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Calixarene-Based Surfactants: Evidence of Structural Reorganization upon Micellization

Nuno Basílio, † Luis Garcia-Rio, *,† and Manuel Martín-Pastor ‡

[†]Departamento de Química Física y Centro Singular de Investigación en Química Biológica y Materiales Moleculares (CIQUS) and [‡]Unidad de Resonancia Magnética (RIAIDT), Universidad de Santiago, 15782 Santiago, Spain

Supporting Information



ABSTRACT: The self-aggregation of five amphiphilic *p*-sulfonatocalix[*n*] arenes bearing alkyl chains at the lower rim was investigated by NMR spectroscopy and electrical conductivity. The critical micelle concentration was determined, and the tendency of this special class of surfactants to self-aggregate in aqueous solution was analyzed as a function of the alkyl chain length and the number of aromatic units in the macrocyclic ring. The structure of the surfactants in the monomeric and micellized states was elucidated by means of ¹H NMR and, in the case of the calix[6] arene derivative, with 2D NMR experiments. While all amphiphilic calix[4] arenes studied here are blocked in the cone conformation, in the monomeric state the calix[6] arene adopts a pseudo-1,2,3-alternate conformation and the calix[8] arene is conformationally mobile. These calixarenes undergo an aggregates were studied by diffusion ordered spectroscopy (DOSY) experiments, and the results indicate that these surfactants self-assemble into ellipsoidal micelles.

■ INTRODUCTION

Calix[n] arenes¹ are macrocyclic oligomers made of phenol units linked by methylene bridges. They can be readily functionalized at the phenolic hydroxyl groups (lower rim) and at the para positions (upper rim) and give rise to wide range of structural derivatives. Research on calixarene chemistry was initially directed to the design of host compounds with receptor abilities. Depending on the functional groups introduced on the calixarene framework, these compounds can display high affinity and selectivity for the inclusion of neutral, cationic, or anionic guests. However, the versatility of the calixarenes is not limited to host–guest chemistry, and when properly functionalized, they can be applied in diverse areas such as self-assembly on monolayers² and nanoparticles,³ formation of rotaxanes and molecular machines,⁴ dendrimers,⁵ and surfactants.⁶

Calixarene-based surfactants can be obtained by introducing hydrophilic groups at the upper or lower rim and hydrophobic groups (generally alkyl chains) at the opposite rim.^{7–16} Shinkai et al.¹⁰ showed that the conformation adopted by the calixarene is a crucial parameter in modulating their aggregation behavior and suggested that the cone shaped conformation is ideal for the formation of globular micelles. However, the conformation is not the only factor that determines the type of self-assembled aggregates formed from amphiphilic calixarenes. The nature of the hydrophilic headgroup is also important to control the aggregation behavior of this type of amphiphile, and it was showed that calixarenes in the cone conformation can also form vesicles.^{11,13,16}

When compared with conventional surfactants, amphiphilic calixarenes have lower critical micelle concentration (cmc) values and tend to present exchange rates between monomers in bulk solution and in the micelles several orders of magnitude slower than that observed for the formers,^{14,15,17} though these properties are not yet well understood. Furthermore, calixarene-based amphiphiles can display special properties such as self-assembling into completely uniform and structurally persistent micelles, as reported by Hirsch's group.¹²

Among the several calixarene-based surfactants described in the literature, *p*-sulfonatocalix[*n*] arenes bearing alkyl groups at the lower rim were the first to be described and are probably the most studied.^{7-9,17} As recognized by Shinkai et al.,⁹ the aggregation properties can be significantly affected by the calixarene architecture, being the length of the alkyl chain the most important factor contributing to the variations on the aggregation

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characteristics of these amphiphiles. Accordingly, these calixarenes (along with other derivatives) were classified⁹ into three categories: (i) nonmicellar calixarenes: those calixarenes that do not bear long alkyl chains (e.g., CH₃) do not forms micelles; (ii) micelle-forming calixarenes: those with moderate alkyl chain length (e.g., hexyl) self-aggregate into micelles; and (iii) unimolecular micelle calixarenes: calixarenes that possess long alkyl chains (e.g., dodecyl) behave as a unimolecular micelle. (The authors suggested that these calixarenes are stable in water as a monomer or oligomer and proposed that a "single molecule having six dodecyl chains can form a stable, micellelike closed shell by itself".) This classification was suggested on the basis of measurements of the critical micelle concentrations (cmc) by surface tension, conductance, and spectroscopic methods. The micellization of calixarenes in the categories ii and iii was confirmed by a small-angle X-ray scattering study.¹⁸ Ise et al. found that *p*-sulfonatocalix[6]arene hexahexyl ether (SC6HH) forms ellipsoidal micelles with aggregation numbers that varies between 7 and 15 depending on the concentration. However, in the same work it was also estimated that *p*-sulfonatocalix[6]arene hexadodecyl ether self-aggregates into micelles with aggregation numbers higher than 80 which is definitively not compatible with the existence of unimolecular micelles. It was suggested by the authors that the different results could be attributed to the higher concentration used in their work in comparison with that used in the work developed by Shinkai et al.7

To get more in depth into the structure–aggregation relationships of surfactant-based calixarenes, in this work, we characterized the self-assembly process of five *p*-sulfonatocalixarenes (Scheme 1) with different alkyl chain length and number

Scheme 1.	Calixarene-Based	Surfactants	Used	l in T	This	Stud	y
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of phenol units in the macrocyclic ring. The cmc and the degree of counterion binding (β) were determined by electrical conductivity experiments, and ¹H NMR spectroscopy was used to investigate possible conformational changes induced by the aggregation process. Finally, the determination of the diffusion coefficients of the calixarenes both below and above the cmc, using diffusion ordered NMR spectroscopy (DOSY) experiments, provided information about the type and geometry of the aggregates present in solution.

