Phase Transitions

An Onion Phase in Salt-Free Zero-Charged Catanionic Surfactant Solutions**

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Mixtures of cationic and anionic (catanionic) single-chain surfactants can readily form bilayers in aqueous solutions,[1] in which uni- and multilamellar onion phases (the so-called vesicle phase) are often observed to be in equilibrium.[2] Since vesicles represent simple model systems for biological membranes and have practical applications (for example, for controlled drug or DNA release),[3] investigations of vesicle phases are of considerable interest in different areas, including surfactants, materials, and life sciences. Recently, two new self-assembled structures of controlled size (nanodisks and regular hollow icosahedra) were observed in dilute catanionic surfactants with H^+ and OH^- counterions by Zemb and coworkers.[4–6] Such so-called "true" catanionic systems, with a nonswelling but finite uptake of water, and with a spacing of the same order as described in the current study were studied and documented by Jokela et al.[7] It was also later established by Rand, Parsegian, and Leiken[8] that the lamellar phase at maximum swelling of salt-free catanionic systems with a zero osmotic pressure, that is, the repulsive hydration interaction is compensated by van der Waals force at that point. The molar ratio r of the anionic to cationic components controls the structural surface charge and, hence, controls the long-range repulsive interaction independently of the weight volume fraction (ϕ), which in turn controls the average colloid–colloid distance. The salt-free catanionic systems can be represented in a ternary phase diagram whose two independent variables are ϕ and r.[6]

Herein we report, for the first time to our knowledge, the discovery of a "true", salt-free concentrated catanionic uni- and multilamellar onion phase that differs from the catanionic surfactant systems with excess salt that are formed by the combination of the counterions, as evident from our freeze-fracture transmission electron microscopy (FF-TEM) observations and small-angle X-ray scattering (SAXS) measurements. This molecular catanionic couple comprises the longest hydrocarbon chains described to date, so it was essential to determine if the carbon chains were in a frozen (gel) or liquid state. The size of the unilamellar vesicles ranges from about 20 to 700 nm and that of the large onions are several micrometers. The interlamellar spacing between the bilayers of onions is about 35 nm, thus suggesting rather compact packing of the bilayers. The high osmotic pressure sustains the highly stable colloidal suspension of the catanionic onion phase. The observations of the onion phase may prove valuable and stimulating to fellow specialists, not least as "true" catanionic surfactant systems do not seem to be exhaustively investigated yet.

The "true" salt-free catanionic vesicle phase was obtained by mixing aqueous solutions of trimethyltetradecylammonium hydroxide (TTAOH) and oleic acid (OA; see Figure 3). The stock solution of TTAOH (pH 12–13) was prepared from the commercial bromide form (TTABr) by anion exchange with a strong base at 40°C until no bromide ions could be detected by precipitation with $AgNO_3$ in excess HNO_3. The resulting ion exchange with hydroxide ions was $>99\%$ (as determined from the detection limit of Br^- ions (Br^- sensitivity)).[9] The critical micelle concentration (cmc) of TTAOH was determined by surface tension measurements to be 1.8 mmol L^{-1}. The phase behavior on mixing 100 mM TTAOH with increasing concentrations of OA (up to 200 mM) was studied (not shown here). OA can dissolve in the aqueous solution containing TTAOH micelles. A single transparent, low viscous solution, which is the L_1 phase (spherical micelle phase), is evident at $c_{OA} = 0$–49.5 mM. The single transparent but viscous L_1 phase (rodlike micelles) is obtained between $c_{OA} = 49.5$ and about 62.5 mM; the viscosity of the samples in this region is much higher, which is evident since the surface of the L_1 phase could not be inclined on tilting the sample tube. A macroscopic phase separation into a birefringent $L\alpha$ phase on top of the viscous L_1 phase at the bottom of the

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tube occurs between 62.5 and approximately 90 mM. From $c_{OA} = 90$ to about 180 mM, a very stable, slightly turbid, bluish, and viscoelastic solution is seen. When $c_{OA} > 180$ mM, this birefringent Lα phase again separates into a two-phase emulsion/viscous layer. The upper phase is an emulsion phase and the lower phase contains the oleic acid component.

