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Catanionic Tubules with Tunable Charge[**]

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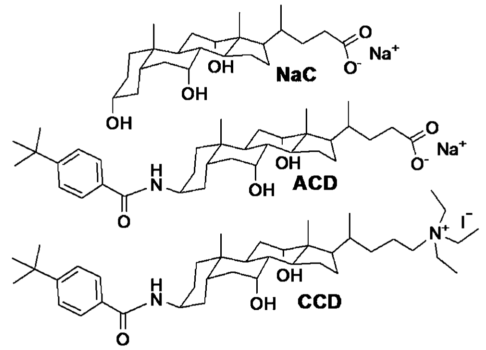
The three-dimensional structures with nanoscopic dimensions that are yielded by the self-assembly of lipids and surfactants are of particular interest for their applications in nanotechnology. In these applications, the possibility of controlling the charge of the particles allows the regulation of fundamental aspects, such as the ability of the particles to load molecules (drugs, DNA, proteins, etc.), to aggregate, and to penetrate membranes. Within the possible surfactant supramolecular architectures, tubular structures have recently drawn much research interest.[1] The main reason is that micro- and nanoscopic tubules have many interesting potential applications in nanotechnology, involving for example catalysis, selective separations,[2] sensors, electronic, electrochemical, and field emission devices,[3] tissue engineering,[4] and preparations of template nanostructured materials[5] or of prospective nanoscopic networks.[6] Because of this interest, several families of compounds have been studied that self-assemble in tubules,[7] for example phospholipids,[8] glycolipids,[9] peptides,[5,10] polymers,[11] bile salts,[12] and rationally designed amphiphiles.[13]

Herein, we report on the preparation of tubules in aqueous solutions of mixed cationic and anionic amphiphiles, whose compositions and charges can be tuned by controlling the stoichiometry of the mixtures. In particular, it was found that a very dilute mixture of anionic (ACD) and cationic (CCD) derivatives of the bile salt sodium cholate (Scheme 1) forms tubules over the whole range of the investigated anionic/cationic surfactant molar ratio. An interval within this wide range was found in which variation of the tubule composition and charge occurs.



Scheme 1. Structures of the anionic (ACD) and cationic (CCD) derivatives of sodium cholate (NaC).

Mixtures of anionic and cationic surfactants in water have been extensively investigated for many years;[14] however, the behavior described herein has never been observed. Commonly, in conditions similar to those adopted in this work, domains of cationic and anionic rich vesicles and micelles can be recognized in the phase diagram of these systems. Two-phase regions and precipitates around the equimolar composition are observed as well.[15]

Details of the syntheses of ACD and CCD are given in the Supporting Information. Two sets of mixtures of ACD and CCD were prepared with total surfactant concentrations (c_T) of 0.80 and 0.40 mM, as reported in the Experimental Section. In view of the thermal stability of the previously studied pure ACD tubules,[16] the samples were measured at 40 °C. The mixture composition is reported as molar fraction of each surfactant defined as $X_i = n_i/n_{tot}$ (where n_i and n_{tot} are the number of moles of the component i (CCD or ACD) and of the total surfactant, respectively).

The 0.4 and 0.8 mM solutions of neat ACD and CCD are transparent (Figure S1 in the Supporting Information). Transmission electron microscopy (TEM) images of these samples did not show any aggregate structure even after aging (3 months). Moreover, critical aggregation concentrations of around 0.4 mM were inferred for ACD and CCD at 40 °C by surface tension measurements (Figure S2 in the Supporting Information). These results suggest that the neat surfactants are present essentially as monomers or as monomers and few small aggregates in 0.4 and 0.8 mM solutions, respectively.

By contrast, the mixtures are slightly turbid, especially in the central region of the X_i values (Figure S1 in the Supporting Information). TEM images show that tubules are formed in these solutions, regardless of the mixing ratio, with

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diameters in the range 200–400 nm (Figure 1 and Figures S3 and S4 in the Supporting Information). In all samples, a wide distribution of the tubule length with values as large as 50–60 μm was observed. Apart from the tubules, no other structure was revealed in a significant amount.

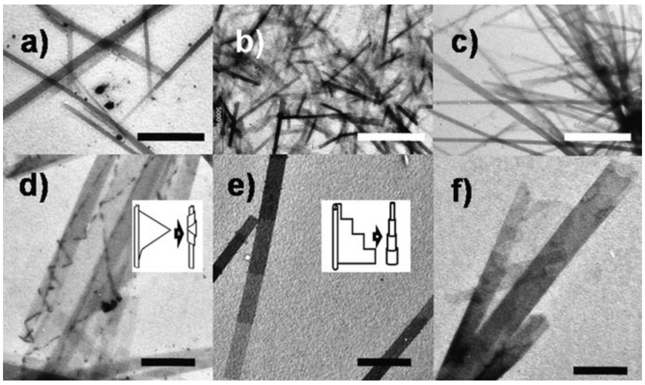

Figure 1. TEM images of mixtures at X_{CCD} values of: a) 0.7, b) 0.6, and c) 0.3 (c_T = 0.8 mM). Details of the tubules are reported in (d), (e), and (f). The insets in (d) and (e) show suggested layer arrangements for the respective tubules. The bars represent 5 μm in (a–c) and 1 μm in (d–f).