EXPERIMENTAL SECTION

All commercial reagents were of the highest purity available and used as received. *p*-Sulfonatocalix[*n*]arenes (SCn) were prepared by ipsosulfonation of the correspondent *p*-tert-butylcalix[*n*]arene in H_2SO_4 at 80 °C. Amphiphilic calixarenes were synthesized from the reaction of SCn with the corresponding alkyl bromide in basic media following literature procedures.⁷ The products were characterized by ¹H NMR and MALDI-TOF mass spectrometry (see Supporting Information). ¹H NMR, COSY, ROESY, and DOSY spectra were recorded at 25 °C in a Varian Inova 400. The DOSY spectra were acquired with the standard stimulated echo pulse sequence using LED and bipolar gradients pulses.¹⁹ Rectangular-shaped pulse field gradients of duration 2 ms were applied with a power level linearly incremented from 2.1 to 64.3 G cm⁻¹ in 20 steps. The gradient power was previously calibrated with the same DOSY experiment with a reference sample of 99% D₂O at 25 °C ($D = 1.872 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$). To obtain reliable results of the diffusion coefficient, the diffusion time Δ of the experiment was optimized for each sample to a value between 60 and 200 ms so as to capture smoothly the attenuation of the signal intensity, while affording the maximum difference in intensity between the traces with the maximum and minimum gradient power. The raw data were processed using the MestreC program (Mestrelab Research Inc.).

Electrical conductivity was measured by using a Radiometer CDM3 conductivity meter with a cell constant of 0.968 cm⁻¹. The conductivity meter was calibrated with two KCl conductivity standard solutions (0.0100 M, with $\kappa = 1413 \ \mu S \ cm^{-1}$ at 25.0 °C; and 0.100 M, with $\kappa = 11.28 \ m S \ cm^{-1}$ at 25.0 °C) supplied by Crison. The temperature was kept constant to within ± 0.1 °C by passing thermostated water through a jacketed vessel holding the solution.

All solutions used in the NMR experiments were prepared in D_2O (99.9%) while the solutions used for conductivity measurements were prepared in deionized and doubly distilled water.

For the molecular modeling calculations an initial model of the SC6HH calixarene in the pseudo-1,2,3-alternate conformation having the alkyl chains completely extended and outside the calixarene cavity was built with ChemOffice 2004 modeling software (CambrigeSoft Corp.). This initial model was exported for refinement with the modeling software Vega-ZZ v.2.4²⁰ using molecular mechanics and dynamics calculations. The module NAMD²¹ incorporated in the Vega-ZZ program was used for molecular dynamics simulations. Both molecular mechanics and molecular dynamics calculations were performed with the CHARMM22_LIG force field and Gasteiger charges using a dielectric constant $\varepsilon = 80$.

The initial conformation of SC6HH was extensively optimized with molecular mechanics minimization until convergence through a maximum of 60K cycles of conjugated gradient optimization. The resulting conformation was used as starting conformation for a 2 ns molecular dynamics simulation at 300 K. During the trajectory the calixarene molecule and in particular the alkyl chains were able to optimize its geometry overcoming small energy barriers. The final conformation obtained in the MD trajectory was finally submitted to extensive molecular mechanics minimization with a maximum of 60K cycles of conjugated gradient optimization. The final conformation obtained using this molecular modeling protocol is discussed in the text.

RESULTS AND DISCUSSION

Conductivity. The cmc's of the different calixarene-based surfactants studied in this work were determined by electrical conductivity (κ) measurements. For all surfactants κ increases as the concentration increases (see Supporting Information for an example); however, the rate of increase in κ , relative to the concentration, is different below and above the cmc. Each plot of κ against the surfactant concentration furnishes two straight lines that intersect at the concentration corresponding to the micelle formation, allowing the determination of the cmc. From the slopes of the lines it is possible to calculate the micelle ionization degree (α). Assuming that the micelles do not contribute significantly to the conductivity, α can be calculated from the ratio of the slopes of the two straight lines above and below the cmc.²² The degree of ionization can be replaced by the degree of counterion binding (β) using the relationship $\beta = 1 - \alpha$. This parameter reflects the ability of the micelles to bind counterions present in solution. The cmc's and β values obtained by electrical conductivity measurements are listed in Table 1. The cmc values obtained for SC4TB, SC4TH, and Table 1. Critical Micelle Concentration (cmc) and Degree of Counterion Binding (β) Obtained from Electrical Conductivity Data for the Five Amphiphilic *p*-Sulfonatocalixarenes Investigated in This Work^{*a*}

surfactant	n	т	cmc (mM)	β
SC4TB	4	4	3.18	0.59
SC4TH	4	6	0.491	0.61
SC4TO	4	8	0.0911	0.59
SC6HH	6	6	0.734	0.70
SC8OH	8	6	0.729	0.78

^{*a*}All data were obtained at 25 °C. n is the number of phenolic units in the macrocyclic ring, and m is the number of carbons per alkyl chain.