The birefringent Lα phase consists of both uni- and multilamellar vesicles, as shown in Figure 1 for two typical samples of TTAOH/OA by means of FF-TEM. The features of the vesicular structures in the two samples are apparent; uni- and multilamellar vesicles can both be seen throughout the samples. The diameters of the unilamellar vesicles, which range from about 20 to over 700 nm, are similar to those formed by single-tailed catanionic surfactants[10] and natural lipids such as lecithine.[11] The vesicles are completely dispersed in solutions and do not tend to associate with each other. Very large multilamellar vesicles with diameters of more than 3 μm can also be seen. The interlamellar spacing between the bilayers is rather small, in the range of about (36 ± 2) nm (Figure 1 c), thus suggesting quite compact packing of the bilayers. An important feature is evident in Figure 1 a and b: the multilamellar onion phase in the system containing excess cationic component (namely with positive charges; $r_{TTAOH}/r_{OA} = 100{:}94$) has less bilayers than those of ones with zero charges (at $r_{TTAOH}/r_{OA} = 100{:}100$), thus suggesting that the imbalance in the molar ratio of cationic and anionic surfactants does not favor the formation of multilamellar "onions" with quite a compact packing of bilayers.

Small-angle X-ray scattering (SAXS) measurements were carried out on one sample with the birefringent, viscoelastic Lα phase (containing 100 mmol L^{-1} TTAOH and 100 mmol L^{-1} OA), and clearly revealed the formation of lamellar aggregate structures (Figure 2). Three scattering peaks at $q = 0.18$, 0.36, and 0.54 nm^{-1} were observed. The relative peak positions $q_1{:}q_2{:}q_3$ are 1:2:3, which is typical for a Bragg scattering pattern from a one-dimensional lamellar structure (corresponding to the 001, 002, and 003 planes). The interlayer distance d ($= 2\pi/q_{max}$) was determined to be around 35 nm (Figure 3), which is completely consistent with the value obtained from the FF-TEM images. Such a lamellar structure has been commonly observed in mixtures of polyelectrolyte/surfactant complexes[12] and cationic/anionic surfactant systems[13] with excess salts; very few observations have been reported of such an onion-phase structure in salt-free catanionic systems. The observation of an onion phase in the salt-free catanionic surfactant system should open up new studies on such catanionic systems, and shed new light on the "true", salt-free catanionic onion phase. Meanwhile, the viscoelastic onion phase which contains no dispersed materials will find many applications where such salt-free viscoelastic phases are required with the morphology (spherical core-shell structure) obtained with our catanionic mixtures, for example, in producing mesoporous materials, to orient solubilized molecules, as well as in pharmaceuticals. The SAXS measurements clearly show that the bilayers of vesicles with zero charge have large spacing. This situation arises since electrostatic effects add to the hydration force to compensate for the attractive force and produce low osmotic pressure, probably about 1000 Pa—as reported by Meister et al.[14]

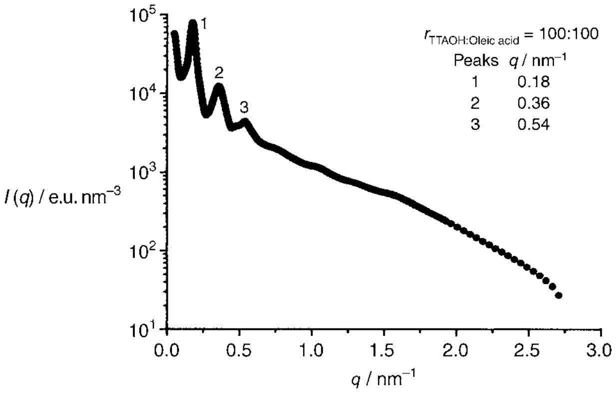

Figure 2. A SAXS profile of one Lα phase in the sample prepared from 100 mmol L^{-1} TTAOH/100 mmol L^{-1} OA. The scattering peaks for the TTAOH/OA mixture can be indexed to a lamellar structure.

