Novel catanionic vesicles from calixarene and single-chain surfactant†

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The mixed system between *p*-sulfonatocalix[4]arene and tetradecyltrimethylammonium bromide forms unilamellar vesicles after sonication of the aqueous dispersion. Furthermore these vesicles can be stored, without use of lyoprotectants, by lyophilization and then rehydration without change in size or shape.

The construction of well-defined structures in the nanometre or micrometre length scale based on molecular self-assembly is one of the most important challenges facing modern chemistry. For instance, noncovalent interactions have been used to obtain a wide-range of structured aggregates such as tubules, fibers, micelles, vesicles, and disks through molecular self-assembly of small organic compounds.[1]

Mixtures of anionic and cationic surfactants (catanionic mixtures) offer an attractive approach for the construction of complex self-assembled nanostructures. The formation of spontaneous vesicles in mixtures of oppositely charged surfactants was first demonstrated by Kaler[2] and since then, intense research has been devoted to the study of self-assembled structures formed in catanionic surfactant systems.[3] Globular micelles, cylindrical micelles, long threadlike micelle, discs, and large lamellar sheets have also been observed in some of the aqueous cationic–anionic systems. The molecular assemblies formed in these systems are mainly attributed to a strong electrostatic association modulated by chain packing interactions, which generally result in a reduced head-group area promoting a dense packing of surfactant molecules in the aggregate.

We have recently demonstrated that when the anionic surfactant is replaced with a non-aggregating and surface inactive hexamethylated *p*-sulfonatocalix[6]arene (SC6HM) the micellization of single chain trimethylammonium amphiphiles is promoted at concentrations below the critical micelle concentration (cmc) of neat surfactant.[4] It was suggested that the complexation of the cationic surfactant with SC6HM yields a species with surface active properties that induce aggregation at lower concentrations than that of neat surfactant. Motivated by this observation, we decided to study the mixed system formed by the most common *p*-sulfonatocalix[4]arene (SC4) with tetradecyltrimethylammonium bromide (TTABr).

SC4 is a well known receptor for organic ammonium cations in water[5] and displays especially strong binding abilities for these guests due to their π-rich cavities and to five negative charges at pH 7. Though ammonium cations are typical guests for SC4 hosts, reported studies on the complexation of alkylammonium cations are scarce.[6]

In contrast to SC6HM, when 2 mM of SC4 is mixed with TTABr, we observe the formation of a white dispersion at TTABr concentrations between 0.1 mM and 50 mM, and a precipitate zone near the charge neutrality. Below and above this gap a clear solution is observed. Since this last behavior is common in some catanionic systems, we carried out further experiments to identify the aggregates formed in that region. In the literature, it is known that the mixture between the above calixarene and some biorelevant molecules, such as aliphatic amines, polyamines and amino acid isomers, is organized in bilayer-type structures in the solid state.[7] In this work we study the host–guest system in liquid state and the use of conventional amphiphilic surfactants, which increase the number of molecules that can form bilayer structures with the calixarene.

When a milky dispersion of 50 mM SC4/TTABr with molar ratio 1 : 2.5 was examined under Nomarski light microscopy between glass and cover slide, a high concentration of giant vesicles (0.5–5 μm) was visible (Fig. 1), with the smaller ones in fast Brownian motion. The presence of these very large vesicles is the reason for the white opaque appearance of the dispersion and was detected throughout the 0.1–50 mM range.

In the diluted region, after sonication of a dispersion containing 2 mM of SC4 and 5 mM of TTABr, the sample was studied by dynamic light scattering (DLS) and transmission electron microscopy (TEM). As shown below our results are

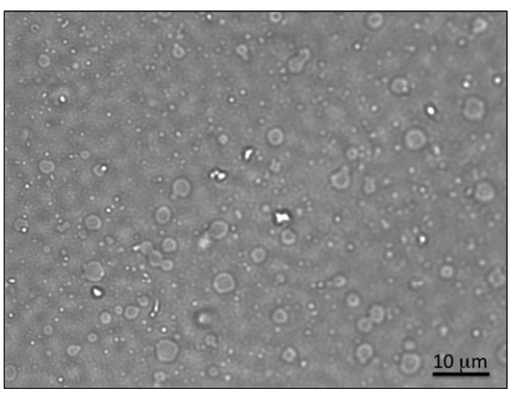

Fig. 1 A light micrograph of a 50 mM white dispersion of SC4/TTABr with a molar ratio 1 : 2.5, showing the presence of polydisperse and very large vesicles. The smallest visible vesicle appears in Brownian motion.