SC6HH are in good agreement with those obtained in previous works.^{7,9,17}

For surfactants derived from calix[4] arene (SC4TB, SC4TH, and SC4TO) the cmc decreases with increasing the number of carbons atoms in the alkyl chains. In this series, all surfactants have the same type and number of heads groups, the same type and number of counterions, and the same number of alkyl chains. The only factor that varies is the number of carbons in the alkyl chains (m). In analogy with gemini surfactants,²³ which have two alkyl chain and two head groups per molecule, the cmc values obtained here are correlated with the number of carbon atoms per alkyl chain (m) instead of the total number of carbon in the alkyl chains. The correlation between log 4cmc (4cmc is the cmc in units of mM of alkyl chain) against m presents a linear behavior with a negative slope as generally observed for conventional surfactants (see Supporting Information). It is worth noting that if log cmc is used instead of log 4cmc, the same slope is obtained but the intercept will be more negative (-0.97 ± 0.08) . From this representation a line of the type log 4cmc = A + Bm was obtained with $B = -0.39 \pm 0.02$ and $A = -0.37 \pm 0.08$. Since log cmc is proportional to the free energy of transfer, ΔG_{t} , of a surfactant molecule from the aqueous phase to the micellized state, $^{23-25}$ the negative *B* value reflects the fact that the hydrophobic interactions contributes favorably to the micellization process making ΔG_t more negative. On the other hand, the A value is generally positive for conventional surfactants²⁵ (e.g., in the case of anionic surfactants the A values usually found varies between 1.3 and 1.9), indicating that the transfer of a hydrophilic group from the aqueous bulk to the micellar phase contributes unfavorably to the micellization process. In the present case A is negative since in this correlation it includes the contribution from the ionic groups, the aromatic rings, the phenolic oxygen atoms, and the CH₂ groups of the methylene bridges. In analogy with conventional surfactants the ionic and polar groups should contribute unfavorably to the aggregation process while the CH₂ groups and the aromatic rings should contribute in a favorable way to the formation of micelles due to hydrophobic, CH- π , and π -stacking interactions.

In this work we also studied the aggregation of three *p*-sulfonatocalix[n] arenes bearing hexyl chains at the lower rim with different number of phenol units in the macrocyclic ring (SC4TH, SC6HH, and SC8OH). In Table 1 it can be observed that the calix[4] arene derivative (SC4TH) presents the lower value for the cmc (higher tendency to self-aggregate), while the calix[6] arene (SC6HH) and calix[8] arene (SC8OH) derivatives present similar values for the cmc. This behavior is contrary to what is expected since, in a general way, the tendency of oligomeric surfactants (i.e., molecules with two or more identical amphiphilic moieties linked at the level, or as close as possible, of the headgroups by spacer groups) to self-aggregate increases as the number of alkyl chains increases.²⁶ In addition, it was observed that for *p*-sulfonatocalixarenes bearing butyl chains at the lower rim the cmc decreases as the number of phenol units increases.⁹

As in the past series, it is useful to compare the obtained values for the cmc in mole of alkyl chain units (n cmc) instead of mole of surfactant units. When the values of log ncmc are represented against the number of phenolic units in the macrocyclic ring (see Supprting Information), it is observed that the tendency to self-aggregate into micelles decreases with the increment of phenolic units in the calixarene structure. Since SC4TH is blocked in the cone conformation¹⁷ and, as we will show later, the cone conformation is preferred in the micellar state, the higher tendency of SC4TH to aggregate can be interpreted in terms of preorganization effects (there is no unfavorable energetic cost related to conformational changes). On the other hand, SC6HH and SC8OH are more flexible (due to the unhindered rotation of the phenol unit through the annulus)²⁷ and are allowed to adopt conformations other than cone in bulk solution (monomeric state). Thus, it is probable that the free energy of micellization of these two calixarenes include an extra and unfavorable term associated with the conformational reorganization into cone in the aggregates. Apparently, in the case of the most flexible SC8OH the entropic cost to fix the cone conformation is higher, and consequently, it presents a lower tendency to self-assemble into micelles. While this supposed preorganization effect is effective in the case of *p*-sulfonatocalixarenes bearing hexyl chains, according to ref 9, it is not observed for their analogues bearing butyl chains. The aggregation of surfactants is a complex process and results from the balance of several weak interactions. In the latter case increasing the number of phenol units (and thus alkyl chains) seems to be more important to the micellization process than the preorganization effect.