Electrostatic and hydrophobic interactions are responsible for the formation of aggregates in all catanionic surfactant systems investigated in the literature. These catanionic surfactant systems contain dissociated small electrolytes from the counterions of both the anionic and cationic surfactants, which lead to some exclusive properties. For example, the mixed solutions have high ionic strength between the aggregates, electrostatic repulsions are screened,

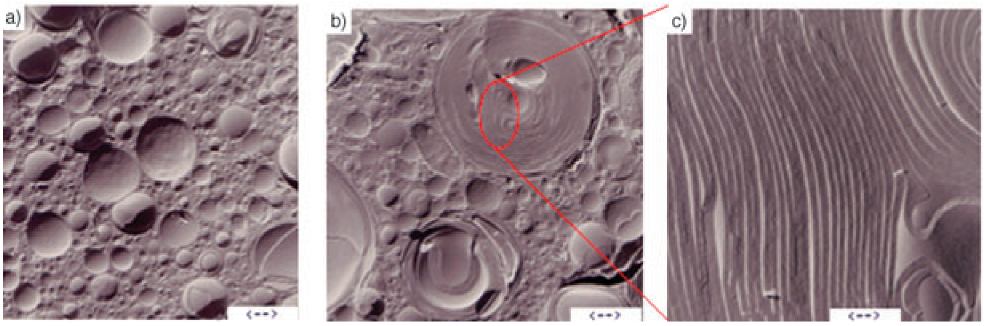

Figure 1. FF-TEM images of two samples: a) 94 mmol L^{-1} OA in 100 mmol L^{-1} TTAOH micellar solution, the scale bar corresponds to 0.350 μm, and b) 100 mmol L^{-1} OA in 100 mmol L^{-1} TTAOH micellar solution, the scale bar corresponds to 0.583 μm. c) Magnification of the multilamellar vesicles to determine the interlamellar spacing between two adjacent bilayers; the scale bar corresponds to 0.14 μm. The sample was frozen from room temperature in liquid propane cooled by liquid N_2: for further details see reference [2].

