, G. *J. Phys. Cond. Mat*. **1992**, *4*, 8649.
(8) Jung, M.; Hubert, D. H. W.; Van Veldhoven, E.; Frederik, P. M.; Blandamer, M. J.; Briggs, B.; Visser, A. J. W. G.; Van, Herk, A. M.; German, A. L. *Langmuir* **2000**, *16*, 968.
(9) Nagarajan, R. *Curr. Opin. Colloid Interface Sci*. **1997**, *2*, 282.
(10) Inoue, T. In *Vesicles*; Rosoff, M., Ed.; Marcell Dekker: New York, **1996**; Chapter 5, pp 151−195.
(11) Lohner, K. *Chem. Phys. Lipids* **1991**, *57*, 341.
(12) Benton, W. J.; Miller, C. A. *J. Phy. Chem*. **1983**, *87*, 4981.
(13) Gomati, R.; Appell, J.; Bassereau, P.; Marignan, J.; Porte, G. *J. Phy. Chem*. **1987**, *91*, 6203.
(14) Hoffmann, H.; Thunig, C.; Munkert, U. *Langmuir* **1992**, *8*, 2629.
(15) Yagle, P. L. *The Structure of biological membranes*, 2nd ed.; CRC Press: Boca Ratton, 2005; p 58.
(16) Benatti, C. R.; Feitosa, E.; Fernandez, R. M.; Lamy-Freund, M. T. *Chem. Phys. Lipids* **2001**, *111*, 93.
(17) Berliner, L. J. *Spin Labeling: Theory and Applications*; Academic Press: New York, 1976.