Table 1 also shows the obtained values for β . As can be observed, for the calix 4 arene derivatives, β is independent of the alkyl chain length. This suggests that the hydration of the surfactant headgroups does not change with chain length. The β values are associated with the capacity of the micelles to bind counterions, and in general, higher values of β arise from a closer packing of the headgroups and a higher surface charge density at the micelle-solution interface.²⁸ So the results indicate that all amphiphilic calix[4] arene derivatives studied here present a similar surface charge density and suggest that the aggregates should present similar geometries. On the other hand, the β values increase with increasing the number of phenol units in the calixarene structure. This increase in the surface charge density indicates that amphiphilic calixarenes with larger macrocyclic ring and higher flexibility form more compact aggregates than those with more rigid frameworks.

¹H NMR study of Calix[4]arene Derivatives. The ¹H NMR spectra of all amphiphilic calix[4]arene derivatives show a pair of doublets for the methylene protons (H_{exo} and H_{endo}) at concentrations below the cmc, indicating that those calixarenes adopt the cone conformation.²⁷ In addition, it had been shown that when the alkyl groups present in the lower rim of tetra-*O*-alkylated calix[4]arenes has 3 (i.e., propyl) or more carbons the rotation of the benzene unit through the annulus is inhibited.²⁷ So the calix[4]arene derivatives are immobilized in the cone conformation. As can be observed in the ¹H NMR spectra of SC4TB (see Supporting Information), when the concentration

increases above the cmc, the chemical shifts of the protons H_{endo} , OCH₂ are displaced upfield while the chemical shift of H_{exo} remains constant. This behavior was previously observed,⁹ and it was suggested that the butyl groups form the micellar core while the calixarene rings form stacks at micellar surface. From the graphical representation of the chemical shifts of the mentioned protons against the concentration of calixarene, a cmc value of 2.3 mM was determined (see Supporting Information). This value is in good agreement with that obtained in an early work (2.5 mM)⁹ and slightly lower than the value obtained by conductivity (3.18 mM, Table 1). This discrepancy can be attributed to the fact that the first was measured in D₂O while the second was obtained in H₂O. It is known that the cmc of ionic and neutral surfactants is generally lower in D₂O since hydrophobic interactions are stronger in this solvent.²⁹

As we showed in an earlier paper,¹⁷ when the concentration of SC4TH is increased to values above the cmc, new signals for the H_{endot} OCH₂ protons appear in the ¹H NMR spectra (at 25 °C) due to the slow exchange between monomers in the bulk and in the micelles. This phenomenon is clearly observed for the proton signals subjected higher chemical shift variations upon micellization. To inspect if the slow exchange is also observed in the case of SC4TB, we decided to study the influence of the temperature on the H_{endo}, OCH₂, and H_{exo} ¹H NMR signals of this compound above the cmc (4 mm, see Supporting Information). The results show that when the temperature decreases from 25 to 5 °C, the peaks broadened, indicating that exchange rate becomes slower at that temperature. At 1 °C the signals started to resolve, but the separation between the signals of the micelle and those of free monomers is not sufficient to obtain the exchange rate constant with 2D EXSY experiments. It is worth noting that the signal corresponding to the H_{exo} protons remains practically unaffected by the exchange process. Though it was not possible to extract the exchange rate constants for SC4TB, this study seems to suggest that the monomer-micelle exchange rate become slower with increasing the length of the alkyl chains (since the magnitude of the

chemical shifts variation upon micellization is approximately the same for SC4TB as for SC4TH).

Analysis of the ¹H NMR spectra of SC4TO below and above the cmc (see Supporting Information) seems to suggests that the chemical shifts are unaffected by the aggregation process, and new signals (due to slow exchange) do not appear in the spectra at concentrations above the cmc. However, careful observation of the ¹H NMR spectra of SC4TO shows that below the cmc (0.06 mM) the signals of the H_{endo} and OCH₂ protons present considerable broadening and appear displaced to higher field (4.45 and 3.9 ppm) when compared with the signals of the same protons in the spectra of SC4TB and SC4TH monomers (4.6 and 4.1 ppm). In addition, the SC4TO signals present approximately the same chemical shifts as those observed for the micelles formed from SC4TB and SC4TH. These results, considered together, strongly suggest the presence of aggregates at premicellar concentrations.

NMR Study of Calix[6]arene Derivative below the **CMC.** The ¹H NMR spectra of SC6HH (Figure 1) show a more complex pattern than that observed for calix[4]arene derivatives. Larger calix[6] arenes display a higher flexibility and are more difficult to block into a given conformation because of the facile "through the annulus" rotation of their aromatic units.²⁷ Calix[6] arenes derivatives can adopt eight theoretical conformations, which differ in the syn or anti orientations of the aromatic units with respect to one another.²⁷ To our knowledge, only the 1,2,4-conformation has not been reported to date. In addition to the eight "up-down" conformations, numerous others in which one or more of the aryl groups project outward from the average plane of the molecule are possible. In the solid state, p-sulfonatocalix[6]arene (SC6) adopts an 1,2,3-alternate conformation³¹ stabilized by intramolecular hydrogen bonding between the three hydroxyl groups in each half of the molecule. Because of the high degree of hydration in the solid state, it is reasonable to think that SC6 adopts a similar conformation in solution. In the case of SC6HH, hydrogen bonding is no longer possible, but the ¹H NMR spectra indicate that this molecule is



Figure 1. ¹H NMR spectra of SC6HH 0.4 mM in D₂O at 25 °C. The inset show the (triangles) ArH, (squares) ArCH₂Ar, and (circles) OCH₂ regions.