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compatible with the presence of unilamellar vesicles in the solution. When the aqueous mixture of components in the sample is examined by DLS two relaxation modes are observed: a fast mode at short relaxation times, related to the diffusion of large aggregates, and (within the low experimental precision because of bad statistics for such slow fluctuations in a limited sampling time) a slow mode at long relaxation times which is independent of the scattering vector q, indicating that it is a "viscoelastic" mode. The average diffusion coefficient of the vesicles is obtained by fitting the angular dependence relaxation times of the fast mode, $D = (4.29 \pm 0.05)\ \mu m^2\ s^{-1}$, which corresponds to an average hydrodynamic radius of *ca.* 57.2 ± 0.7 nm (see the ESI†).

The size distribution was also investigated by TEM, and the experimental results are in agreement with the average size obtained by DLS, showing that the vesicles are generally smooth and spherical (see Fig. 2). We have also measured the charge of these vesicles in Z-Sizer equipment and a ζ-potential about -23 ± 5 mV was obtained at 25 °C. This value met our expectations since we worked with an excess of negative charge due to the calixarene. The diameter obtained by the Z-Sizer is 135 nm and it is in accordance with TEM and DLS data.

To obtain more information on the structure of the aggregates NMR spectra of neat SC4, neat TTABr and mixed vesicles were performed. The assigned chemical shifts are listed in Table 1. The terminal protons of the surfactant alkyl chain are unaffected in the complex. However, in contrast, the $N(CH_3)_3$ and the protons bonded to the alpha carbon (C_α) show large changes in chemical shifts, compatible with an inclusion complex where the surfactant polar head is located in the aromatic cavity of the calixarene (Scheme 1). In Scheme 1 the calixarene is represented in the simplified shape, "cone" conformation, but the ^{1}H NMR spectrum indicates that the calixarene is exchanging rapidly in the NMR time-scale between several possible conformations, since the $ArCH_2Ar$ methylene protons give one singlet.

In order to study the stability of the vesicles, the evolution of the relaxation time spectra was studied by DLS (see the ESI†). No change was observed within 4–5 days from preparation; however, after 7 days, a new relaxation mode is observed at longer times than the fast diffusive mode. The relative amplitude of the new mode increases, while that of the corresponding fast mode decreases; this feature is an indication that the vesicles are either coalescing and growing in size or flocculating.

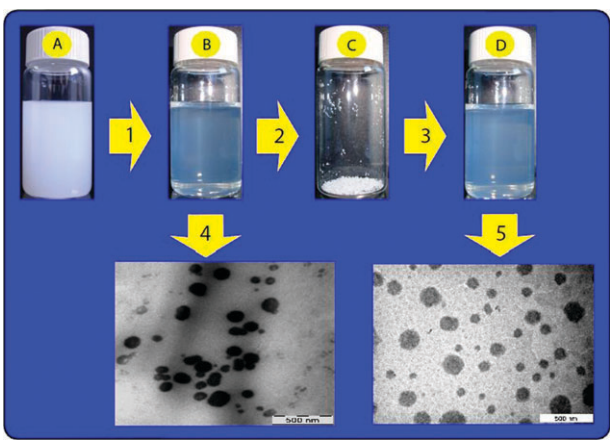
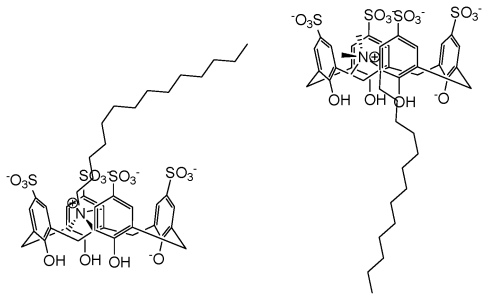


Fig. 2 Schematic representation for vesicle storage method and rehydration. Steps 1, 2 and 3 correspond to sonication, lyophilization and rehydration, respectively. The TEM images (negatively stained 2% phosphotungstic acid, pH = 7) are before step 4 and after the lyophilization (step 5).