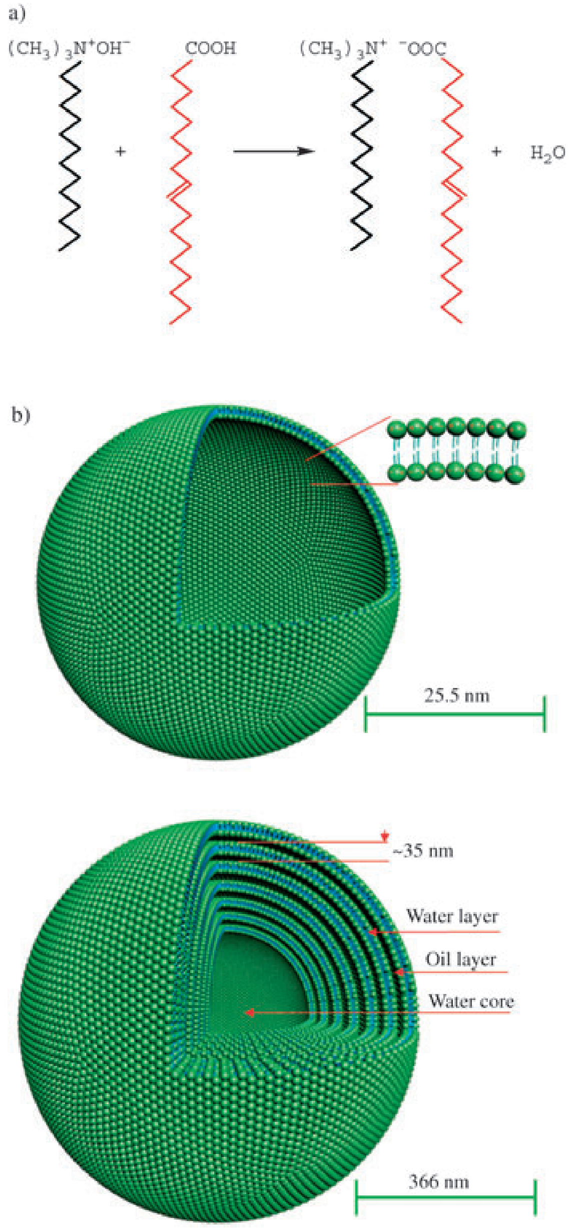

Figure 3. a) Formation of the salt-free catanionic surfactants. b) Models of unilamellar vesicle (top) with 25.5-nm radius and multilamellar vesicles (below) with 366-nm radius. The analysis of experimental data is shown for the multilamellar vesicles. The hydrophobic double-chain hydrocarbons are shown as blue rods and the hydrophilic-charged cationic-anionic groups as green spheres. A sector in each model has been cut out to enhance the visibility. Seven bilayers comprise the multilamellar onion phase.

and osmotic pressure of aggregates is < 100 Pa. Moreover, when mixing cationic and anionic surfactants in solutions, the strong reduction in area per head group resulting from ion pairing induces lamellar structures. Thus vesicles, flexible cylinders, as well as flat lamellae can be prepared and observed.[10,13]

In the current study, a low osmotic pressure is produced by unscreened electrostatic repulsion, which sustains the highly stable colloidal suspension, the "onion phase". However, it is quite different from the excess-salt catanionic surfactants, because solid surfactant precipitates do not form in the salt-free systems at relatively high surfactant concentrations (100 mmol$\,L^{-1}$, or 3–5 wt% surfactants) and a 1:1 cationic/anionic surfactant ratio (zero charge). Thus, the observation of an onion phase in the current study constitutes a salt-free concentrated catanionic surfactant system with the longest hydrocarbon chains having an unsaturated bond to date. Such a well-defined salt-free onion-phase system should be much more useful than the excess-salt catanionic surfactant systems, because of the interactions and effects controlling the assembly of the surfactants and/or the formation of different new surfactant phases, the more theoretically predictable behavior, and the fact that solid surfactant precipitates do not form at a 1:1 cationic and anionic surfactant ratio.

Experimental Section

Materials: Trimethyltetradecylammonium bromide (TTABr) was purchased from Acros Organics, New Jersey, USA and recrystallized three times from the mixed solvent of diethyl ether/ethanol. The TTAOH stock solution was prepared from the TTABr solution (120 mM) by anion exchange with a strong base (Ion exchanger III, Merck) at 40 °C. Bromide ions could not be detected by $AgNO_3$ in the TTAOH stock solution ($Ag^+ + Br^- \rightarrow AgBr\downarrow$), so the ion exchange with hydroxide is > 99%.[9] The critical micelle concentration (cmc) of TTAOH (0.0018 mol$\,L^{-1}$) was determined by using surface tension measurements. Oleic acid (OA) was obtained from Shanghai Chemical Co. with ionic impurities of sodium (0.1% molar fraction) and calcium (0.05% molar fraction). The fatty acids are quasi-insoluble in water, however, they can be mixed with TTAOH solution to provide clear solutions by heating to 40 °C.

Methods: The phase diagram of 100 mM TTAOH with the variable oleic acid concentrations was established by observing the solutions in test tubes at 25.0 ± 0.1 °C. The samples were homogenized by mixing and heating to about 40 °C for a few minutes, and then the hot solutions were cooled to room temperature with permanent shaking during cooling. The solutions were allowed to equilibrate for at least four weeks at 25.0 ± 0.1 °C and did not contain any trace of insoluble materials. All the experiments described were done at 25 ± 0.1 °C unless specified otherwise.