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stabilized in a specific conformation, probably by hydrophobic interaction between the alkyl chains.

While the protons of the methylene bridges (ArCH₂Ar) connecting adjacent aromatic units with syn orientations appear as a pair of doublets in the ¹H NMR spectra (where the low field doublet corresponds to H_{endo} protons and the upfield doublet to H_{exo} protons), when the adjacent aromatic units are anti oriented, those protons appear as a singlet.³⁰ The ¹H NMR spectra of SC6HH were analyzed before,^{7,9} but it was not specified if the spectra were acquired at concentrations below or above the cmc. According to the authors, the signals corresponding to the ArCH₂Ar protons afforded a broadened singlet resonance assigned to a mobile conformation. As can be observed in Figure 1, our results are quite different: the ArCH₂Ar signals of SC6HH present a pattern that consists of two pairs of doublets signals (2:2:2:2) and two singlets (2:2), suggesting that the conformation adopted by SC6HH presents four methylene bridges connecting adjacent aromatic units with syn orientations (the doublet at 3.65 ppm appears overlapped with one pair of OCH₂ protons) and two methylene bridges connecting adjacent aromatic units with anti orientations. Between the eight possible conformations of SC6HH only the 1,2,3-alternate, the partial cone, and the 1,2-alternate are compatible with this combination of syn and anti methylene bridges. The H_{endo} (low field) and H_{exo} (high field) protons (of the syn methylene bridges) were assigned by COSY (Figure 2). This experiment reveals one pair of methylene bridges with higher syn character ($\Delta \delta_{obs}$ = 0.9 ppm) and another one with lower syn character ($\Delta \delta_{obs} = 0.5$ ppm).

The splitting pattern observed for the ArH (2:2:2:2:2:2) signals is characteristic of a C_s symmetrical conformation. The cross peaks in ArH region of the COSY spectrum (Figure 2) exclusively reflect correlations between protons in the same aromatic unit. The results indicate that there are three different types of aromatic rings in SC6HH, and therefore each signal represents two equivalent protons in different aromatic units. This observation combined with presence of two nonequivalent anti methylene bridges strongly suggests that the molecule should have a symmetry C_2 axis.

The signals corresponding to the OCH₂ protons (2:2:2:2:2:2) (see Figure 1) indicate that each OCH₂ group experiment a different chemical environment and that the conformation adopted by the calixarene is somewhat distorted. Besides, the appearance of a high-field and relatively broadened signals at 2.9 ppm suggests that at least one of the alkyl chains is self-included in the SC6HH cavity. Conformational stabilization by self-inclusion of alkyl groups have been observed for other calix[6]arene derivatives.³²

In order to account for the ¹H NMR splitting patterns, we propose that SC6HH adopts a pseudo-1,2,3-alternate conformation where two adjacent aromatic units, oriented in anti configuration relative to each other, are pointing outside of the average plane of the molecule in such a way that one or two alkyl chains can be (partially) self-included in the calixarene cavity (alternatively, the dynamic motions of these units could bend fast on the NMR time scale giving rise to equivalent peaks in the spectra). This structure is characterized by a C_2 axis that passes thru the anti methylene bridges (Scheme 2).

The proposed conformation for SC6HH was further supported by NOESY and ROESY experiments. The NOE cross peaks were all negative (i.e., same sign as the diagonal), and the ROESY spectra (see Supporting Information) served to rule out the possibility that any of the relevant cross peaks observed



Figure 2. Part of the 2D ¹H, ¹H COSY spectra of SC6HH 0.4 mM in D₂O at 25 °C corresponding to the ArCH₂Ar signals (up) and to the ArH signals (down). Signals corresponding to the three different types of aromatic rings are labeled in the horizontal inset corresponding to the 1D ¹H spectrum.

Scheme 2. Structure of SC6HH in the Proposed Pseudo-1,2,3-Alternate Conformation and Simplified Scheme Representing This Conformer to Facilitate the View^a



^{*a*}The circles represent aromatic units and are code-colored to identify NMR equivalent aromatic units.

in the NOESY spectra were due to chemical exchange. Figure 3 shows part of the 2D NOESY spectra of SC6HH corresponding to the regions where the ArH–ArH and ArH–ArCH₂Ar



Figure 3. Selected regions of the NOESY spectra of SC6HH (0.4 mM in D_2O , mixing time = 500 ms) showing NOE cross signals between the ArH–ArH protons (up) and between ArH and ArCH₂Ar protons (down). The labeling of the ArCH₂Ar protons in the horizontal inset uses letters S for singlet and D for doublet.

correlations appears. In Scheme 3, it is shown a model of SC6HH in the pseudo-1,2,3-alternate conformation (see Scheme 2) that was considered for the assignment of the ¹H NMR spectra. In this scheme circles with the same color represents equivalent aromatic units. The NOE cross-peaks labeled in the NOESY spectra of Figure 3 were all nicely compatible with the proposed pseudo-1,2,3-alternate conformation.