Scheme 1 Two possible inclusion modes of TTABr in SC4.

Table 1 Chemical shift changes ($\Delta\delta$, ppm) for the inclusion complex formed between the surfactant TTABr and the *p*-sulfonatocalix[4]arene. Negative values indicate up-field shift

	ArH aromatic	$ArCH_2Ar$	RCH_3	$RCH_{2\alpha}$	$N^+(CH_3)_3$
p-SC4	7.58	4.01	—	—	—
TTABr	—	—	0.87	3.41	3.17
Complex	7.61	4.04	0.85	2.15	1.31
$\Delta\delta$	0.03	0.03	−0.02	−1.26	−1.86

This behavior is quite usual since often the high curvature vesicles are metastable aggregates and consequently, the size distribution evolves with time to larger structures (*e.g.* lamellar sheets). The initial aqueous dispersions may even show phase separation. In addition, chemical or biological degradation may also develop. Due to this issue, dispersions that contain vesicles must be freshly prepared just prior to use. Since this process is often poorly defined and difficult to control,[8] this procedure presents some disadvantages (*i.e.*, when preparing liposome/DNA complexes).

To circumvent colloidal instability and/or avoid degradation and to allow for long-term storage of vesicles, water may be removed through the most common and frequent method to dehydrate, that is the freeze-drying technique.[9] The complete process to stabilize and store our vesicles can be summarized in the following four points: (A) *p*-Sulfonatocalix[4]arene (SC4) and tetradecyltrimethyl-ammonium bromide (TTABr) are dissolved in water yielding a whitish dispersion. (B) The aqueous cloudy dispersion is sonicated for approximately 30 min, and a homogenous clear solution is obtained. (C) The solution is then frozen with liquid nitrogen, causing ice crystals to nucleate and grow. Sublimation of ice yields the freeze-dried powder. (D) The dried power is rehydrated again with water (Fig. 2).

After sample lyophilization (step C), a white powder is obtained that completely redisperses in water forming again the vesicles, without any need of sonication. Usually simple hydration of the dried vesicles powder does not completely redisperse them in water, but produces a mixture of suspended vesicles and larger aggregates and therefore requires the sample to be sonicated again.[10]

Consequently we can deduce that the structure of vesicles formed when the solution is sonicated for the first time maintains their organization when water escapes and later enters the structure again. Also we can conclude that this process does not significantly change the size of the vesicles as one might expect. Usually the conventional vesicle structure is lost during the freeze-drying process, and as a result the use of carbohydrates is introduced.[11] The sugar coating on the surface of the vesicles results in a low molecular mobility, which minimizes damage caused by the fusion process or crystal formation after drying.[10] To confirm that these vesicles do not need any cryoprotectant we have performed a new set of DLS measurements after rehydration (see the ESI†). From the linear fit, the average diffusion coefficient obtained is $D = (3.44 \pm 0.06)\ \mu m^2\ s^{-1}$, which corresponds to an average hydrodynamic radius of 71 ± 1 nm. This value confirms that when these vesicles are lyophilized and hydrated again they do not yield the thermodynamically preferred lamellar phase domains. With respect to the process of water leaving and entering the mixed amphiphilic film, the effect cannot be considered very remarkable since the water permeability of usual lipids is very high, for instance, almost 10 orders of magnitude larger than that of sodium ion.[12] We can derive from this fact that the inclusion complex between *p*-SC4 and TTABr does not significantly change the transport of water across the vesicle bilayer.

This work shows a new type of catanionic vesicle, of which the principal feature is its potential or ability to be stored and rehydrated on demand without any significant change in size. Further investigations need to be done related to other water-soluble calixarene and surfactants, as well as in the combined properties of calixarenes as macrocyclic hosts and self-organizing systems able to form vesicles. The lack of toxicity and immune response of calixarene derivates enable new applications of these macrocycles in biomedical and pharmaceutical sciences.[13]

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