Most of the tubules have homogeneous walls. Images of partially assembled tubules point to a rolled layer structure (Figure 1 d,f). In some cases, a peculiar architecture resembling a telescopic structure was observed. For these structures, arrangements based on the rolling of layers with stair shaped borders can be hypothesized (Figure 1 e). Some tubules were also detected showing typical markers of the layer edges, as expected when a triangular layer is rolled along one of its sides (Figure 1 d). The various shown architectures were observed for all mixture compositions.

Scanning electron microscopy (SEM) images confirm the presence of tubules in the mixtures and their typical rolled layer structure (Figure 2 and Figure S5 in the Supporting Information). As shown later below, circular dichroism (CD) and optical microscopy measurements confirm the presence of tubules in the mixture solutions. However, because the images are collected from dried samples (see the Supporting Information), some drying effects on the tubule morphologies provided by TEM and SEM cannot be neglected.

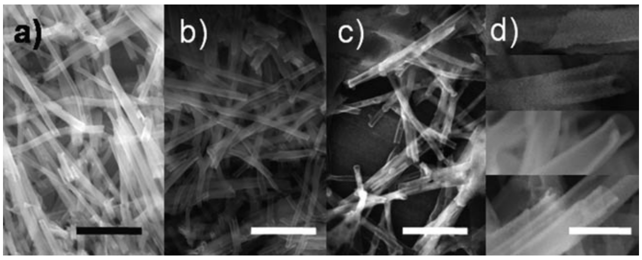

Figure 2. SEM images of the tubules of the mixtures at X_{CCD} values of: a) 0.4, b) 0.5, and c) 0.7 (c_T = 0.8 mM). Tubule details are reported in panel (d). The bars represent 2 μm in (a–c) and 500 nm in (d).

The electrophoretic mobility (μ) values, obtained by laser Doppler velocimetry (LDV) measurements on the mixtures, vary with the composition following a typical sigmoid pattern (Figure 3). At the extremes of the analyzed range of molar ratios, the mobilities are almost constant. For mixtures with $c_T = 0.8$ mM, at low CCD molar fractions ($X_{CCD} \leq 0.4$, region I), values within -5.8 ± 0.4 μm cm V^{-1}s^{-1} were obtained, suggesting the formation of negatively charged tubules, formed by a larger fraction of the anionic surfactant. Conversely, for samples rich in cationic derivative ($X_{CCD} \geq$ 0.8, region III), values within 3.2 ± 0.3 μm cm V^{-1}s^{-1} were found, pointing to the formation of tubules containing a larger fraction of the cationic derivative (positively charged). The invariance of the mobility values in each of the two regions suggests that tubules with constant composition and similar size are formed. The behavior of the electrophoretic mobility is different in the range $0.5 \leq X_{CCD} \leq 0.7$ (region II), where a progressive variation from negative to positive values of μ was observed with increasing CCD fraction, pointing to a variation of the tubule composition. The μ pattern is preserved in the mixture series at $c_T = 0.4$ mM.

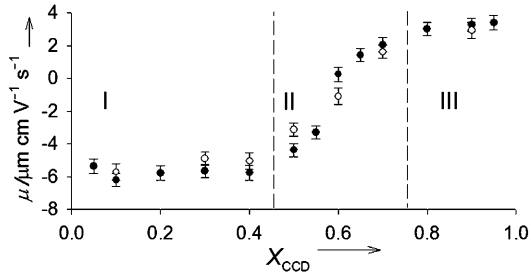

Figure 3. Electrophoretic mobility (μ) as a function of mixture composition at total surfactant concentrations of 0.8 (solid circles) and 0.4 mM (open circles). Composition regions separating the plateaus (I and III) and the variable region (II) of μ are outlined.

For a rough verification of the μ results, the electrophoretic migration was observed by optical microscopy inside a microscopy adapted cuvette for LDV measurements (Figure S6 in the Supporting Information). The results are described in the Supporting Information and recorded in the Supporting Information Movies S1–S3.