Molecular modeling calculations based in unrestrained molecular mechanics and molecular dynamics provided an optimized conformation for the pseudo-1,2,3-alternate conformation of SC6HH (see Supporting Information) (the details of this calculation are given in the Experimental Section). The interproton distances in this model were found to be fully compatible with the qualitative analysis of the NOE intensities for those cross peaks labeled in Figure 3. The model has one alkyl chain selfincluded in the cavity, a characteristic that had been discussed above. The structure is defined by two half-cavities, one of them being highly symmetric, defined by the 1, 2, and 3 aromatic rings, and the other, defined by rings 4, 5, and 6, distorted. It is worth noting that the alkyl chain that is included in SC6HH cavity is the one corresponding to the aromatic ring 4 instead of the ring 5. If the alkyl chain attached to aromatic ring in position 5 (or 2) was self-included into the cavity, the structure of the molecule should have a symmetry plane instead of a rotation axis C_{2} , resulting in different ¹H NMR spectra. To account for the 2-fold symmetry observed in the ¹H NMR spectra of SC6HH, molecular flexibility should permit the fast interconversion on the NMR time scale between the half-undistorted and half-distorted parts of the molecule, leading to the average structure depicted on Scheme 3. Such a type of dynamic equilibrium is consistent with the possibility of the aromatic unit undergo rotation through the annulus and with the observation that will be described in the next section that increasing the concentration of SC6HH toward values above the cmc produces substantial changes in the ¹H NMR spectra. On overall, the single structural model of SC6HH was found to provide a very consistent

Scheme 3. Simplified Scheme, Equivalent to That Depicted in Scheme 2, Representing the Pseudo-1,2,3-Alternate Conformer^a



^{*a*}This scheme is exposed to help the reader to visualize the correlations observed in the NOESY spectra. Small circles represent protons or groups or protons. Ar stands for aromatic, S for singlet, and D for doublet.

explanation for most of the characteristics observed in the NMR spectra.

¹H NMR Study of Calix[6]arene Derivative above the CMC. The effect of increasing the concentration of SC6HH toward values above the cmc (see Supporting Information) produces substantial changes in the ¹H NMR spectra. At intermediate concentrations it is possible to observe the appearance of new peaks in the spectra assigned to the aggregates and the presence of the signals of the monomer, suggesting that for SC6HH exchange between monomers in the bulk and in the micelles is slow on the NMR time scale. At sufficiently high concentrations (10 mM) only the signals corresponding to the aggregates are observable. Judging by the simplicity of the spectra, it is evident that in the aggregates SC6HH adopts a more symmetrical conformation. The two signals that appear in the ArCH₂Ar and OCH₂ regions were found to have relative areas of 1:3. Considering this pattern, the first signal can be assigned to the H_{endo} protons while the second is assigned to the H_{exo} and OCH₂ protons, indicating that SC6HH adopts the cone conformation in the aggregates. This conformation is expectable since in the micelles the alkyl chains should point to the hydrophobic interior while the sulfonate groups remain in contact with the solvent. When SC6HH changes from the pseudo-1,2,3-alternate conformation, in bulk solution, to cone, in the micelles, the aromatic units should undergo a "through the annulus" rotation. This conformational conversion demonstrates that SC6HH is not blocked in a pseudo-1,2,3-alternate conformation though this structure is stabilized, probably, by hydrophobic interactions between the alkyl chains. Conformational changes induced by self-assembly have been observed for other calix[6] arenes derivatives such as in the case of hydrogenbonded molecular cages.³³

¹H NMR Study of Calix[8]arene Derivative. The influence of the concentration on the ¹H NMR of SC8OH (see Supporting Information) shows that below the cmc (0.30 mM) the spectra of this highly flexible calix[8]arene derivative present a pattern compatible with a conformational mobile structure. A broadened signal at 4.15 ppm is assigned to the ArCH₂Ar protons and is compatible with rapid exchange (on the NMR

time scale) between the several possible conformations adopted by SC8OH.

As was observed for SC4TH and SC6HH, increasing the concentration of SC8OH above the cmc leads to the appearance of new signals in the ¹H NMR spectra of this molecule, again compatible with slow exchange (on the NMR time scale) between monomers in the bulk and in the aggregates. At concentrations well above the cmc (4.0 mM) it can be observed two signals (1:1) for the methylene protons separated by 0.7 ppm, indicating that in the micelles SC8OH adopts the cone conformation. Scheme 4 summarizes all the conformations adopted by the amphiphilic calixarenes studied herein both in bulk solution and in the micellized state.