The microstructure of the samples with birefringence between the polarizers was examined by freeze-fracture transmission electron microscopy (FF-TEM). A small amount of sample (ca. 4 μL) was placed on a 0.1-mm thick copper disk covered with a second copper disk. The copper sandwich with the sample was frozen by plunging it into liquid propane which had been cooled by liquid nitrogen. A freeze-fracture apparatus (Balzer BAF 400, Germany) was used at a temperature of −140 °C for fracturing and replication. Pt/C was deposited at an angle of 45°. The replicas were examined with a Zeiss CEM 902 transmission electron microscope operated at 80 kV.

SAXS measurements were carried out at room temperature on a modified Kratky compact camera. The evacuated camera was mounted on a sealed X-ray tube equipped with a copper target. The scattering intensities were measured with a linear position-sensitive, gas-filled detector (Mbraun, Germany) by monitoring the scattering curves in the q range ($q = 4\pi/\lambda \sin\theta/2$, where θ is the scattering angle and λ is the wavelength of radiation). The sample solutions were injected into a 1 mm diameter quartz capillary mounted in a steel cuvette. The data collection time for each scattering curve amounted to about 15 h.

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[1] a) E. Sackman, R. Lipowsky, *Handbook of biological physics, Vol. 1B* (Ed.: A. J. Hoff), North-Holland, Amsterdam, **1995**; b) H. T. Jung, S. Y. Lee, E. W. Kaler, B. Coldren, J. A. Zasadziski, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 15318; c) H. T. Jung, B. Coldren, J. A. Zasadzinski, D. J. Iampietro, E. W. Kaler, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 1353.
[2] J. Hao, H. Hoffmann, K. Horbaschek, *J. Phys. Chem. B* **2000**, *104*, 10144.
[3] *Vesicles*, *Vol. 62* (Ed.: E. Rosoff), Marcel Dekker, New York, **1996** (Surfactant Science Series).
[4] Th. Zemb, M. Dubois, B. Demé, Th. Gulik-Krzwicki, *Science* **1999**, *283*, 816.
[5] M. Dubois, B. Demé, Th. Gulik-Krzwicki, J. C. Dedieu, C. Vautrin, S. Désert, E. Perez, Th. Zemb, *Nature* **2001**, *411*, 672.
[6] M. Dubois, V. Lizunov, A. Meister, Th. Gulik-Krzywicki, J. M. Verbavatz, E. Perez, J. Zimmerberg, Th. Zemb, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15082.
[7] a) P. Jokela, B. Jonsson, A. Khan, *J. Phys. Chem.* **1987**, *91*, 3291; b) P. Jokela, B. Jonsson, H. Wennerstrom, *Prog. Colloid Polym. Sci.* **1985**, *70*, 17; c) P. Jokela, B. Jonsson, B. Eichmuller, K. Fontell, *Langmuir*, **1988**, *4*, 187.
[8] a) R. P. Rand, V. A. Parsegian, *Biochim. Biophys. Acta* **1989**, *988*, 351; b) S. Leikin, V. A. Parsegian, *Annu. Rev. Phys. Chem.* **1993**, *44*, 369, and references therein.
[9] Q. Cai, Z. Guo, C. Yu, W. Zhang, Z. Yang, *Anal. Bioanal. Chem.* **2003**, *377*, 740.
[10] E. W. Kaler, A. K. Murthy, B. E. Rodriguez, J. N. Zasadzinski, *Science* **1989**, *245*, 1371.
[11] J. M. Gebicki, M. Hicks, *Nature* **1973**, *243*, 232.
[12] a) C. K. Ober, G. Wegner, *Adv. Mater.* **1997**, *9*, 17; b) D. G. Kurth, P. Lehmann, M. Schütte, *Proc. Natl. Acad. Sci. USA* **2000**, *99*, 5704.
[13] J. Hao, H. Hoffmann, *Curr. Opin. Colloid Interface Sci.* **2004**, *9*, 273, and references therein.
[14] A. Meister, M. Dubois, L. Belloni, T. Zemb, *Langmuir* **2003**, *19*, 7259.