Typical CD profiles were recorded for the mixtures, which indicate a chiral arrangement of the chromophores in the tubules. The curves resemble those previously observed for the tubules of ACD at high concentration and ionic strength.[16] For the mixtures of regions I and III, the CD profiles are very similar in shape and vary only in their intensities. This result suggests that the packing of the two surfactants in the tubules does not change with the mixture composition, and only a variation of the tubule fraction takes place. In particular, an increase of the CD intensities was detected by increasing the fraction of the minority surfactant, suggesting that growth of the tubule fraction is promoted (see Figures S7 and S8 and the discussion of the CD Spectra in the

Supporting Information). UV profiles as a function of the mixture composition also support the interpretation of the CD evolution (see Figure S9 and the discussion of the UV Spectra in the Supporting Information).

Statistical analysis of the TEM images shows a correlation between the diameters and the compositions of the tubules (Figure S10 in the Supporting Information). The diameter distribution in each sample is wide and the standard deviations are about 50 nm. However, within each of the regions I and III, very similar average diameters were measured for the different mixtures (250 nm for region I and 350 nm for region III). Intermediate values were determined for the mixtures of region II.

To assay the tubule stability, the CD spectra were monitored over three months for the set of samples at $c_T = 0.8$ mM at 40 °C. Different evolutions of the spectra occurred depending on the mixture composition (Figure S11 in the Supporting Information). For $X_{CCD} \geq 0.4$, the CD pattern ascribed to the original tubules is roughly preserved. Accordingly, TEM images show that tubules remain in solution. At $X_{CCD} \leq 0.3$, a continuous variation of the curves takes place that is faster for lower X_{CCD} values. At the lowest X_{CCD} value (0.05), the variation seems to be complete, and an equilibrium state with a completely changed CD profile is reached. TEM images collected after storage of the samples show the presence of platelike structures with parallelogram shapes, probably obtained from unrolling of the tubule.[13] These structures coexist with tubules in the mixtures at $0.2 \leq X_{CCD} \leq 0.3$ (Figure S12 in the Supporting Information). It is important to remark that, in all the solutions in which the evolution to platelike structure is observed after aging, the tubules can be re-formed by repeating the heating–cooling process described in the sample preparation (see the Experimental Section).

Despite the large number of catanionic mixtures reported so far, no data are available on systems of anionic and cationic forms of bile salts and derivatives. These amphiphiles represent very interesting building blocks for supramolecular aggregates, since they have a structure that is very different from that of conventional surfactants. In fact, because of their structures, the bile salt self-assembly cannot be explained on the basis of the conventional geometric rules of surfactant packing, and it is expected that they aggregate in very peculiar and ordered arrangements.[16] In the mixture of ACD and CCD, the tubular structure probably results in a very stable arrangement of the surfactant molecules in a well-defined packing order similar to crystal packing, leading to the formation of tubules at all the mixture compositions. The packing stability implies a favorable interaction between the *tert*-butylphenyl amide substituted steroid skeletons and the charged heads. The latter is achieved when comparable fractions of the two surfactants are involved in the tubule formation. Accordingly, regardless of the mixture composition, the ratio of the two derivatives in the tubule is expected to change in a narrow interval of favorable values. This result could explain the fact that, in the presence of a large excess of one of the two derivatives (regions I and III), positively or negatively charged tubules at a fixed composition are formed. The surfactant molar ratios in these tubules are expected to be the richest in ACD (region I) or in CCD (region III), for which a favorable packing energy is still fulfilled. Conversely, in region II, the tubule surfactant ratio changes within the favorable values, thus determining the charge variation. In this range of compositions, tubule stability is preserved because the two bile salt based amphiphiles are completely identical, except for the charge heads, and the variation of the charge involves small changes of the anionic/cationic head ratio.

Our results suggest that new catanionic structures can be prepared by mixing anionic and cationic forms of rigid nonconventional amphiphiles. In this particular case, tubules with tunable charge were obtained that could show interesting properties for applications such as the controlled loading of charged molecules and macromolecules by changing the mixture composition.

Experimental Section

Sample preparation: The two sets of mixtures with c_T values of 0.80 (0.047–0.060 wt %) and 0.40 mM (0.023–0.030 wt %) were prepared by mixing the relevant amounts of ACD and CCD solutions (0.9 mM and 0.5 mM for the two different sets) directly in vials and adding water to give the final concentrations. Slightly turbid samples were obtained, which were heated until they became transparent (ca. 00 °C for 10 s) to guarantee the best surfactant mixing, and then cooled to 40 °C, at which they again became turbid (Figure S1 in the Supporting Information). TEM and CD results show that the tubules form already upon mixing, although they are shorter and in lower amounts (Figure S13 in the Supporting Information) and are probably broken and spontaneously re-formed in the heating–cooling process. The solutions were kept at 40 °C for 1 h before the measurements were performed (details are reported in the Supporting Information). The presence of 10 wt % of water in solid CCD, determined by thermogravimetric measurements, was taken into account in the preparation of the solutions to the desired concentration.

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