DOSY. Diffusion ordered NMR spectroscopy (DOSY) experiments were used to determine the self-diffusion coefficients (D) of the five calixarene-based surfactants studied here. The experiments were performed for several concentrations of surfactant below and above the cmc. The signal attenuation obtained during the influence of a pulsed field gradient in the stimulated echo based DOSY experiment is a function of the molecular motion. For single diffusing species and when square-shaped pulsed field gradients are applied, the attenuation of the signal intensities (I) is given by

$$\ln \frac{I}{I_0} = -D \left[\gamma^2 G^2 \delta^2 \left(\Delta - \frac{\delta}{3} \right) \right] \tag{1}$$

where γ is the magnetogyric ratio of the nucleus under observation (in this case ¹H), *G* is the gradient strength, δ is the duration of the gradient, and Δ is the diffusion time. If several spectra with different gradient strengths are acquired and all other parameters are kept constant (δ and Δ), *D* can be directly obtained by representing the signal intensities against ($\gamma G \delta$)²-($\Delta - \delta/3$) and fitting data to eq 1. Above the cmc the surfactant molecules are in dynamic equilibrium between monomeric and micellar states. When the exchange kinetics is fast on the diffusion time scale, the observed diffusion coefficient (D_{obs}) is the molar fraction weighted average of the diffusion coefficients of the monomer (D_m) and the micelle (D_M):

$$D_{\text{obs}} = \frac{\text{cmc}}{[S]_0} D_{\text{m}} + \frac{[S]_0 - \text{cmc}}{[S]_0} D_{\text{M}}$$
(2)

 $[S]_0$ being the total surfactant concentration. When the exchange is slow, each signal shows the attenuation expected for appropriate diffusion coefficient, and the effects of the exchange may be neglected. In the intermediate case, for two-site exchange, the signal attenuation no longer shows a exponential dependence on G^2 but rather a sum of two exponentials.^{34–37} In this work we used the signals of the ArH protons for the determination of the $D_{\rm obs}$ since they are not subjected to variations on their chemical shifts due to the aggregation process and thus not broadened out due to slow exchange. In the case of SC6HH all signals are subjected to significant broadening above the cmc, and in this case we have used the ArH signals below the cmc and the OCH_2 + H_{exo} signals above the cmc. In all cases we observed good monoexponential decays, suggesting that eq 2 can be applied (i.e., fast exchange in the diffusion time scale). For further confirmation, the D_{obs} were obtained using distinct diffusion times, and the same value (within the experimental error) was obtained (see Supporting Information).

Figure 4 shows the obtained values for D_{obs} plotted against the concentration of surfactant. The data for SC4TH (Figure 4b) was already published in a early paper and is showed again



Scheme 4. Conformations Adopted by the Calixarene-Based Surfactants in Bulk Solution and in the Micelles

for comparison.¹⁷ In the case of SC6HH, due to slow exchange for all ¹H NMR signals, it was not possible to obtain accurate values of D_{obs} for intermediate concentrations (ca. 1–5 mM). The variation of D_{obs} with the concentration of surfactant presents the expected tendency and (except in the case of SC4TO) allows the determination of the cmc, $D_{\rm m}$, and $D_{\rm M}$. Below the cmc the solution contains only surfactant molecules in their monomeric state, and thus D_{obs} is constant and equal to D_m . The cmc is indentified in the point where the D_{obs} abruptly drops due to the presence of aggregates in solution. The cmc's determined by this technique are presented in Table 2. As can be observed, the values obtained in D₂O are, in all cases, lower than that obtained in H₂O using conductivity measurements, and this difference can explained by the fact that hydrophobic interactions are stronger in $D_2O_2^{29}$ as it was already mentioned in this paper.

In the case of SC4TO it was not possible to determine the cmc due to the lack of sensitivity of the technique at dilute concentrations, required to reach constant values of $D_{\rm obs}$. However, as can be observed, we have been able to measure the $D_{\rm obs}$ for a concentration (0.03 mM) well below the cmc determined by conductivity (cmc = 0.09 mM), and the value obtained for $D_{\rm obs} = (1.5 \pm 0.3) \times 10^{-6}$ cm² s⁻¹ is considerably lower than what is expected for monomers ($\approx 2.5 \times 10^{-6}$ cm² s⁻¹) based on the values obtained for SC4TB and SC4TH. This result can be attributed to the formation premicellar aggregates and

is in line with the observations made from the ¹H NMR experiments.

Using eq 2 along with the cmc and the $D_{\rm m}$ values (the average of the $D_{\rm obs}$ obtained below cmc), it is possible to obtain the $D_{\rm M}$ value. In this work we only calculated $D_{\rm M}$ for concentrations well above the cmc to avoid any complications that could arise from the monomer-micelle exchange process. In the case of SC4TO we assumed that for concentrations much higher than the cmc $D_{\rm obs} \approx D_{\rm M}$. Once the $D_{\rm M}$ values are known, it is possible to calculate the "effective" hydrodynamic radius of the aggregates $(r_{\rm h})$ using the Stokes-Einstein equation for spherical particles

$$D = \frac{k_{\rm b}T}{6\pi\eta r_{\rm h}} \tag{3}$$

where k_b is the Boltzmann constant, T the temperature, and η the viscosity of the solution. The calculated r_h values are presented in Table 2. As can be observed, these values are typical of micellar aggregates. Simple calculations of the length of the monomeric unit of the amphiphilic calixarenes, using ChemOffice Chem3D, with the alkyl chain fully extended give the values (*b*) presented in the Table 2. As can be observed, the calculated r_h for the micellar aggregates are much higher than the length of the surfactants. This observation suggests that the micelles have geometries other than spherical. If we assume that the micellar assemblies present ellipsoidal geometries, the



Figure 4. Variation of the observed self-diffusion coefficient (D_{obs}) against the concentration of the amphiphilic *p*-sulfonatocalixarenes studied in this work. (a) SC4TB, (b) SC4TH, (c) SC4TO, (d) SC6HH, and (e) SC8OH.

following equation applies³⁸

$$D = \frac{k_{\rm b}T}{6\pi\eta b} f(\rho) \tag{4}$$

where *b* is the minor semiaxis length of the micelle (and assumed to be equal to the length of the surfactant); $\rho = a/b$ is the axial ratio, with *a* being the length of the major semiaxis; and $f(\rho)$ is a function representing the shape factor.

For a prolate ellipsoid (rodlike), $f(\rho)$ is defined by eq 5 and for an oblate ellipsoid (disklike) by eq 6.

$$f(\rho) = \frac{\ln(\rho + \sqrt{\rho^2 - 1})}{\sqrt{\rho^2 - 1}}$$
(5)

$$f(\rho) = \frac{\arctan(\sqrt{\rho^2 - 1})}{\sqrt{\rho^2 - 1}}$$
(6)

Table 2. Critical Micelle Concentration (cmc) in D_2O at 25 °C Obtained from DOSY Experiments, Hydrodynamic Radius (r_h) Considering Spherical Micelles, and Length of the Minor (b) and Major (a) Semiaxis Considering Ellipsoidal Micelles with Prolate and Oblate Geometries

				<i>a</i> (nm)	
surfactant	cmc (mM)	$r_{\rm h}~({\rm nm})$	<i>b</i> (nm)	prolate	oblate
SC4TB	2.3	2.7 ± 0.2	1.15	6.6	3.5
SC4TH	0.32	3.5 ± 0.2	1.40	8.9	4.6
SC4TO		3.4 ± 0.1	1.65	7.8	4.3
SC6HH	0.56	2.9 ± 0.2	1.40	6.6	3.7
SC8OH	0.50	3.1 ± 0.1	1.40	7.3	4.0

By applying eqs 4-6, we have calculated the length of the major semiaxis (*a*) of the micelles assuming prolate and oblate ellipsoids (Table 2). However, the results obtained here do not permit to distinguish between prolate and oblate geometries. As commented in the Introduction, it was proposed,¹⁸ on the basis of small-angle X-ray study, that SC6HH self-aggregates into

ellipsoidal micelles. These results are in agreement with those obtained here, supporting the evidence that at concentrations well above the cmc the micelles formed from amphiphilic *p*-sulfonatocalixarenes adopt ellipsoidal geometries. We plan to study this system in more detail using DOSY experiments and a hydrophobic probe molecule, such hexamethyldisilane,³⁹ in order to obtain detailed information about the structure of these aggregates.

CONCLUSIONS

Amphiphilic *p*-sulfonatocalix[*n*]arenes self-assembled into globular micelles probably with ellipsoidal geometry as revealed by DOSY experiments. The cmc of *p*-sulfonatocalix[4]arenes derivatives blocked into the cone conformation decreases when length of the alkyl chains is increased, due to stronger hydrophobic interactions, as generally observed for conventional surfactants. Analysis of the ¹H NMR spectra of SC6HH, supported by 2D NMR experiments indicates that, in the monomeric state, SC6HH adopts an asymmetric pseudo-1,2,3-alternate conformation with a self-included alkyl chain. Since for this calixarene the aromatic units can undergo "through the annulus" rotation, it is likely that this conformation is stabilized by hydrophobic interactions between the alkyl chains. On the other hand, the higher flexible calix[8]arene derivative (SC8OH) is conformationaly mobile and exchange rapidly, on the NMR time scale, between several possible conformation. However, in the micellized state both SC6HH and SC8OH adopt the cone conformation. This aggregation induced conformational change is expected due to the globular structure of the micelles. This geometry favors the cone conformation where all alkyl chains are directed inward and the hydrophilic sulfonate groups are in contact with the bulk aqueous solution.

For *p*-sulfonatocalixarenes bearing hexyl chains at the lower rim, the cmc (in moles of alkyl chain units) increases when the number of aromatic units in the macrocycle increases. This behavior is unexpected and can be explained taking into account the conformations adopted by these amphiphilic molecules. SC4TH shows a higher tendency to micellize since it is preorganized in the cone conformation, and thus there are no conformational changes (and thus energetic costs associated with it) when the monomers are transferred from the bulk aqueous phase to the micelle. SC6HH and SC8OH change their conformation when they are transferred from the monomeric to the micellized state, and this presents an extra energetic cost that results in a relative destabilization of the micelles and cmc increase. When the aggregation tendency of SC6HH is compared with that of SC8OH, the last is disfavored, probably, due to a decrease in degrees of freedom, since SC8OH changes from his highly flexible monomeric state to a micellar environment where the molecule is conformationally constrained into the cone conformation.

ASSOCIATED CONTENT

S Supporting Information

Characterization data, typical electrical conductivity against surfactant concentration plot, graphs of the cmc plotted against the alkyl chain length and the number of phenolic units, NMR spectra, plot of the chemical shifts against surfactant concentration, energy-minimized molecular models of SC6HH, and a typical echo attenuation plot. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: luis.garcia@usc.es.

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